Tissue Collection for Systematic Phenotyping in the Mouse

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ABSTRACT

In this unit, a procedure for post-mortem examination of mice and tissue collection is provided. This procedure is performed for post-mortem analysis of anatomical defects (necropsy) and histological analysis and/or tissue collection destined for molecular biology applications. In both cases, tissue preservation is the major issue, but the way to achieve it depends on the objective. When histological analysis is the aim, tissue preservation is achieved by rapid transfer into fixative solutions. In contrast, molecular biology applications require rapid freezing of tissue samples to preserve mRNA integrity. Consequently, performing both procedures simultaneously may be at the expense of the final product quality. *Curr. Protoc. Mol. Biol.* 80:29A.4.1-29A.4.23. © 2007 by John Wiley & Sons, Inc.

Keywords: phenotyping • mouse • tissue collection • histology • necropsy

INTRODUCTION

A dissection for the purpose of post-mortem analysis or necropsy is undertaken to identify, at a macroscopic level (by the naked eye or with a dissecting microscope), morphological defects that characterize the mutant mouse and identify gross lesions that may contribute to morbidity and mortality. Tissues that are collected for subsequent analyses require appropriate handling and preservation to prevent their deterioration. Once death has occurred, tissues undergo a process of self-digestion (autolysis) that is accelerated by heat and post-mortem colonization of bacterial flora from the gut, respiratory system, and urinary tract (putrefaction). One major aim of necropsy is to avoid any unnecessary delay in collecting tissue samples so that they can be optimally preserved.

Incompatibilities between the requirements of tissue processing for histological purposes and tissue collection during molecular biology applications can appear when the procedures are performed simultaneously. For example, intestines are injected with fixative solution at the beginning of the necropsy procedure to preserve morphology. To avoid incompatibilities between the two procedures, it is advised to use di fferent mice for necropsy and tissue collection for molecular biology applications.

This unit provides strategic information for collecting tissues for a full histological analysis of the mouse. Tissue collection during necropsy (see Basic Protocol 1) is provided with images illustrating proper dissections at each step. Tissue collection for molecular biological analyses is presented separately (see Basic Protocol 2). A detailed description of histological analyses is presented in *UNIT 29B.4*.

CAUTION: General laboratory procedures should be followed, including not eating, chewing gum, drinking, or applying cosmetics in the work area. Laboratory coats must be worn at all times in the work area. Formalin is a carcinogen, and an eye, skin, and respiratory irritant. Avoid contact and inhalation. Work under a fume hood and wear disposable gloves. Collect used fixatives containing formalin in an appropriate waste

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container for disposal. For further information consult a local center for occupational health and safety. Clean the instruments and workstation immediately after necropsy work has been completed.

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and must follow officially approved procedures for the care and use of laboratory animals. Regulations concerning the persons allowed to sacrifice a mouse vary between countries. Local authorities should be consulted for the proper procedure.

BASIC PROTOCOL 1

NECROPSY FOR HISTOLOGICAL EXAMINATION

Mouse identification and sample labeling. Confirm the identity of the mouse, and make sure this matches the corresponding necropsy request. Use a unique mouse identification number for labeling samples and reporting findings.

External examination. The purpose of external examination is to easily record information regarding the state of health for the mouse. This information concerns the general aspects of the mouse (e.g., obese, thin, or malformed), the state of superficial tissues and organs (e.g., skin, eyes), and the natural orifices which may be informative about some inner-body pathology (e.g., the mouth: pallor of the oral mucosa is indicative of anemia).

Organ weights. Heart, liver, spleen, genital fat pads, kidneys, and total body weights are systematically determined.

Tissue dissection. Tissue dissections are not presented in complete detail, and it is assumed that the investigator or technician performing these dissections has proper training and experience in mouse dissection before attempting these analyses. Although some directions are provided for dissection, the focus of this protocol is the strategy for dissection and proper grouping of tissues. To preserve organs, use attached tissues such as ligaments and mesentery to handle the organs. Trim off adherent tissues before tissue/organs are weighed. Do not attempt to isolate small gross lesions. Additional details for trimming tissues and defining planes of section can be found in the appendix at the end of this unit.

