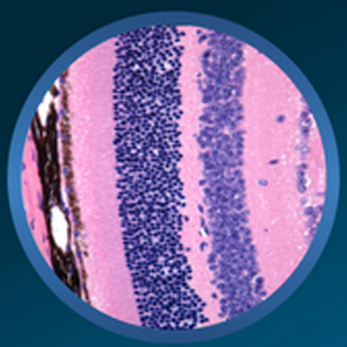
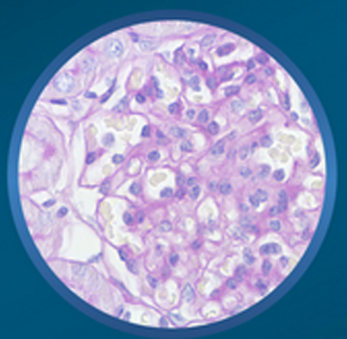
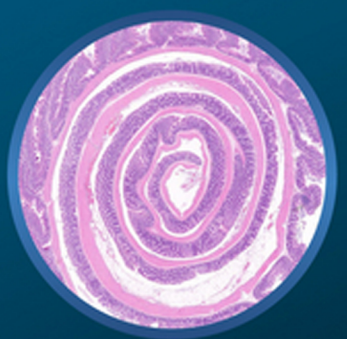
A Practical Guide to the Histology of the Mouse

Cheryl L. Scudamore







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Foreword

Mice are crucial partners in contemporary translational science and may be our most valuable species for genetic modelling of mammalian disease due to the genetic characterization of many inbred and recombinant inbred mice and of increasing numbers of genetically engineered mice (GEM). Recognizing and characterizing deviant phenotypes and developing genetically and pathophysiologically valid models of disease conditions require sophisticated knowledge of mouse anatomy and pathology. A lack of comprehensive and practical contemporary resources on mouse anatomy and pathology and scarcity of experienced mouse pathologists has hindered extensive application of histomorphology and pathology in phenotyping and other biomedical research involving mice and has led to concerns regarding adequate and accurate characterization of mouse disease phenotypes by pathology. *A Practical Guide to the Histology of the Mouse* is an ambitious and comprehensive combination of practical information for the application of mouse histology in research and phenotyping, with detailed and illustrated information on macroscopic and microscopic mouse anatomy. I expect this book to become an essential resource for diverse laboratories and programmes that use mouse models.

Now 40 years after the first transgenic mice, genetic model mice continue to increase, and ongoing international efforts to expand their genetic and phenotypic data and improve access to those data should further enhance their utility as genetic and phenotypic translational research models. The international knockout mouse consortium (IKMC) is creating genetically defined mice that carry mutations in every functional gene, aiming for about 15 000 new lines by 2020. The Collaborative Cross is another international initiative to create genetically diverse recombinant inbred strains, also for genetic modelling. Intentionally outbred mice offer additional genetic modelling options. Phenotyping of these genetic models provides the key to understanding gene functions in a complex mammalian system. The international mouse phenotyping consortium (IMPC) is conducting baseline phenotyping on all of the IKMC-generated mice, to enhance their utility to the global research community. The mouse phenome database (MPD) at the Jackson Laboratory is another important internationally contributed phenotype resource. The application of histopathology in these initiatives, to date, has been limited. This book will facilitate efforts to improve application of histopathology in research involving mice for (i) troubleshooting unexpected morbidity or mortality in research projects, (ii) characterizing disease phenotypes, and (iii) validating genetic models.

A Practical Guide to the Histology of the Mouse presents each organ system in the context of its development, its anatomic structures and functions, with specimen collection and sampling options, and common strain-, sex-, or age-related expected (background) findings. The level of detail, excellent illustrative images and insights on common artefacts and misinterpretations will be appreciated by experienced pathologists as well as those who are new to mouse pathology, or to research-specific histomorphology assessments in mice. A unique feature of this book is the valuable guidance for study design, handling specimens, records and data, which provides important insights for optimizing pathology results and pathology data in any biomedical research setting.

