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

A Practical Guide to the Histology of the Mouse, First Edition. Cheryl L. Scudamore.

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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

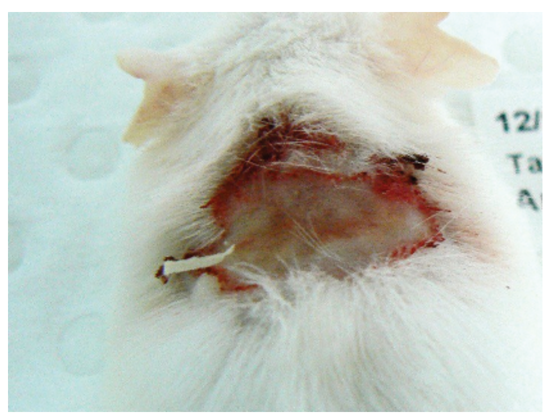

Figure 1.3 Focal ulcerative lesion in the skin noted in scapula region of mouse.

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.

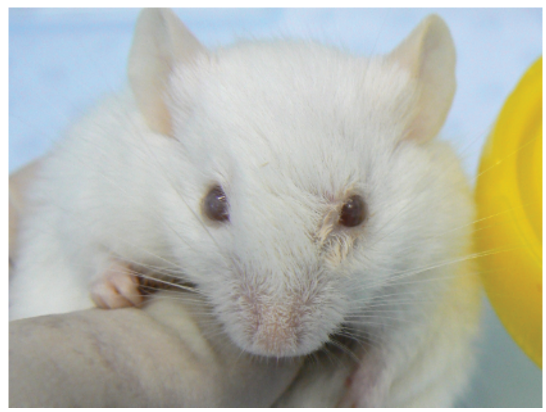

Figure 1.5 A cataract in the right eye is characterisation by opacification of the crystalline lens of the eye.

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

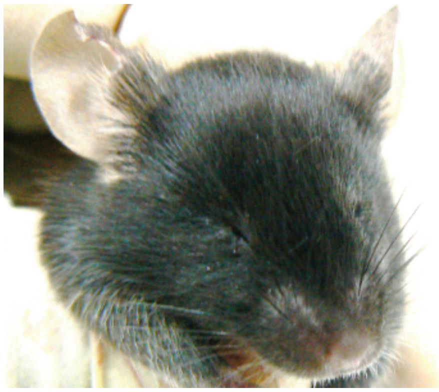

Figure 1.6 Micro-ophthalmia in the right eye of a C57 black mouse.

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.

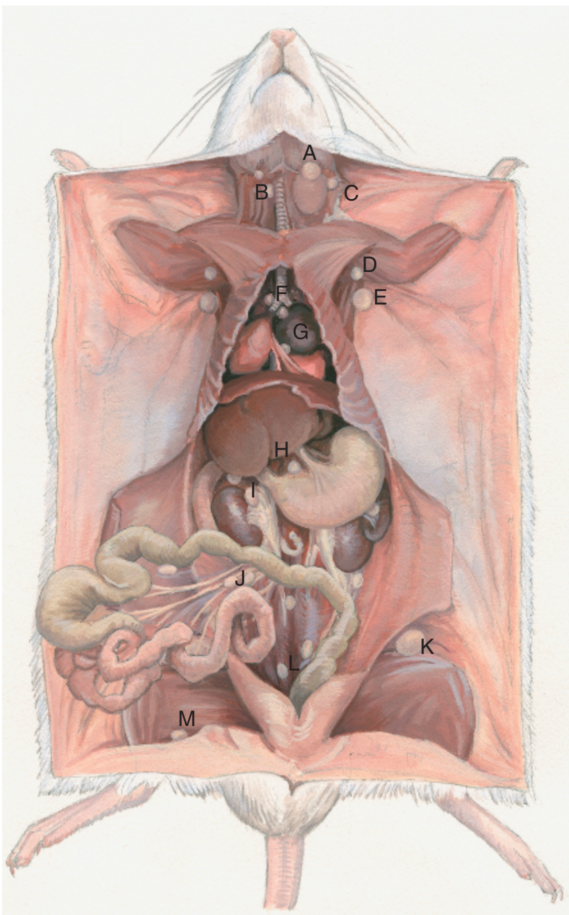

Figure 1.9 The position of the mandibular (A), deep cervical (B), superficial parotid (C), axillary (D), accessory axillary (E), tracheobronchial (F), caudal mediastinal (G), gastric (H), renal (I), mesenteric (J), inguinal/subiliac (K), medial iliac (L) and popliteal (M) lymph nodes is indicated on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

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

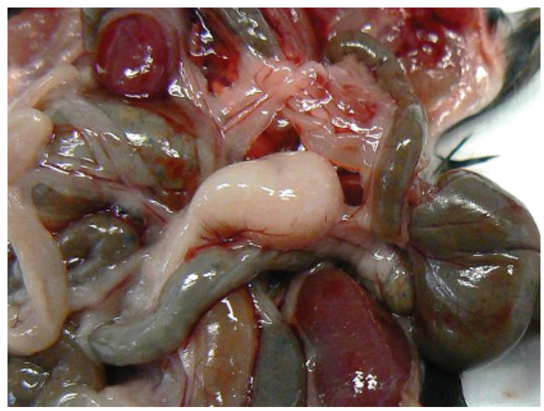

Figure 1.10 Enlarged lymph nodes as a result of lymphoma.

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

Figure 1.12 The salivary glands are paired organs found in association with submandibular lymph nodes below the chin.

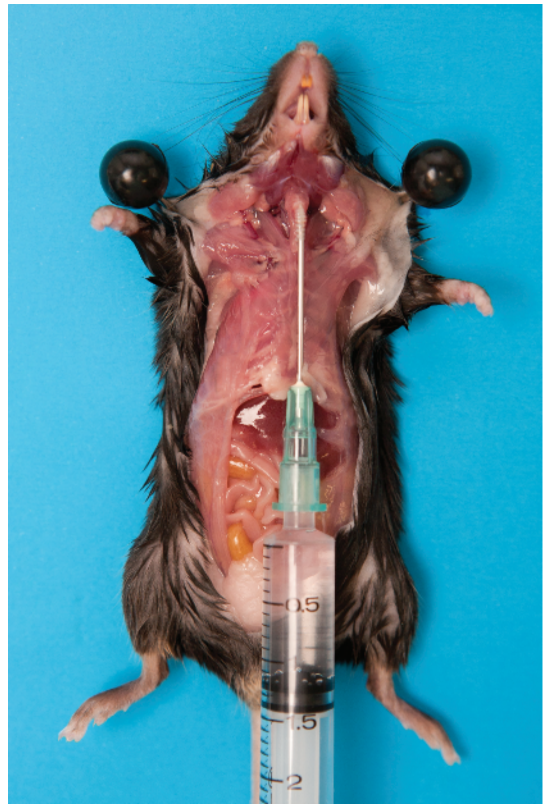

Figure 1.13 A nasotracheal wash should be performed during routine health monitoring.