Fixation. One important function of the fixative is to prevent autolysis and putrefaction. Immediately place fresh tissues in plastic embedding cassettes and immerse in fixative. Use a volume of fixative that is 10 to 20 times the volume of the tissue. Fix most tissues for 24 hr in 10% neutral buffered formalin before trimming and processing to paraffin embedding. For head and long bones, fix for 48 hr in 10% neutral buffered formalin; for testis, fix in Bouin's fluid for 24 hr. For further details on fixation, see *UNIT 29B.4*. Be aware that special techniques such as histochemistry, including detection of enzymes, immunohistochemistry (*UNIT 14.6*), and in situ hybridization (*UNIT 14.3*), require special fixatives and short fixation periods for optimal results.

Order of dissections. The order in which organs are dissected in the systematic necropsy procedure described below, and the grouping of organs in embedding cassettes, is summarized in Table 29A.4.1. This protocol is indicated for a mouse that has no overt clinical phenotype. However, any organ that, based on gene expression patterns or clinical findings, represents a likely target of the mutation should be proprietarily protected from autolytic damage. For example, if the hypophysis is a likely target, the necropsy should start with the head, not the subcutaneous glands as proposed below. The entire necropsy procedure should be completed in ~30 min.

Reporting necropsy findings. Use correct anatomical and medical terminology to describe the location, size, color, consistency, and distribution of any tissue abnormalities, including absence of organs (see mouse anatomy dictionary at *http://www.informatics.jax.org/searches/anatdict_form.shtml*). Keep descriptions accurate and avoid over interpretation.

Table 29A.4.1 Organs Systematically Analyzed in a First-Line Histopathological Screen[a]

Cassette	Organs
1 (male)	Preputial glands, salivary glands, pancreas
1 (female)	Mammary glands, salivary glands, pancreas
2	Stomach
3	Duodenum, distal ileum, proximal colon
4	Liver[b]
5	Spleen[b], kidney[b]
6A (male)	Right testis and epididymis (fixed in formalin)
6B (male)	Left testis and epididymis (fixed in Bouin's fluid)
7 (male)	Prostates, seminal vesicles, urinary bladder
6 (female)	Ovaries, oviducts
7 (female)	Vagina, uterus body, uterine horns, urinary bladder
8	Adrenal gland, mesenteric lymph nodes, thoracic aorta
9	Trachea, thyroid glands, oesophagus, thymus
10	Heart[b], entire lung
11	Leg muscle, tongue, BAT[c], WAT[b,c]
12	Dorsal, tail, footpad, and snout skin
13	Eye, Harderian gland
14	Knee joint
15	Brain
16	Hypophysis

[a]Organs listed according to sequence of dissection and grouping in embedding cassettes. For more information on histological screens, see *UNIT 29B.4*.
[b]Organs to be weighed before fixation.
[c]BAT, brown adipose tissue; WAT, white adipose tissue (paragenital fat pad).

A general term such as a mass is preferable to either tumor or abscess, because these more-refined diagnoses require microscopic confirmation. Diagrams should be used to provide extra information or clarity, and photographs should be taken of all grossly abnormal organs and external lesions. A ruler should also be included in the photograph for scale.