The editor and other contributing authors are highly experienced, published authorities in rodent pathology. They provide a wonderfully detailed, systematic and comprehensive approach that reflects their pathology expertise and their extensive experience in discovery, safety, toxicity and carcinogenicity studies. I expect this book to be widely used and recommended for explaining and teaching mouse anatomy and pathology for many years and to be one of the most useful mouse resources in many research laboratories.

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Preface

Mouse models of disease are used extensively in biomedical research with many hundreds of new models being generated each year. In addition, global consortia are working to knock out every gene in the mouse genome. Full phenotypic analysis of mouse models requires the morphologic analysis of tissue samples, which, in turn, requires an understanding of the basic histology and common background variations in the histology of mice. However there is a global shortage of pathologists trained to evaluate mouse tissues and many researchers have to attempt this analysis themselves. While it would be most appropriate to have guidance from an experienced pathologist, in reality this is not always possible.

This book is therefore aimed at veterinary and medical pathologists who are unfamiliar with mouse tissues, and scientists who wish to evaluate their own mouse models. It aims to provide practical guidance on the collection, sampling and analysis of mouse tissue samples in order to maximize the information that can be gained from these tissues. Getting the most information from each individual animal is good practice scientifically, financially and from a welfare point of view, as a contribution, by reduction, to the 3Rs.

As well as illustrating the normal microscopic anatomy of the mouse, some of the common anatomic variations, artefacts associated with tissue collection and background lesions are described and explained to help the observer distinguish these changes from experimentally-induced lesions. Methods for recording and analysing the data gained from the pathological analyses are also described. Histology is a relatively old discipline and there are many adjunct techniques (for example special stains and immunohistochemistry) over and above the standard haematoxylin and eosin (H&E) stain, which can be used to add value to the information gained from fixed tissues. In addition new histological techniques are emerging all the time. It is not possible to cover all these techniques comprehensively in this book but examples have been given where appropriate. There are also often many alternative methods for achieving similar end points and this book does not aim to be comprehensive but rather to present tried and tested methods that the authors know to work, particularly in a high throughput environment.

Inevitably, in a book of this kind there will be some omissions, some points for debate and some errors. The first two are unfortunate consequences of a book where space is limited and the nature of pathology is subjective, but we apologize for the errors. The authors would welcome constructive comments and suggestions for future editions. Readers may find some additional books helpful to fill in some of the omissions. A basic primer in mammalian histology, of which there are many, will be useful for complete novices and a recently published, comparative atlas of mouse and human histology (Treuting and Dintsiz 2011) is recommended for more detailed coverage. Although this book attempts to point out some of the common artefacts and background findings in the mouse, it is not possible to cover all of the common spontaneous pathology seen in mouse strains and additional books will also be useful in a library (Maronpot 1999; McInnes 2012).

It is important to acknowledge that this book has come about from the knowledge distilled from the numerous histologist and pathologist colleagues with whom we have worked with over the years. These colleagues are too numerous to mention individually but they know who they are and we thank them for their training, advice and wisdom. One person who does deserve a special mention is the artist from Veterinary Path Illustrations who

produced the beautiful drawings for this book. Finally we must thank our current colleagues, friends and family for supporting us in this effort over the last few years.

Cheryl L. Scudamore
Liz McInnes
Aude Roulois
Ian Taylor

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About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/scudamore/mousehistology

The website includes:

- Powerpoints of all figures from the book for downloading
- PDFs of tables from the book for downloading

Chapter 1

Necropsy of the mouse

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Necropsies on mice are a fundamental part of the research process (Fiete and Slaoui 2011) and it is vital that, in every laboratory where animal research is conducted, prosectors (persons who perform necropsies or post mortem examinations) are trained to perform a complete mouse necropsy.