cranial trachea (Figure 1.12). The submandibular salivary glands are large and are situated in the central region of the neck (Fiette and Slaoui 2011). The sublingual glands are situated above the submandibular glands and the parotid salivary glands are located laterally and extend to the base of the ear (Fiette and Slaoui 2011). In intact adult male mice, the submandibular salivary gland is twice the size of the salivary gland in female animals (Frith and Ward 1988). Once the salivary glands have been removed, the trachea is exposed. The prosector may wish to perform a nasotracheal wash at this point if an upper respiratory tract infection is suspected. Scissors or forceps should be used to raise the trachea for a nasotracheal wash. A nasotracheal wash should be performed (Figure 1.13) during routine mouse health monitoring and also if infection is suspected in the lungs or upper respiratory tract. A detailed method for performing a nasotracheal wash is described in McInnes *et al.* (2011).

There are five pairs of mammary glands in the female mouse and these are situated within fat pads, laterally, on both sides of the animal, immediately in front of and behind the forelimbs and immediately cranial to the hindlimbs and in the inguinal canal region (Figure 1.14). The mammary glands are easy to see when the female mouse is lactating (Knoblaugh *et al.* 2011). When the mammary glands are completely developed, they may extend through to almost all of the subcutaneous tissues apart from some areas of the back (Fiette and Slaoui 2011) (Figure 1.15). Mammary tumours that manifest as hardened or cystic masses may be observed at a high incidence in some strains of mice.

The clitoral glands in the female animal and the preputial glands in the male mouse should

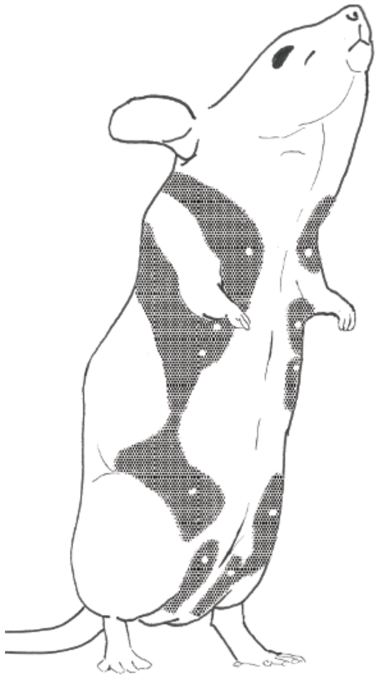

Figure 1.14 Location of mammary tissue in the female mouse. There are five pairs of mammary glands in the female mouse and these are situated within fat pads, laterally, on both sides of the animal, immediately in front of and behind the forelimbs and immediately cranial to the hindlimbs and in the inguinal region.

Figure 1.15 Lactating mammary tissue may extend to almost all of the subcutaneous tissues.

be examined at this point. Clitoral or preputial glands are modified sebaceous glands, which are leaf-shaped, grey in colour and soft in consistency (Slaoui and Fiette 2011). Abscessation of the preputial glands is common in male mice (Suwa *et al.* 2001) (Figure 1.16). The preputial glands are situated between the penis and rectum in the male animal. In the female mouse, the clitoral glands are found in the same region, cranial to the vulva.

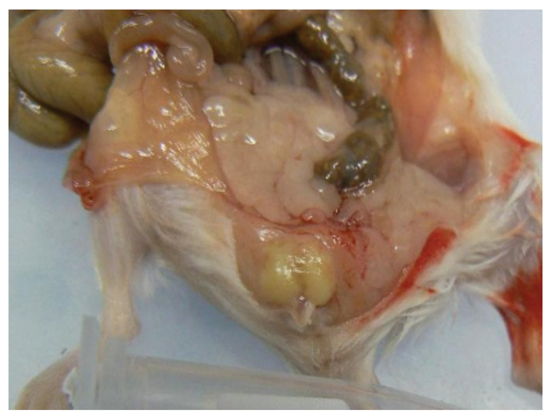

Figure 1.16 Abscessation of the preputial glands is common in the male mouse.

1.7 Opening the abdominal cavity and exposing organs

The abdominal cavity is opened by grasping a small piece of muscle in the midline of the abdomen with the forceps. The muscle is then lifted and a small incision is made with the scissors. One blade of the scissors is then inserted into the abdominal cavity and the incision is continued along the midline to the xiphoid bone cranially and the pubis caudally. It is advisable to split the pelvis in two in order to expose the reproductive organs,

Figure 1.17 The abdominal musculature may be sectioned on either side to expose the abdominal organs.

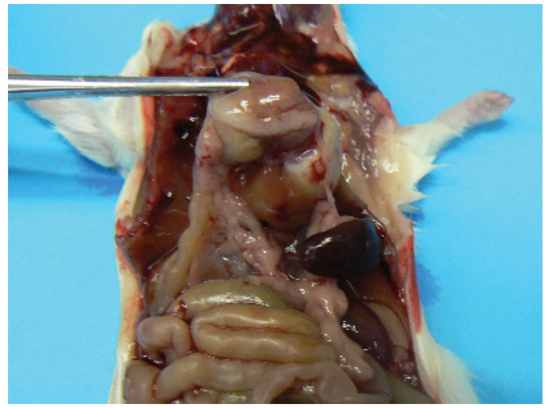

Figure 1.18 Lift the stomach and examine the spleen and pancreas found beneath the stomach.

rectum and urinary bladder. The abdominal musculature may be sectioned on either side, adjacent to the ribs, in order to expose the abdominal organs (Figure 1.17). Before removing any abdominal organs, the prosector should examine the abdominal cavity for the presence of organs in unusual positions, distension of the intestinal tract and for the presence of fluids, blood or adhesions between organs. Adhesions between organs are generally as a result of a previous inflammatory process such as peritonitis. Acute peritonitis tends to manifest with the presence of a yellow, often cloudy fluid with occasional strands of nonadherent, yellow material (fibrin) present. The presence of clear fluid in the abdominal cavity may indicate either kidney, heart or liver failure and may also be observed in cases of lymphoid tumours. The presence of blood or bloodstained fluid within the abdominal cavity may indicate rupture of a major blood vessel, haemorrhage from ovarian cysts in ageing female mice, rupture of the urinary bladder in male mice with urethral obstruction secondary to traumatic damage to the external genitalia or rupture of the spleen due to trauma.