Materials

Mice
CO_2 source
70% ethanol
10% (v/v) neutral buffered formalin (equivalent to 4% [w/v] formaldehyde; e.g., Carlo Erba)
Bouin's fixative solution (e.g., Carlo Erba)
1× phosphate-buffered saline (PBS; e.g., Sigma-Aldrich)

Box with transparent walls and/or lids
Ruler
Electric shaver
Dissecting microscope equipped with a digital camera
Large containers with lids to hold fixing fluids (e.g., Labonord)
Dissection instruments, e.g., forceps, scissors (one pair dedicated to cutting bones), scalpels, razor blades

1.5-ml microcentrifuge tubes
Cork boards and needles
Plastic embedding cassettes (e.g., Labonord)
5-ml syringe and 25-G needles
Balance (e.g., Fisher Bioblock Scientific)
50-ml plastic tubes with caps (e.g., Eppendorf)
Biopsy capsules
Petri dishes
Mouse coronal brain matrices (e.g., stainless-steel for long-term use or acrylic for sporadic use; Harvard Apparatus)

Euthanize mouse by carbon dioxide inhalation

1. Place the mouse in a box with transparent walls and/or lid so that it can be observed during exposure to the carbon dioxide. Pipe CO_2 into the chamber progressively so that the mouse will be exposed to a slowly rising concentration of gas. Before proceeding, ensure death has occurred by testing for the absence of vital signs, including respiration and corneal reflex.

   The slowly rising concentration of gas ensures that the mouse will loose consciousness because of the effects of the CO_2 on the central nervous system, rather than suffocating because of immediate exposure to 100% CO_2.

Perform external examination

2. Using a ruler, measure the length of the mouse from the snout to the tail base. Record findings on each of the following:

   a. State of nutrition and dysmorphologies
      Thin mouse
      Obese mouse
      Dysmorphologies: e.g., craniofacial abnormality, polydactyly, shortened limbs or curly tail
   b. Skin and subcutaneous tissue
      Traumatic wounds
      Ulcers
      Infectious lesions
      Skin masses
      Mammary gland masses
      Oedema: swollen, smooth and glossy skin
      Condition of fur: rough, dry and hirsute in a serious chronic disease, or depilated areas
   c. Natural orifices and eyes
      i. Mouth
         Oral mucosa: pale mucosa in case of anaemia; petechia (haemorrhages of the submucosa) in infectious diseases; erosions of the mucosa, ulcers, or vesicles
         Teeth: loss, erosion or fracture of teeth (prevent adequate animal feeding and can be a cause of death). Excessive incisor growth can also prevent mice from feeding normally and can cause malnutrition.
      ii. Nasal openings
         Discharges
         Haemorrhages (epistaxis)
      iii. Eyes
         Narrowed eyelid opening (blepharophymosis)
         Corneal and conjunctival exudates and ulcerations.
      iv. Ear pinnae and external ear canals
         Discharges

v. Anal opening
Smeared by feces in animals affected by diarrhea
Rectal prolapse
vi. External genitalia
Discharges

Prepare mouse

3. Wet the fur with 70% ethanol and shave a portion of the back skin with an electric shaver (Fig. 29A.4.1A).

Shaving with a razor blade may damage superficial skin layers.