Necropsies on mice are performed for a number of reasons including harvesting of tissues for research, health surveillance and investigation of disease. The process may involve collection of tissues for pathology (e.g. for phenotyping or analysis of research models), but also collection of appropriate samples for microbiological and parasitological examinations for disease identification. Autolysis after death begins immediately after the onset of hypoxia as a result of cessation of blood flow (Slauson and Cooper 2000). Autolysis of the small intestine will commence within 10 minutes of the death of the animal, resulting in the swelling of villus tips and epithelial denudation of the villi (Pearson and Logan 1978a,b). Bone marrow and adrenals are also susceptible to rapid autolysis. Storage of mouse carcases in a refrigerator (2–4 °C) is recommended to avoid rapid autolysis, which may occur in the warm atmosphere of an animal house.

A systematic approach to the mouse necropsy, which allows the examination of all the tissues in the animal in the most expedient manner, is recommended (Slaoui and Fiette 2011). In this chapter, the authors describe a recommended protocol, but variations on this method may exist, depending on the target organ or disease model. It is important to conduct a complete necropsy and to avoid the temptation of just looking at the organs of interest or selection of organs (Seymour *et al.* 2004). Necropsy results should always be viewed in conjunction with ante mortem clinical signs and haematology and biochemistry results. It is advisable to prepare all instruments, sample collection materials, camera and forms before beginning the necropsy (Knoblaugh *et al.* 2011). Necropsy personnel should always have access to the experimental study plan so that they can collect particular tissues that are pertinent to the study (Fiette and Slaoui 2011).

Mouse necropsies carry risks of zoonoses, allergen exposure and exposure to hazardous materials

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such as formalin (Fiette and Slaoui 2011). Appropriate equipment is thus necessary to conduct the mouse necropsy procedure. This includes equipment to conduct appropriate and ethical methods of euthanasia, if the mouse is still alive. Different methods of euthanasia of laboratory mice include carbon dioxide asphyxiation, barbiturate overdose and cervical dislocation (Seymour *et al.* 2004). Carbon dioxide asphyxiation is a rapid and humane form of euthanasia for mice over the age of seven days (Seymour *et al.* 2004), but can result in significant agonal haemorrhage, which can complicate microscopic examination of the lungs. Only one mouse should be placed in the perspex container at a time and carbon dioxide gas slowly added to the chamber. A flow rate of 20% V/min CO_2 as a gradual fill or slow filling method for the chamber results in least evidence of stress in mice (Valentine *et al.* 2012). Barbiturate overdose is an effective and efficient form of euthanasia and requires the use of pentobarbital sodium (Seymour *et al.* 2004). Decapitation of adult mice should be avoided because the method has welfare concerns and may not be accepted by the ethics committee of an institute or the Home Office (United Kingdom) (Seymour *et al.* 2004). Cervical dislocation was first approved for mouse euthanasia in 1972 by the AVMA Panel on Euthanasia (Carbone *et al.* 2012). The disadvantages of cervical dislocation are that although it may be a quick and efficient method of euthanasia, it causes damage to the tissues in the cervical area and may cause the release of large amounts of blood into the body cavities (Seymour *et al.* 2004). In addition, Carbone and co-workers (2012) examined spinal dislocation and noted that of the 81 mice that underwent cervical dislocation, 17 (21%) continued to breathe and euthanasia was scored as unsuccessful.