After the initial viewing of the exposed abdominal organs, it is advisable to lift the stomach and to examine the spleen and pancreas found beneath the stomach (Figure 1.18). The spleen in the mouse is generally shaped as an elongated oval and is connected to the stomach. The spleen in immunodeficient mice may be very small in comparison with that of an immunocompetent mouse. The spleen should be grasped with forceps and removed. Occasionally ectopic splenic tissue may be observed in the mesentery of the gastrointestinal tract. Black mice of the C57BL/6 strain may demonstrate a blackened portion of the spleen (Figure 1.19) due to the presence of melanin in the red pulp (Suttie 2006). In the mouse, enlargement of the spleen (splenomegaly) is observed commonly and may indicate a tumour process (commonly lymphoma, but other tumour types also occur e.g. haemangiosarcoma), lymphoid hyperplasia or an increase in extramedullary haemopoietic cells (granulocytes or precursor erythrocytes) in response to systemic inflammatory processes or red blood cells (in response to anaemia) in the red pulp (Figure 1.20).

Figure 1.19 The mouse spleen is generally an elongated oval and in black mice of the C57BL/6 strain may demonstrate focal areas of darkening.

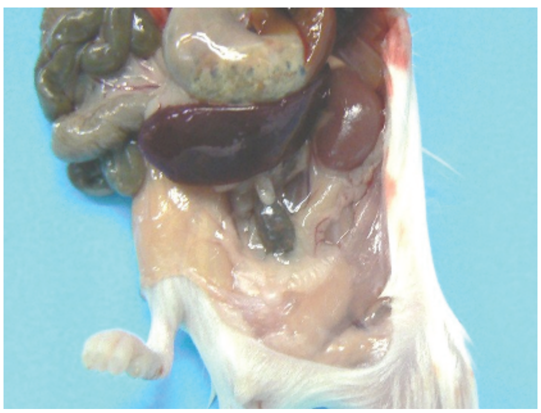

Figure 1.20 Splenomegaly is observed commonly and may indicate a tumour process or an increase in immature haemopoietic cells.

The pancreas is located throughout the mesentery adjacent to the cranial duodenum and may be removed separately or be left attached to the duodenum. The pancreas is a pink, diffuse tissue and is covered with the mesentery. The pancreas may display oedema (fluid present within the interstitium of the pancreas) (Figure 1.21) or atrophy.

The mouse liver is made up of four lobes, right, left, medial and caudate (Knoblaugh *et al.* 2011) (Figure 1.22). It is advisable to remove the entire liver and gallbladder from the abdominal cavity

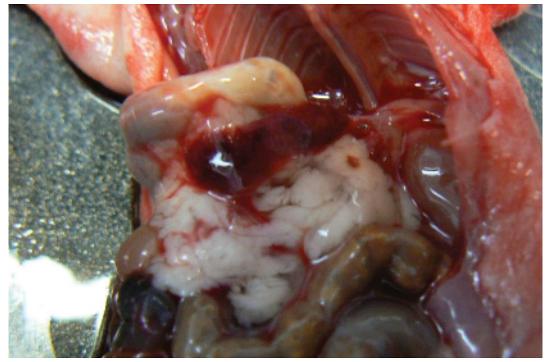

Figure 1.21 The pancreas may occasionally display oedema (fluid present within the interstitium of the pancreas).

Figure 1.22 The mouse liver is made up of four lobes, right, left, medial and caudate with the gall bladder present between the medial lobes.

to fix it whole. The normal colour of the liver is reddish brown. The liver should be examined for abnormal colour, a firm or soft consistency (a firm consistency is often indicative of scarring or fibrosis) and enlargement with rounded edges. Common lesions in the livers of older mice include a diffuse, yellow colour (fatty or lipid vacuolation of the hepatocytes), enlargement, a pitted, granular surface (generally an indication of inflammation) (Figure 1.23) and tumour masses (Figure 1.24). The gallbladder is present between the medial lobes and is generally filled with a watery, yellow fluid. A distended gall bladder may indicate anorexia and prolonged illness. Lesions in the gallbladder include

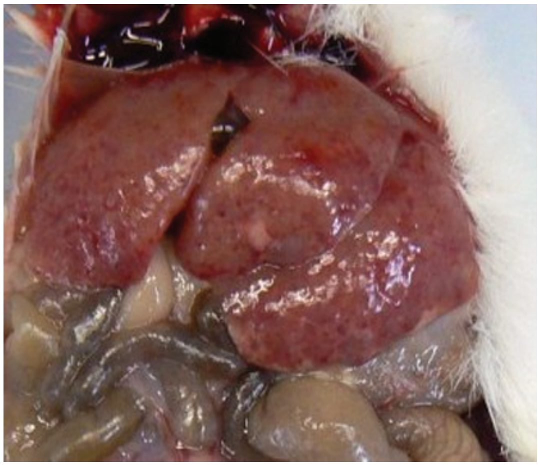

Figure 1.23 Enlargement and a pitted, granular surface of the liver are generally an indication of inflammation.

Figure 1.24 Mass in liver.

severe dilatation, which is a common change in older mice (Lewis 1984).

The gastrointestinal tract should be removed *in situ* from the abdominal cavity. This involves cutting through the oesophagus cranially, close to the insertion of the oesophagus into the stomach and cutting through the rectum caudally and gently separating the intestines from their attachments to the mesentery of the peritoneal cavity. The gastrointestinal tract should then be laid out for inspection (Figure 1.25). The prosector should examine the serosal intestinal surfaces and the intestinal contents