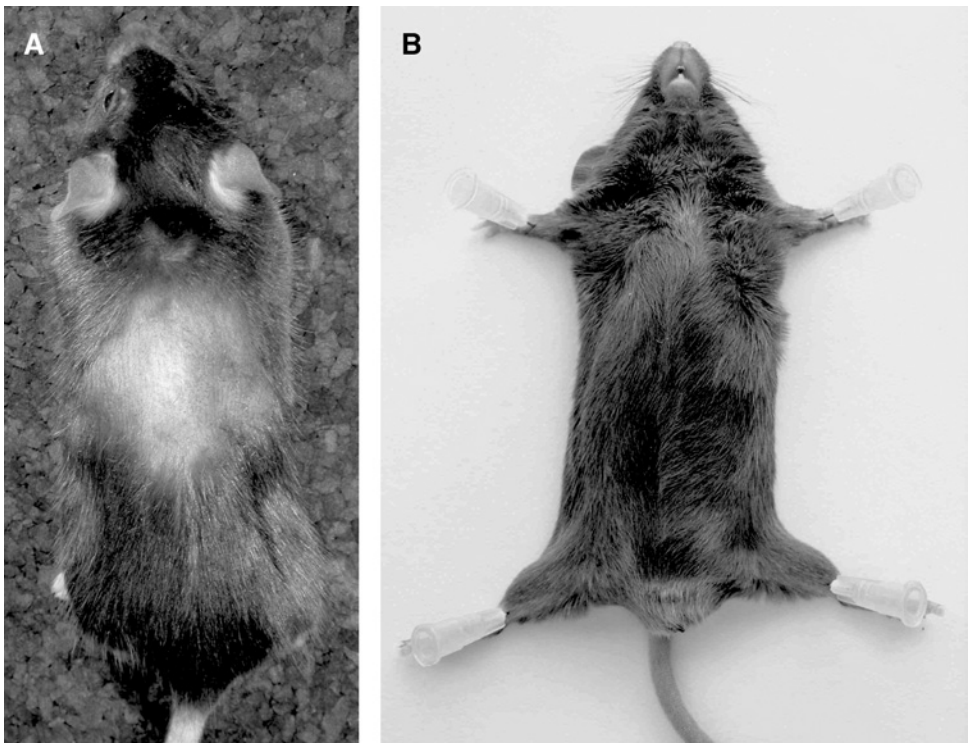

Figure 29A.4.1 Mouse with shaved back (**A**). Mouse pinned ventral-side-up to cork board (**B**).

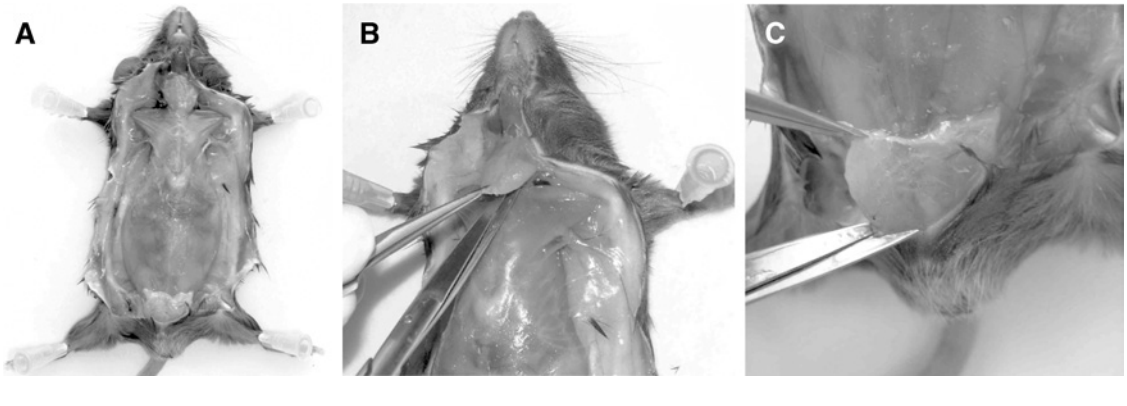

Figure 29A.4.2 Midline incision with skin reflected (**A**). Dissection of salivary glands (**B**) and male preputial gland (**C**).