Further equipment for mouse necropsy includes a controlled air-flow cabinet or down draft table and plastic containers of formalin and syringes. The controlled air-flow cabinet is not always available in all facilities and is not essential, however it does reduce the risk of noxious substance inhalation (e.g. formalin), or allergen exposure and it is also essential to control the spread of known pathogens or zoonotic agents from the mouse carcase. Cover slips and glass slides may also be required for the preparation of cytology and parasitology specimens. It is important to have a flat and contained area in which to perform the necropsy. A flat board made of rubber or plastic is advisable so that it can be decontaminated or autoclaved if necessary. A metal tray to hold the mouse carcase may also be useful. Furthermore, two pairs of forceps, scalpel blades and scalpel blade holder, one pair of sharp-edged scissors, disinfectant spray and racks for Eppendorf containers and other plastic containers as necessary depending on the sampling protocol will be required. In addition, paper towels are necessary throughout the necropsy procedure (Knoblaugh *et al.* 2011). Some prosectors will prefer to pin the mouse carcase to a flat cork board while others may prefer to move the mouse during the necropsy procedure. A metric ruler is important for measuring organs and lesions (Knoblaugh *et al.* 2011). Plastic containers of 10% neutral buffered formalin (NBF) or 4% paraformaldehyde as well as containers of other fixatives (such as modified Davidson's for the fixation of testes and eyes) and containers for microbiology and molecular biology samples should be present at the start of the necropsy process and should all be labelled with the correct mouse identification number. A syringe and plastic cannula or needle (22G) for perfusing the lungs with formalin should also be present at the start (Braber *et al.* 2010). Mouse adrenals, pituitary gland and lymph nodes are notoriously small and difficult to handle and the use of cassettes with foam pads or biopsy bags to store them in so that they are not mislaid is highly recommended (Knoblaugh *et al.* 2011).

1.1 Recording of findings

During the necropsy the prosector must record, in some form, all the observations made during the necropsy examination. This will provide a valuable aid at the end of the necropsy and after the histopathological examination of the slides has been conducted, to form conclusions about what abnormalities were observed and may form part of the data set for the experimental group (Chapter 2).

The observations may be written down on a specific form designed for that purpose or they may be entered into a computer data-collection program. It is a good idea to develop a checklist for the mouse necropsy procedure, which is referred to each time and on which organ systems may be ticked off as they are inspected and collected.

The correct identification of the animal is very important. The researcher must examine the information on the cage lid, the ear tag of the animal or the ear notches (Figure 1.1) or scan the microchip in order to confirm the exact identification of the animal to be necropsied. The prosector may have to consult a specific key indicating what number each ear notch represents as ear notch keys are usually specific to particular research institutions. It is also important to label all samples generated from the necropsy with the same mouse identification number. The age, sex, strain, genotype, reason for submission and study number (if appropriate) should also be recorded if they are known (Seymour *et al*. 2012) the body weight, in grams, at the time of necropsy should be recorded. Retaining the identification (ear tag, ears or chip) with the fixed organs is good practice and acts as a safeguard to ensure that organs can be accurately identified if the external labelling becomes damaged.

During the necropsy process, the prosector should make a note of the characteristics of the abnormalities observed. The abnormal organs should be identified and information about the size, site, shape (for example wedge-shaped lesion or rounded edges of organ), colour (see Chapter 2), the consistency (whether the organs are hard or soft to the touch) and borders (sharp demarcation between normal and abnormal tissue or diffuse borders) of the lesion should be recorded. In addition, the appearance of the cut surface of the abnormality should be described and the normal or abnormal contents of some of the hollow organs such as the urinary bladder and the small and large intestines should be mentioned. Information on whether the lesion is focal, multifocal, focally disseminate or diffuse should be included.

Figure 1.1 Ear notch used to identify mouse.

1.2 Bleeding technique

There are a number of efficient methods for collecting blood from mice (Hoff 2000). If blood samples are required at necropsy, cardiopuncture for blood collection may be performed by inserting the needle 2 mm right of the xiphoid bone to the level of hub and gently withdrawing the plunger (Figure 1.2) but this method can cause artefactual increases in enzymes due to damage to the cardiac muscle. Retro-orbital bleeding under anaesthesia and tail tip amputation may also be used to collect blood (Seymour 2004). Bleeding may also be performed via the anterior thoracic aperture (Frankenberg 1979). A 1 ml syringe and 23 G needle should be used and the blood should be transferred quickly to anticoagulant treated plastic 1 ml containers (for haematology) or nontreated plastic 1 ml containers for the production of serum from the clotted blood. Proficient prosectors should be able to collect between 0.6 to 0.8 ml of blood. Some workers have recommended exsanguination from the abdominal aorta (Fiette and Slaoui 2011).