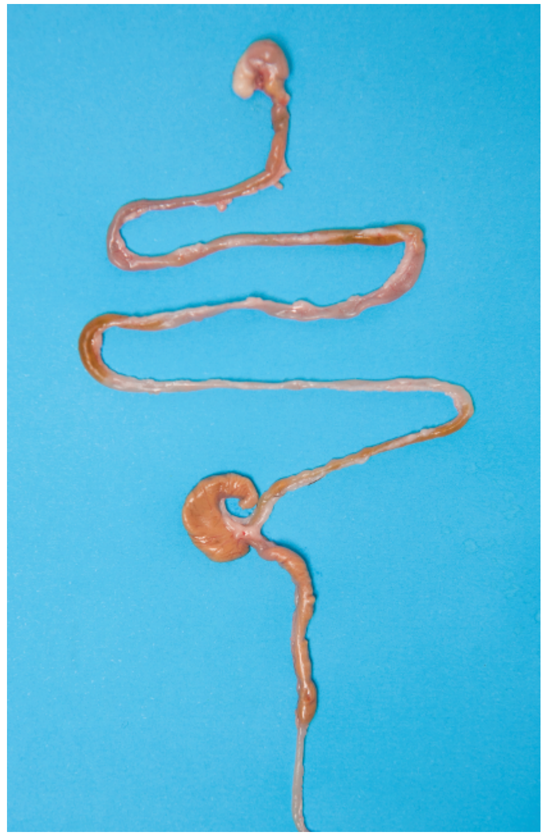

Figure 1.25 The gastrointestinal tract should be laid out for inspection.

carefully looking for evidence of dilatation, haemorrhagic and liquid contents (particularly in the colon and rectum), thickening of the intestinal walls and rupture of the tract and possible adhesions. Merkel's diverticulum is a congenital defect which causes an out pouching of the small intestine generally in the region of the jejunum (Figure 1.26). The excessive accumulation of gas in the gastrointestinal tracts resulting in severe gastric and intestinal dilatation (Figure 1.27) may be caused by obstruction of the nasal passages (Nakajima and Ohi 1977). In these cases, histopathological examination of the nasal turbinates often results in the diagnosis of a severe purulent rhinitis.

The larger mesenteric lymph nodes (situated adjacent to the junction between the caecum and colon) (Figure 1.28) should be inspected for

Figure 1.26 Merkel's diverticulum is a congenital defect which causes an out pouching of the small intestine generally in the region of the jejunum.

Figure 1.27 Excessive accumulation of gas in the gastrointestinal tract of a mouse suffering from purulent rhinitis.

enlargement and colour changes. The mesenteric lymph nodes may demonstrate the presence of lymphoma, which is very common in certain strains of mice. There is an incidence of up to 50% of lymphoma in female animals and 22% in male animals of the CD1 strain of Swiss mice (Son 2003). Phosphate-buffered saline may be used for the rinsing of the gastrointestinal tract before fixation (Knoblaugh *et al.* 2011). If a faecal flotation is to be performed later, flushing of intestinal contents with formalin (thus enabling faecal contents to be stored) using a 10 ml syringe and a 21G needle is advised.

Figure 1.28 The larger mesenteric lymph nodes situated adjacent to the junction between the caecum and colon.

The entire stomach and intestinal tract may be fixed whole, or the stomach and small and large intestines may be opened and then the entire intestinal tract laid out on cardboard (serosa side down) and fixed to prevent distortion. The prosector may also wish to inflate the intestines with formalin before fixation. Swiss rolls of intestine are created by rolling the inflated intestine in concentric centrifugal circles on piece of cardboard (Moolenbeek and Ruitenberg 1981). If the intestines are opened before fixation, the prosector should examine the intestines paying careful attention to the contents, the colour of the mucosa, the thickness of the wall and the presence of ulceration and perforation. The intestinal wall and mucosa will display multifocal, raised nodules at various points through the small and large intestine. These firm, yellowish-white nodules are Peyer's patches or aggregates of lymphoid tissue and may appear quite prominent (Figure 1.29) and are often mistaken for ulceration. In the case of lymphoma, the Peyer's patches may be enlarged.

The organs of the urinary system consist of the paired kidneys, urinary bladder, paired ureters and urethra. The kidneys are situated on either side of the vertebral column and will be observed once the abdominal organs have been removed (Figure 1.30). The adrenal glands are present at the cranial pole of each kidney (Figure 1.30). The adrenals of female

Figure 1.29 The firm, yellowish-white nodules are prominent Peyer's patches or aggregates of lymphoid tissue.

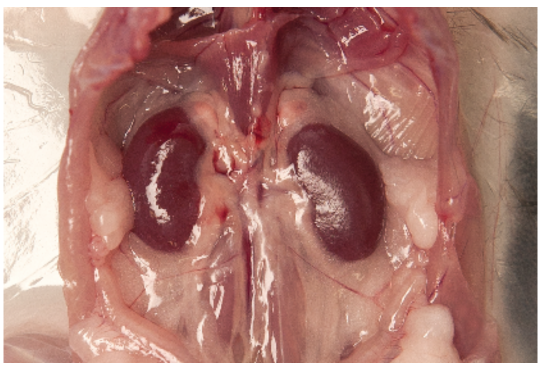

Figure 1.30 The kidneys in the mouse are situated on either side of the vertebral column and the adrenal glands are present at the cranial pole of each kidney.

mice are larger than those of male mice and are generally a pale yellow colour (in contrast to adrenal glands in the male, which are a reddish brown colour). The right kidney is always situated in a slightly more cranial position than the left kidney. The kidneys should be removed. It may be easier to remove the adrenal glands with the kidneys and to fix them together, however it is preferable to fix the adrenals separately using biopsy bags (Knoblaugh *et al.* 2011). It may be useful to use a scalpel to make a longitudinal section in the left kidney and a transverse section in the right kidney in order to distinguish left from right later in the processing procedure (Knoblaugh *et al.* 2011). The

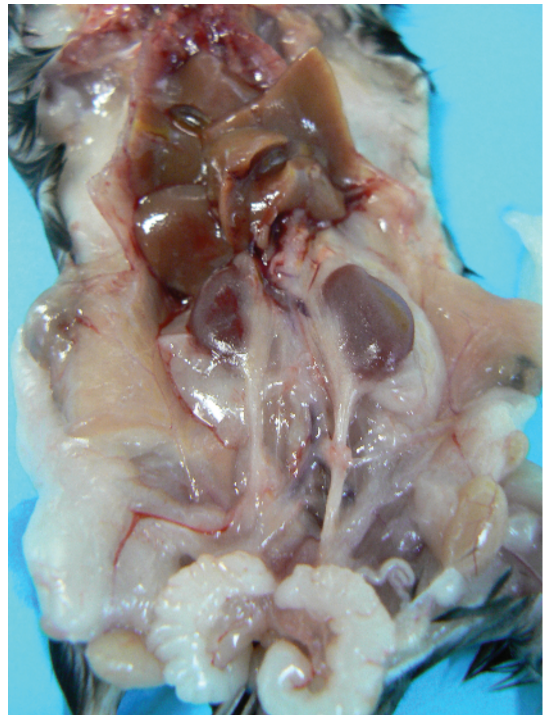

Figure 1.31 The ureters are extremely small, but resilient and may be sampled by lifting the adipose tissue on either sides of the vertebral column.