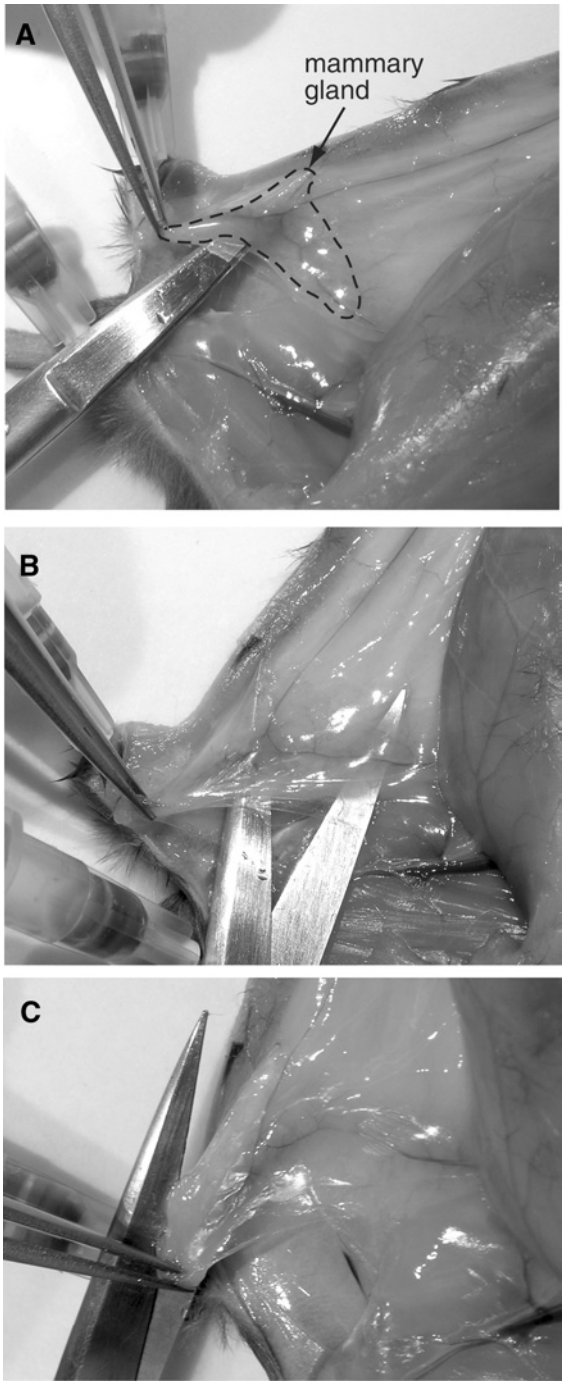

Figure 29A.4.3 Dissection of mammary gland. Insertion of scissors between the skin and the mammary gland (**A**), spreading scissors to dissect the mammary gland from the skin (**B**), and sectioning remaining adherences (**C**).

4. Collect a piece of tail in a 1.5-ml microcentrifuge tube and store at –20°C for regenotyping.

5. Lay the mouse on its back and pin the forelimbs and hindlimbs onto a cork board (Fig. 29A.4.1B).

   Access to the back will be needed later, but it is easier to shave the back before starting the dissections.

6. Wet the fur with 70% ethanol to minimize contamination with hairs and other potential allergens.

Dissect subcutaneous glands

7. Perform a midline incision of the ventral skin, with scissors, from the pubis to the chin. Extend the incision laterally towards inguinal areas to form an upside-down Y, taking care to accurately separate the skin from the underlying musculature (Fig. 29A.4.2A).

8. Dissect the three salivary glands (Fig. 29A.4.2B) and place them into cassette no. 1 (Table 29A.4.1).

9a. *Dissecting a male:* Separate one lobe of the preputial gland (Fig. 29A.4.2C) and place it into cassette no. 1 with the salivary glands.

9b. *Dissecting a female:* Detach the inguinal mammary gland from the overlying skin by scraping gently with scissors (Figs. 29A.4.3A-C) and place it into cassette no. 1 with the salivary glands.

Dissect abdomen

10. Make a midline incision through the abdominal wall muscles. Dissect the pancreas away from its insertion on the spleen and duodenum (Fig. 29A.4.4), place it into cassette no. 1, and transfer the cassette to 10% neutral buffered formalin.

11. Working in a fume hood, take up 5 ml of the formalin fixative into a 5-ml syringe. Using a 25-G needle, slowly inject into the lumen 3 ml fixative into two or three areas along the intestine (Fig. 29A.4.5), taking care not to burst it. Examine different segments of the intestine and inject additional fixative in any regions that have not been reached by the fixative. Puncture the stomach and inject fixative. Proceed with other tissue dissections while allowing stomach and intestines to fix for 24 hr.