1.3 Perfusion

Perfusion is recommended for certain indications to ensure minimal autolytic change and to maximize morphology and retention of antigens in the tissues.

Figure 1.2 If blood samples are required, cardiopuncture may be performed.

The decision to perfuse animals should be made after a cost/benefit analysis of the procedure. Perfusion requires more time, usually more equipment and, if not performed carefully by experienced personnel, can create more artefacts than it prevents (Chapter 4). Protocols also have to be followed carefully to ensure perfusion is completed in a humane manner (Seymour *et al.* 2004). There are a number of options for perfusion, which require varying amounts of additional equipment (Hayat 2000). The following describes a simple technique that can be used with minimal additional equipment. Briefly, the prosector should prepare two 10 ml syringes with one containing saline and the other containing the fixative of choice (usually 10% neutral buffered formalin). The mouse should be anaesthetized using a peritoneal injection of pentobarbitone (0.1 ml/10 g body weight) (Seymour *et al.* 2004). The jugular veins should then be exposed below the salivary glands and the thoracic cavity should be opened. The jugular veins are then cut and the needle of the syringe containing saline should be inserted into the left ventricle of heart. The heart should be perfused with saline and saline should soon be visible exiting from the severed jugular veins. After injecting 4–8 ml of saline, the procedure should be repeated with the fixative. The organs will stiffen and become grey in colour (Seymour *et al.* 2004).

1.4 External examination

The external examination is the first procedure to carry out on the animal's body. The prosector should examine the animal's general condition, whether it is obese, emaciated or normal and to establish whether there is evidence of skeletal muscle atrophy (Fiette and Slaoui 2011). Overgrooming or barbering and whisker plucking is common in C57BL6/J mice and may be evidence of dominance behaviour in cage mates (Sarna *et al.* 2000). It is also important to look for the presence of skin ulceration (Figure 1.3), loss or abnormalities of fur or any superficial lesions. The presence of a rough, dry, scaly skin may indicate the presence of parasites. It is advisable to examine the external openings – that

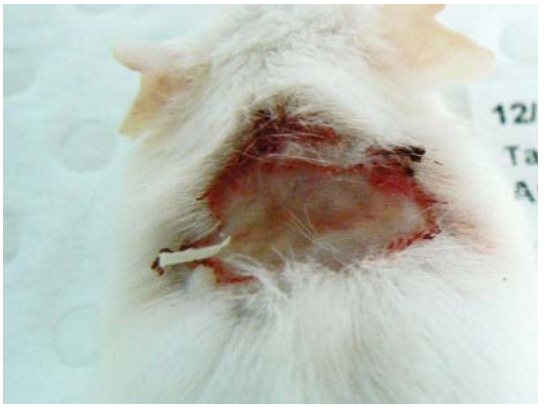

Figure 1.3 Focal ulcerative lesion in the skin noted in scapula region of mouse.

is the eye, ear, mouth and urogenital orifices – for the presence of blood or discharge. Mice of the C57BL/6 strain are susceptible to ulcerative dermatitis (severe skin lesions) and may show an incidence of greater than 20% (Sundberg and King 1996). If phenotyping of the mouse is required, the prosector should always collect a skin sample from the same area of the body such as the thorax (Seymour *et al.* 2004) and the skin should be placed on cardboard before being placed in 10% neutral buffered formalin to avoid curling and distortion.

Examination of the skin should include a search for the presence of traumatic wounds (which are common in some strains of group-housed male mice) including abscesses of the face and retrobulbar region, which are common in mice and are generally caused by bite wounds becoming infected with *Staphylococcus aureus* (Clarke *et al.* 1978). Distension of the abdomen and the presence of skin or mammary gland tumours should also be noted.