ureters are extremely small, but resilient and may be visualized by lifting the adipose tissue on either sides of the vertebral column (Figure 1.31). The kidneys are normally a reddish-brown colour and abnormalities of the kidneys include small, shrunken, firm, pale-yellow kidneys with a roughened, granular surface (Figure 1.32). This may indicate chronic glomerulonephropathy, which is common in older mice (Frith and Ward 1988). Tumour masses such as lymphoma may also be present in the kidneys. Hydronephrosis or pelvic dilatation (Figure 1.33) is a common finding in mice (Goto *et al.* 1984) and is characterized by a large, fluid-filled cyst in the normal position of the kidney and the attached ureter may also be distended with fluid. The kidneys from male animals are larger than the kidneys from female mice. Some strains of ageing C57BL/6 mice are reported to demonstrate a high incidence of chronic progressive nephropathy in both male and female mice (Zurcher *et al.* 1982).

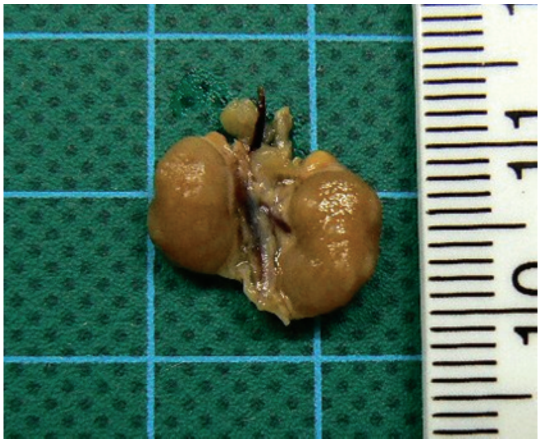

Figure 1.32 Small, shrunken, firm, pale-yellow kidneys with a roughened granular surface.

Figure 1.33 Hydronephrosis in the mouse kidney.

The urinary bladder is situated above the prostate gland in the male animal and above and attached to the vagina in the female animal. When removing the urinary bladder, it is important to look for evidence of distension, which may indicate a blockage in the urinary system. In some cases, it may be easier to remove the urinary bladder while it is still attached to the male and female reproductive organs. A urine sample can be taken from the urinary bladder using a 23G needle and 1 ml syringe, if the urinary bladder is full. Urinary bladder distension (Figure 1.34) is a common finding in the mouse and may be related

Figure 1.34 Distended urinary bladder in the mouse.

Figure 1.35 Swelling of the prepuce in the male mouse.

to uroliths or inflammation of the penis or prepuce and is often secondary to traumatic damage in group housed males (Figure 1.35). The prosector may wish to inflate the urinary bladder with formalin using a syringe and needle and to ligate the urethra to prevent the creation of artefactual folds.

The female reproductive system consists of the uterus, the paired ovaries, the oviducts and the cervix and vagina. The uterus has two horns (Figure 1.36), which are situated on either side of the vertebral column and which arise from the cervix, which is generally below the urinary bladder. The ovaries are small, round organs attached to the tips of the uterine horns and also attached to the abdominal cavity by the mesovarium – see Vincenzo

Figure 1.36 The uterus has two horns and two ovaries situated on either side of the vertebral column.

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). The ovaries may display multifocal nodules which represent follicles or corpora lutea. The ovaries may also demonstrate tumours and cysts (Figure 1.37), which will cause enlargement. The oviducts are too small to visualize but connect the uterine tip to the ovary. At necropsy, it is common to see dilated uterine horns filled with turbid fluid (Figure 1.38). Slight dilatation may be due to cyclical change related to oestrous but massive dilatation can be related to imperforate vagina with consequent hydrometra or mucometra which has been seen in 7% of virgin female BALB/c mice (Sheldon *et al.* 1980). In older mice

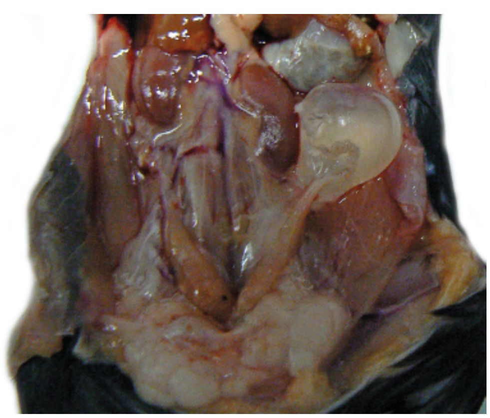

Figure 1.37 Cystic left ovary in the female mouse.

Figure 1.38 Bilateral mucometra in the female mouse uterus.

enlargement and convolution of the uterine horns is common and usually due to endometrial hyperplasia. The vagina is the short, tubular organ that leads from the cervix to the exterior. It is useful to remove the ovaries, uterus, urinary bladder and vagina together. The prosector should separate the ovaries from their attachments to the abdominal wall and pull the ovaries and uterine horns back to the level of the vagina and make an incision through the vaginal tube. Occasionally the vagina may be removed separately incorporating the external vulva.

The male reproductive organs consist of paired testes, epididymis, paired seminal vesicles and coagulating glands, prostate and penis. The mouse testes can move between the scrotum and the abdominal cavity. The male reproductive organs may all be removed together or they may be removed separately. The preputial glands are situated between the penis and rectum in the male animals and, in the female mouse, the clitoral glands are found in the same region, cranial to the vulva (Figure 1.39). Common macroscopic lesions noted at necropsy in the mouse

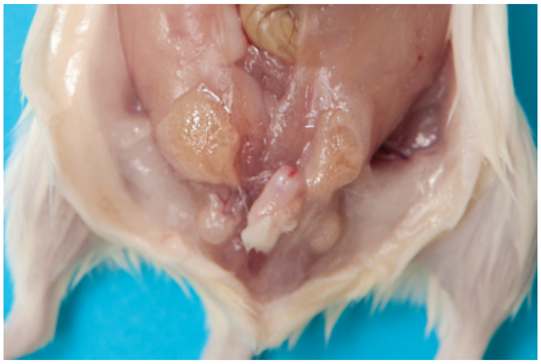

Figure 1.39 The preputial glands are situated between the penis and rectum in the male animals and in the female mouse, the clitoral glands are found in the same region, cranial to the vulva.