A volume of 5 ml of fixative is usually enough to inject the entire digestive tract.

12. Dissect the liver. Cut the ligaments and omentum that connect the liver to the stomach, diaphragm, and the right kidney (Fig. 29A.4.6A-B). Weigh the liver. Collect the median lobe and half of the left lateral lobe, place them into cassette no. 4, and transfer into formalin. Place the remaining liver in a 50-ml plastic tube containing 40 ml formalin for storage.

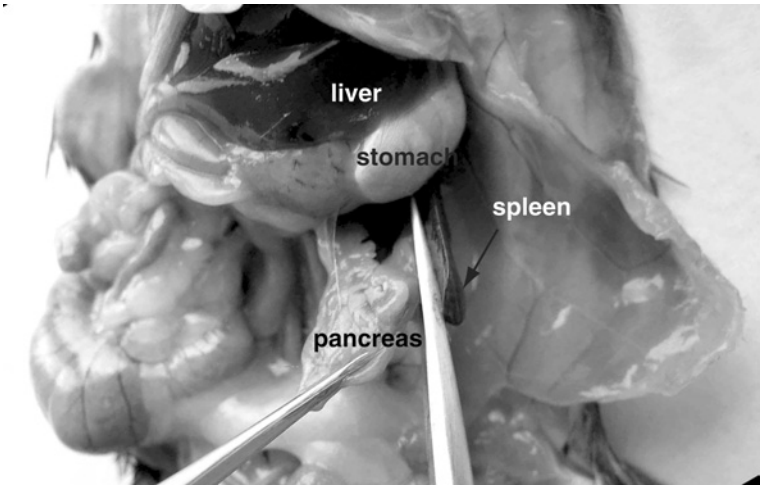

Figure 29A.4.4 Dissection of pancreas.

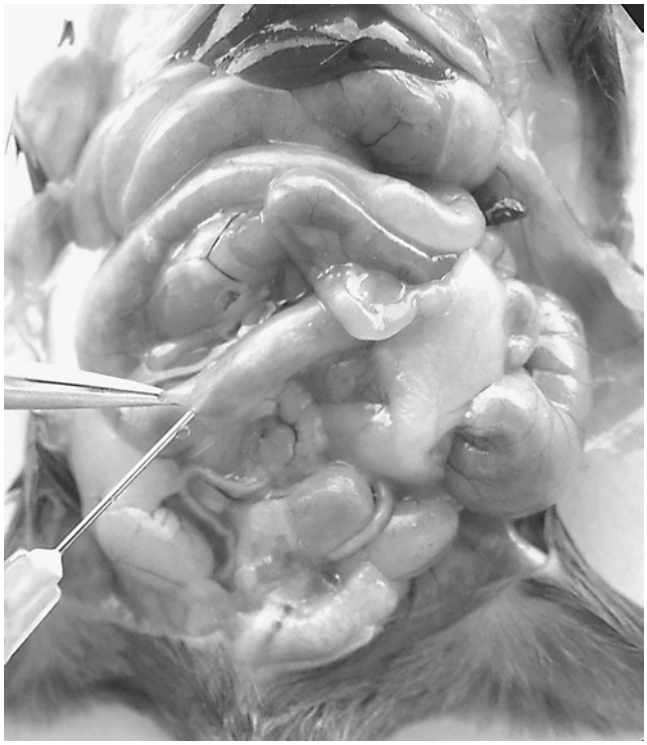

Figure 29A.4.5 Fixation of intestine.