Examination of the inside of the mouth is important and the prosector should make a careful note of the state of the tongue, the oral mucosa, the lips and upper teeth. White mucous membranes in the mouth, may indicate anaemia. Small haemorrhages of the mucosa may be noted on the gums and this may indicate the presence of an infectious disease or toxaemia. The prosector should also examine the oral mucosae for the presence of ulcers and blisters or vesicles. Abnormalities of the teeth include loss, overgrowth, erosion, discolouration and fractures and are all common in mice (Figure 1.4). Severe emaciation in the mouse is often linked to dental abnormalities. The presence of blood at the nares or nostrils is important and should be noted and may indicate rupture of a wall of a blood vessel within the pulmonary system.

Cataracts (opacification of the crystalline lens of the eye) (Figure 1.5) are observed in up to 25% of Swiss CD-1 mice by 28 months of age (Taradach and Greaves 1984). This lesion may be seen at necropsy and is characterisation by the cloudy-white colour of the eye. Small eyes i.e. microophthalmia (Figure 1.6) and anophthalmia are noted commonly in C57BL/6 mice (Smith *et al.* 1994). The Harderian gland is a bilobular pink, horseshoe-shaped gland located in

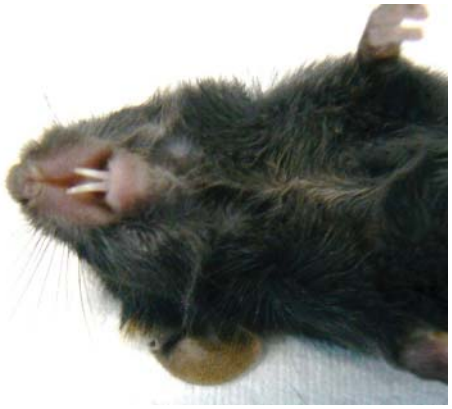

Figure 1.4 Overgrown and misaligned incisor teeth in the mouse.

Figure 1.5 A cataract in the right eye is characterisation by opacification of the crystalline lens of the eye.

the orbit of the eye of all nonprimate vertebrates and in mice this gland characteristically produces high concentrations of porphyrin (brown pigment) under hormonal control (Margolis 1971). Genetically different strains of mice manifest different amounts of porphyrin in their Harderian glands (Margolis 1971). There are marked sex differences in the Harderian gland of the C3H/He strain of mice. Female (but not male) glands contain large amounts of porphyrin (Shirama *et al.* 1981). In addition, the prepuce should be examined for the presence of inflammation, purulent material and penile prolapse, or ulceration. The scrotum should

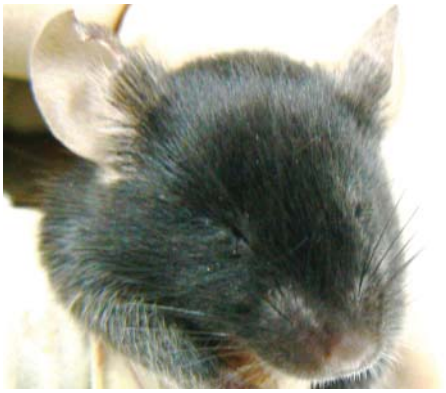

Figure 1.6 Micro-ophthalmia in the right eye of a C57 black mouse.

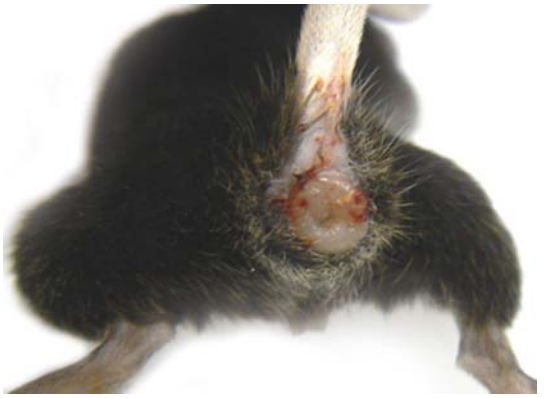

Figure 1.7 Rectal prolapse in the mouse may be observed in the perineal area.

be examined for skin lesions and enlargement and the vulva should be examined for haemorrhage and purulent discharges.