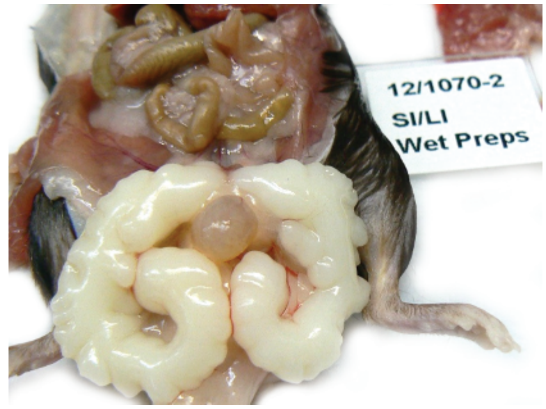

Figure 1.41 The seminal vesicles are situated on either side of the bladder and may be distended in older animals.

include bite wounds to the penis with resultant balanoposthitis (infection of the penis and prepuce), haemorrhage into the bulbourethral glands and often urinary bladder distension. This is referred to as mouse urologic syndrome and this condition may be increased in AKR mice housed in wire cages (Everitt *et al.* 1988). Preputial gland abscess is a common finding at necropsy in the older mouse (Figure 1.16). The testes are light tan in colour and may be pulled out of the scrotal sac for examination (Figure 1.40). Atrophy of one testis is common in male mice. The seminal vesicles are situated on either side of the urinary bladder and communicate with the urethra. They are generally white in colour, although older mice may show tan-yellow discoloration as well as distension (Figure 1.41) (Finch and Girgis 1974). The testes should be removed with the epididymis intact. The seminal vesicles should be removed with an incision as low as possible to avoid the loss of seminal vesicular fluid. The urinary bladder and the prostate may be removed together and the penis and associated skin structures should also be removed separately. Fixation of the testes is complex and modified Davidson's fixative is recommended over Bouin's fixative (Lanning *et al.* 2002; Latendresse *et al.* 2002) or formalin. Modified Davidson's fixative is recommended because the morphological detail is good and there is minimal shrinkage of central tubules in the testes.

Figure 1.40 The testes are light tan in colour and may be pulled out of the scrotal sac for examination.

1.8 Removing the ribcage to expose lungs and heart

The prosector should now open the thoracic cavity by lifting the sternum and making a small incision in the diaphragm and then cutting through the ribs on both sides at the point where the ribs attach to the costal cartilage (Figure 1.42). The hyoid bones at the base of the larynx should now be incised. The thoracic cavity should be carefully examined for the presence of fluid (hydrothorax) or pus (pyothorax)

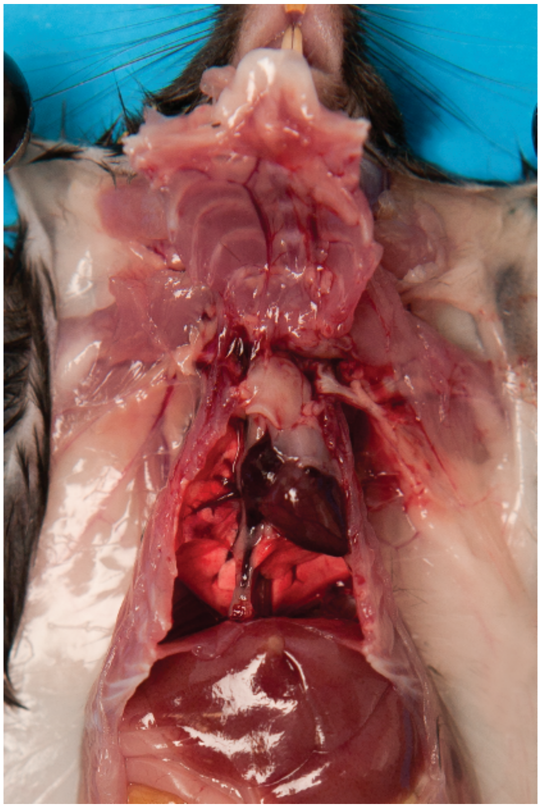

Figure 1.42 The prosector should now open the thoracic cavity by lifting the sternum (bone to which ribs are attached) and making a small incision in the diaphragm and then cutting through the ribs on both sides at the point where the ribs attach to the costal cartilage.

as well as adhesions between the lung lobes and the pleural cavity. Diaphragmatic hernias can result in strangulated liver lobes being present in the thoracic cavity.

Lung tissue consists of 90% air and 10% lung tissue (Braber *et al.* 2010). If the lung is placed, unperfused, into fixative, then collapse, deflation and disruption of the lung tissue occurs resulting in a histopathological section which resembles interstitial pneumonia and gives the impression of alveolar wall thickening and hypercellularity (van Kuppeveldt *et al.* 2000). For this reason, intratracheal instillation of 10% NBF (Braber *et al.* 2010) is recommended for optimal lung histopathological detail. Braber *et al.* (2010) have demonstrated that intratracheal instillation of 10% NBF and paraffin embedding is superior to plastic embedding and Carnoy's instillation via tracheal instillation or fixed volume fixation. The tracheal instillation may be performed after removing the lungs from the thoracic cavity or *in situ* (which prevents the collapse of the lungs which occurs when the thoracic cavity is opened and the negative pressure is removed) (Braber *et al.* 2010). In general, perfusion is performed by inserting a plastic cannula or 22G needle (attached to formalin-filled, 1 ml syringe) into the trachea. The trachea may be pinched closed around the needle or cannula or a ligature may be tied around the trachea to keep the cannula in position. The syringe plunger is then depressed to inflate the lungs with formalin. The lungs are adequately inflated when the formalin reaches the margins of all lung lobes and the lungs fill the chest cavity. The prosector should see the lungs increase in size (Figure 1.43). The trachea is then grasped with forceps to keep the lumen closed while the lungs are immersed in formalin or if necessary the ligature is tied off.

Removal, of the tongue, trachea, oesophagus, lungs and heart as a unit is recommended (Seymour *et al.* 2004; Knoblaugh *et al.* 2011). This is performed by cutting through the muscles between the mandibles, pulling the tongue through to the outside of the animal and using forceps to gently remove the entire tongue, larynx, pharynx, trachea, oesophagus, heart, thymus and lungs from the neck area and the thoracic cavity. The heart and thymus may now be removed separately from the removed tissues, by cutting the heart at its base. The thymus may be extremely small in immunocompromised mice (such as severe combined immunodeficiency (SCID) mice) or due to atrophy in older mice making it difficult to locate. In this case tissue in the expected region of the thymus at the entrance to the thoracic cavity should be harvested and kept in a separate pot of formalin or cassette so that it can be identified later.

The heart is conical in shape and is divided by the septum into right and left areas each made up of an atrium and ventricle. Occasionally, the prosector may wish to bisect the heart at necropsy

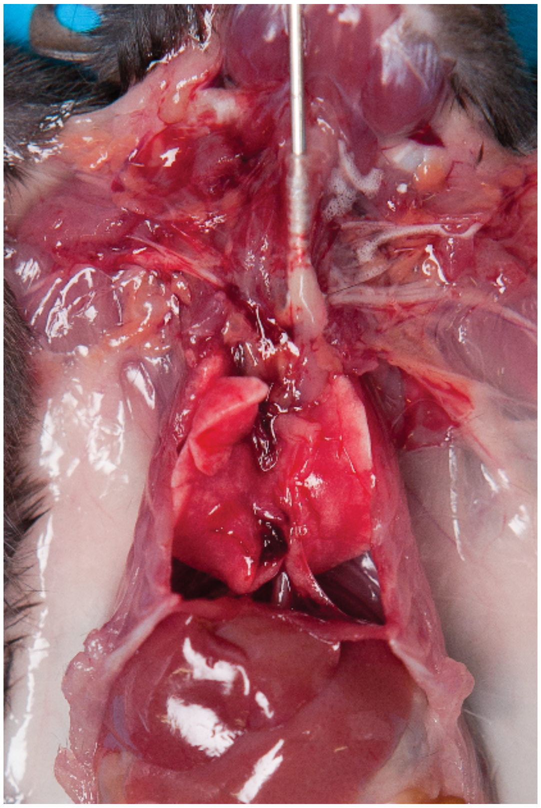

Figure 1.43 During the lung inflation procedure, the prosector should see the size of the lungs increase.

but generally the heart is fixed whole in order to avoid damage to the endocardium and valves. The pericardium should be examined for purulent exudate and fluid and the heart should be examined while attached to the blood vessels and lungs to look for congenital defects. Tumours are occasionally observed in the myocardium. Enlargement of the heart may be observed in cardiomyopathy, which is common in older mice. Cardiomyopathy is a diagnostic term used to describe a spectrum of spontaneous, age-related, degenerative changes, including degeneration, necrosis and increased interstitial fibrous tissue. The inflammatory component of these changes varies (Elwell and Mahler 1999). Left auricular thrombosis (blood clot in the heart chamber) may occur and is reportedly more common in older, breeding female mice with an

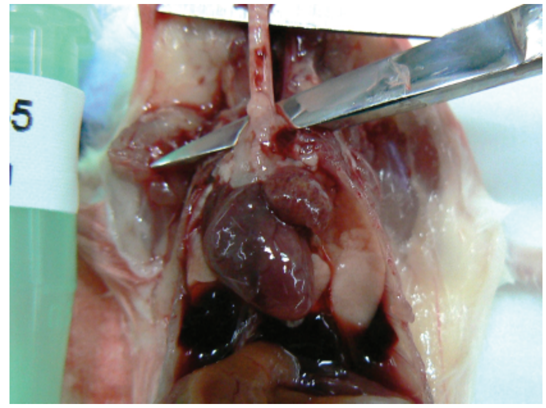

Figure 1.44 The mouse heart is conical in shape and in this animal there is a thrombus in the left atrium.

incidence of up to 66% (Meier and Hoag 1961). The thrombosis is visible at necropsy as a yellow mass within an enlarged left atrial chamber of the heart (Figure 1.44). Occasionally, the pericardium may be filled with blood due to the rupture of the atrium because of the presence of a large thrombus. BALB/c mice show epicardial mineralization (heart calcification) which increases with age with an incidence of 11% in males and 4% in female mice (Frith *et al.* 1975). The lesion may be recognized at necropsy by the presence of white, hard deposits on the epicardial surface of the heart (Figure 1.45).

The lungs are made up of four right lobes and one left lobe. The lungs should be spongy and

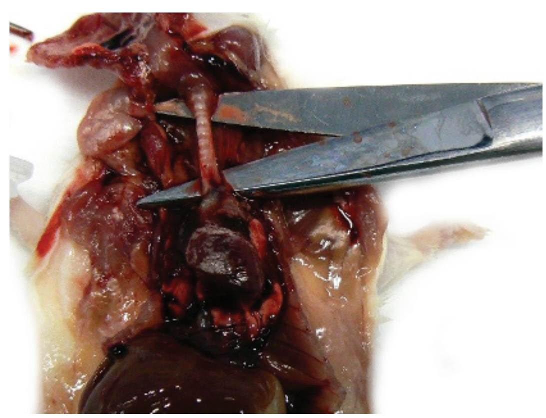

Figure 1.45 White cardiac mineralization of the epicardium of the mouse heart.

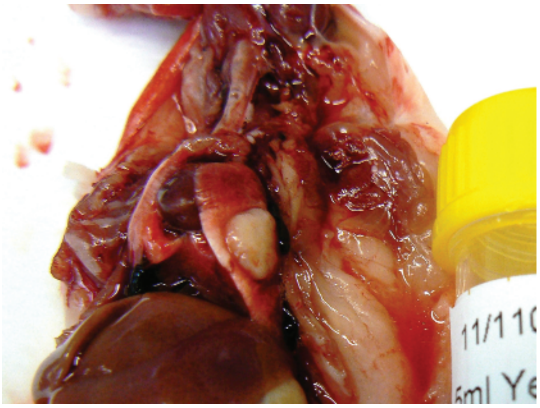

Figure 1.46 Lung adenoma is a common benign tumour of the mouse lung.

elastic in consistency. The lobes should be examined carefully for the presence of atelectasis (collapse), oedema (proteinaceous fluid within the alveoli) and pneumonia (inflammation of the lung causing a firm consistency). Forty-four percent of Swiss mice more than six months old have lung tumours (Lynch 1969). These are generally benign tumours called adenomas and are made up of the alveolar walls of the lung (Figure 1.46).

Acidophilic macrophage pneumonia is an unusual form of disease within the lungs of the black C57BL/6 mice, which results in a cellular reaction around distinctive, red crystals. Ten per cent of C57BL/6 mice are susceptible (Murray and Luz 1990). At necropsy, the acidophilic pneumonia lungs are very prominent and do not collapse upon opening of the thoracic cavity (Figure 1.47). *Pneumocystis carinii* infection is common in immunocompromised mouse strains and may result in macroscopic lesions consisting of a rubbery consistency of the lungs, a failure of the lungs to collapse and the presence of multifocal, white areas in the lungs (Treuting *et al.* 2012).