It is important to examine the perineum, which is the skin adjacent to the rectum. If the mouse has suffered from diarrhoea, the perineal area often contains small flecks of faeces, which may be blood-stained (Sundberg *et al.* 1994). Rectal prolapse can also be observed in this area (Figure 1.7). Rectal prolapse is fairly common in mice and is characterized by the presence of intestinal tissue at the rectum, often with ulceration and infection (*Helicobacter spp.* are a potential cause of rectal prolapse).

1.5 Weighing of organs

Various authors have published recommendations on the weighing of organs at necropsy (Sellers *et al.* 2007; Michael *et al.* 2007). In all cases, the organs should be weighed free of surrounding fat and connective tissues (Fiette and Slaoui 2011).

1.6 Positioning of mouse for necropsy and removing the skin

The mouse is placed on its back after spraying the abdominal surface with 95% ethanol or a disinfectant spray. The mouse limbs may be pinned to a cork board or left unpinned, as preferred by the prosector. A small cut is made at the level of the pubis using a scalpel blade or scissors, and then a longitudinal cut is made along the central midline, through to the chin. The skin is then dissected away from the body leaving the abdominal wall intact (Figure 1.8). The subcutis, superficial lymph nodes, mammary glands, penis and skeletal muscles will be apparent and should be examined at this point.

The subcutaneous tissues may display gelatinous fluid and this indicates the presence of widespread oedema (anasarca). This condition may be observed

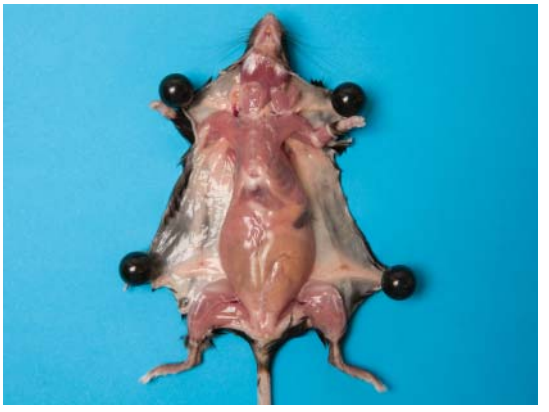

Figure 1.8 The mouse is placed on its back and a longitudinal incision is made from chin to pubis before dissecting the skin away from the body leaving the abdominal wall intact.

in cases of severe chronic renal, heart or liver failure. At this point in the necropsy procedure, it is advisable to look for generalised colour changes such as pale, white subcutaneous tissues, which may indicate widespread anaemia or yellow tissues which indicate the presence of jaundice or icterus. The lymph nodes are small, bean-shaped structures and are not easy to locate in a healthy mouse. Murine lymph nodes may become haemorrhagic and cystic with age. The position of the most commonly harvested lymph nodes in the mouse are indicated in Figure 1.9 and described by Van den Broek *et al.* (2006). Lymph nodes may become enlarged due to inflammatory processes in organ systems or due to the presence of tumours (Figure 1.10). In general, the cervical lymph nodes are situated above the submandibular salivary glands, the axillary lymph nodes are present in the axillary fossa of the forelimbs and the inguinal lymph nodes are present in the fat pad in the fossa of the hind limbs – see Vincenzo Covelli's *Guide to the Necropsy of the Mouse*, http://eulep.pdn.cam.ac.uk/Necropsy_of_the_Mouse/printable.php (accessed 17 July 2013) and Figure 1.11.