The thyroids are located on either side of the lateral and dorsal surfaces of the trachea. If the salivary glands are removed, the thyroids are visible; however each thyroid is only 2–3 mm in length and they are generally not dissected out but are left attached to the trachea. Occasionally tumours may be noted within the thyroids. The parathyroids

Figure 1.47 The lungs do not collapse upon opening the thoracic cavity in acidophilic macrophage pneumonia in the mouse.

are located within the thyroids but are not visible macroscopically (Fiette and Slaoui 2011).

A sample of the gastrocnemius muscle should be taken from the lower hind limb. The femur should be removed and bone marrow may be collected from the femur for bone marrow cytological examination (Reagan et al., 2011). In addition, the entire femoral tibial joint should be removed and fixed for examination of the joint if required.

1.9 Removing the brain and spinal cord

The head should be separated from the body by flexing the head and cutting through the muscles and spinal cord at the level of the first cervical vertebra. The prosector should then remove the eyes from the head and should ensure that a section of optic nerve is included with the eye (Fiette and Slaoui 2011). The Harderian glands should be visible in the orbital cavity behind the eyeball and are usually

Figure 1.48 The head is separated from the body before removal of the brain. The Harderian glands should be visible in the orbital cavity behind the eyeball and are usually a pinkgrey colour.

Figure 1.49 Ocular discharge in the left eye and nasal barbering.

a grey to pink colour (Figure 1.48). The eyes can be fixed in formalin, but improved morphology may be obtained with Davidson's fixative (Chapter 12). Benign and malignant tumours of the Harderian glands are common in CD-1 mice (Maita *et al.* 1988) and may cause keratitis and prolapse of the eye. Some strains and stocks of mice have a high incidence of corneal opacities (DBA/2 (29.1%), C3H (16.2%), CF1 (16.2%) and BALB/c (10.0%)) whereas others have a lower incidence (CD-1 (4.3%) and C57BL/6 (4.1%)) (van Winkle and Balk 1986). In addition, severe inflammation of the eye – inflammation of the cornea and conjunctiva – may be caused by *Staphylococcus aureus* infection (Figure 1.49). Zymbal's glands are modified sebaceous glands situated at the base of the external ear (Fiette and Slaoui 2011) which secrete into the auditory canal. A sample of the sciatic nerve should be taken after locating the nerve within the biceps femoris muscle (Fiette and Slaoui 2011).

Small, pointed scissors should be inserted into the opening at the back of the brain (foramen magnum) and two incisions made through the occipital bone and then around the edge of the brain on either side, above the opening of the ear. The incisions are extended towards the nose and a cut is made across the frontal bone to form a flap, which may be lifted to visualize the brain. The brain can be gently removed by cutting the nerves and vessels at the base of the brain, turning the skull upside down (Seymour *et al.* 2004) and allowing the brain to fall into a fixative container placed immediately below. Artefacts consisting of muscle and bone fragments inserted into the brain occur at this stage, thus caution is required. For some techniques optimal fixing of the brain requires perfusion (Knoblaugh *et al.* 2011), but this is not necessary for standard morphological assessment and the decision to perfuse must take into consideration the benefits to be gained versus the likelihood of inducing additional artefacts (see chapter 8). The brain should be examined *in situ* for the presence of inflammation (purulent material), haemorrhage, the presence of tumours and hydrocephalus (enlargement of the ventricles with thinning of the cerebral cortex). Once the brain has been removed it is important to visualize the pituitary gland (Figure 1.50). The pituitary gland remains in the sella turcica of the skull after the brain has been removed (Hagan *et al.* 2011). The gland is extremely friable and it may be advisable to section the bone on either side of the pituitary gland and to remove the pituitary gland with the bone attached and to fix the whole structure.

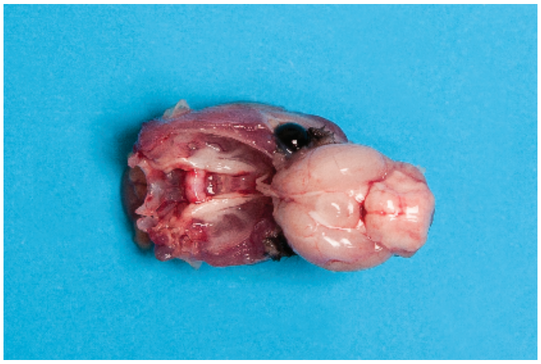

Figure 1.50 Once the brain has been removed it is important to visualize the pituitary gland.

1.10 Collecting and fixing tissue samples

In general, the histological examination of some or all tissues is necessary for the final diagnosis and thus it is important to know how to deal with taken during the necropsy. Slaoui and Fiette (2011) have reviewed the histopathology procedures from tissue sampling reviewed to histopathological evaluation. If the prosector is unable to find any gross abnormalities in a mouse that died suddenly, then it is advisable to collect small and large intestine, stomach, salivary glands, kidney, spleen, heart, lung, brain and liver into 10% neutral buffered formalin. These tissues will give the pathologist a reasonable idea about what might have happened in the animal.

All tissues should be fixed as soon as possible. Recommended fixatives include 10% neutral buffered formalin or 4% paraformaldehyde (Cardiff *et al.* 2000) or modified Davidson's solution for the fixing of testes and eyes. Approximately 20 times the volume of fixative should be used to the volume of the tissues to obtain optimal fixation (Seymour *et al.* 2004). Retaining the remainder of the carcass in formalin is good practice and will allow further analysis if necessary, for example examination of the nasal cavity if rhinitis is suspected or examination of tympanic bullae for otitis media.

Trimming is the process whereby fixed tissues collected during the necropsy are further dissected in order to fit into the embedding cassettes (Knoblaugh *et al.* 2011). If the tissue is too big for the cassette, then the cassette lid will cause impression marks on the tissue surface (Knoblaugh *et al.* 2011). Fresh tissue samples may also be frozen in OCT compound (Tissue Tek, UK) for immunohistochemistry or in situ hybridization. Excellent trimming and blocking patterns indicating how to section each tissue and which tissues should be placed together in a cassette are contained in Ruehl-Fehlert *et al.* (2003), Kittel *et al.* (2004), and Morawietz *et al.* (2004).

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