The salivary glands (submandibular, sublingual and parotid) are paired organs found in association with the paired submandibular lymph nodes in the region below the chin and adjacent to the larynx and

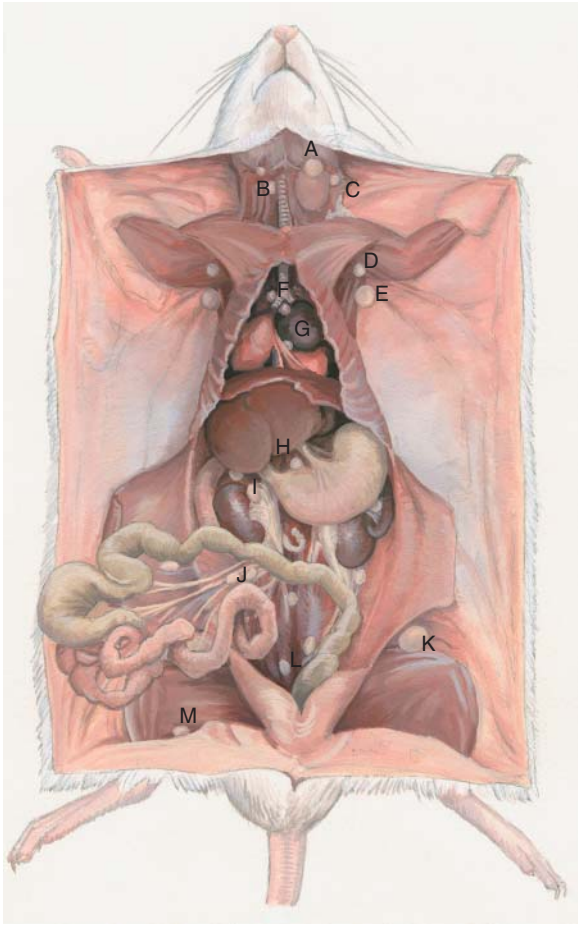

Figure 1.9 The position of the mandibular (A), deep cervical (B), superficial parotid (C), axillary (D), accessory axillary (E), tracheobronchial (F), caudal mediastinal (G), gastric (H), renal (I), mesenteric (J), inguinal/subiliac (K), medial iliac (L) and popliteal (M) lymph nodes is indicated on the diagram.

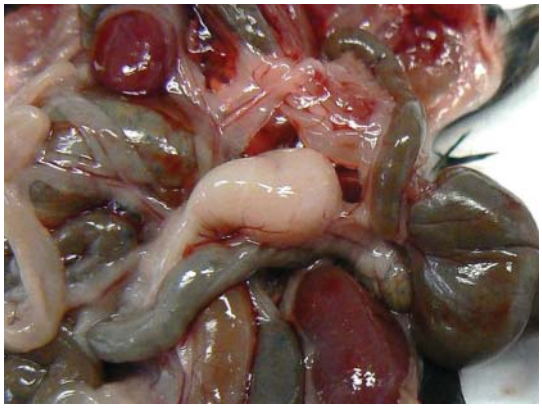

Figure 1.10 Enlarged lymph nodes as a result of lymphoma.

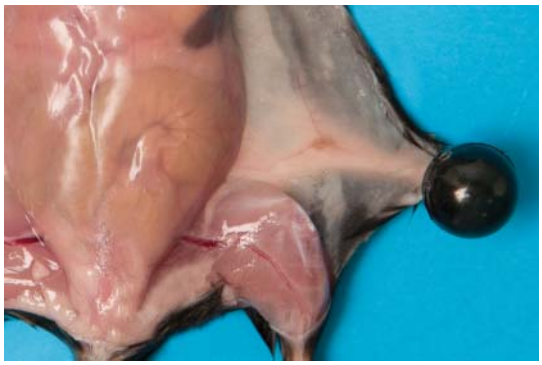

Figure 1.11 Inguinal lymph node in fat pad in the inguinal area.