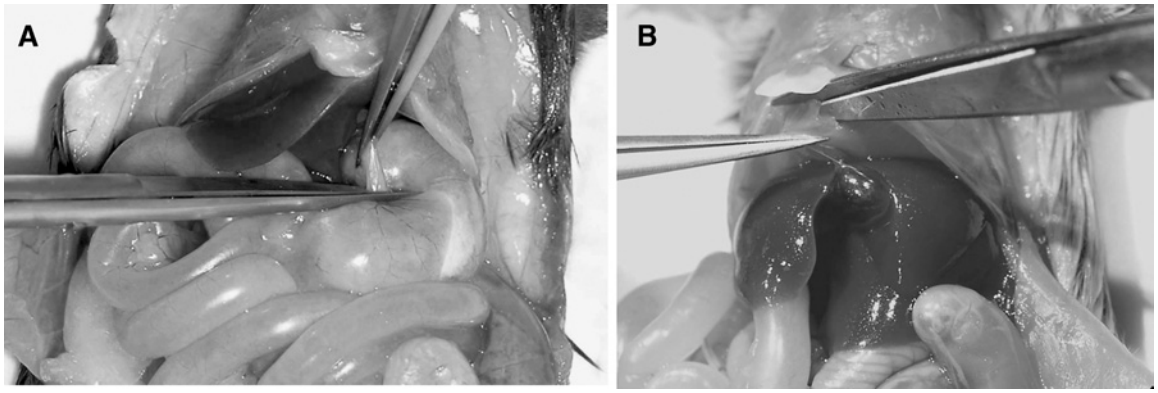

Figure 29A.4.6 Dissection of liver.

13. Remove the spleen, weigh it, and place it into cassette no. 5.

14. Make a transverse cut through the hilum of the kidney (Fig. 29A.4.7A) and separate the organ from the dorsal abdominal wall, while leaving in place the dorsal half of its capsule and the adrenal gland. Weigh the kidney. With a razor blade, make a longitudinal cut through the hilum (Fig. 29A.4.7B) and place the two moieties into cassette no. 5 with the spleen. Close the lid and transfer to formalin.

Dissecting a male

15a. Elevate the right testis from the scrotal sac by gently pulling on the paragenital fat (Fig. 29A.4.8A). Dissect and weigh the paragenital fat and place it into the same 50-ml plastic tube containing formalin.

The paragenital fat will be placed in cassette no. 11, together with brown adipose tissue, skeletal muscle, and tongue.

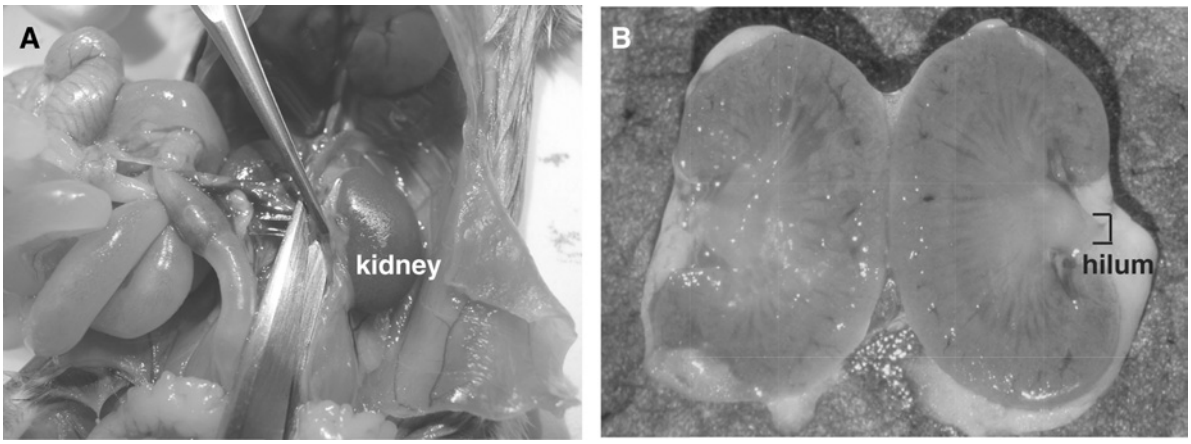

Figure 29A.4.7 Dissection of kidneys.

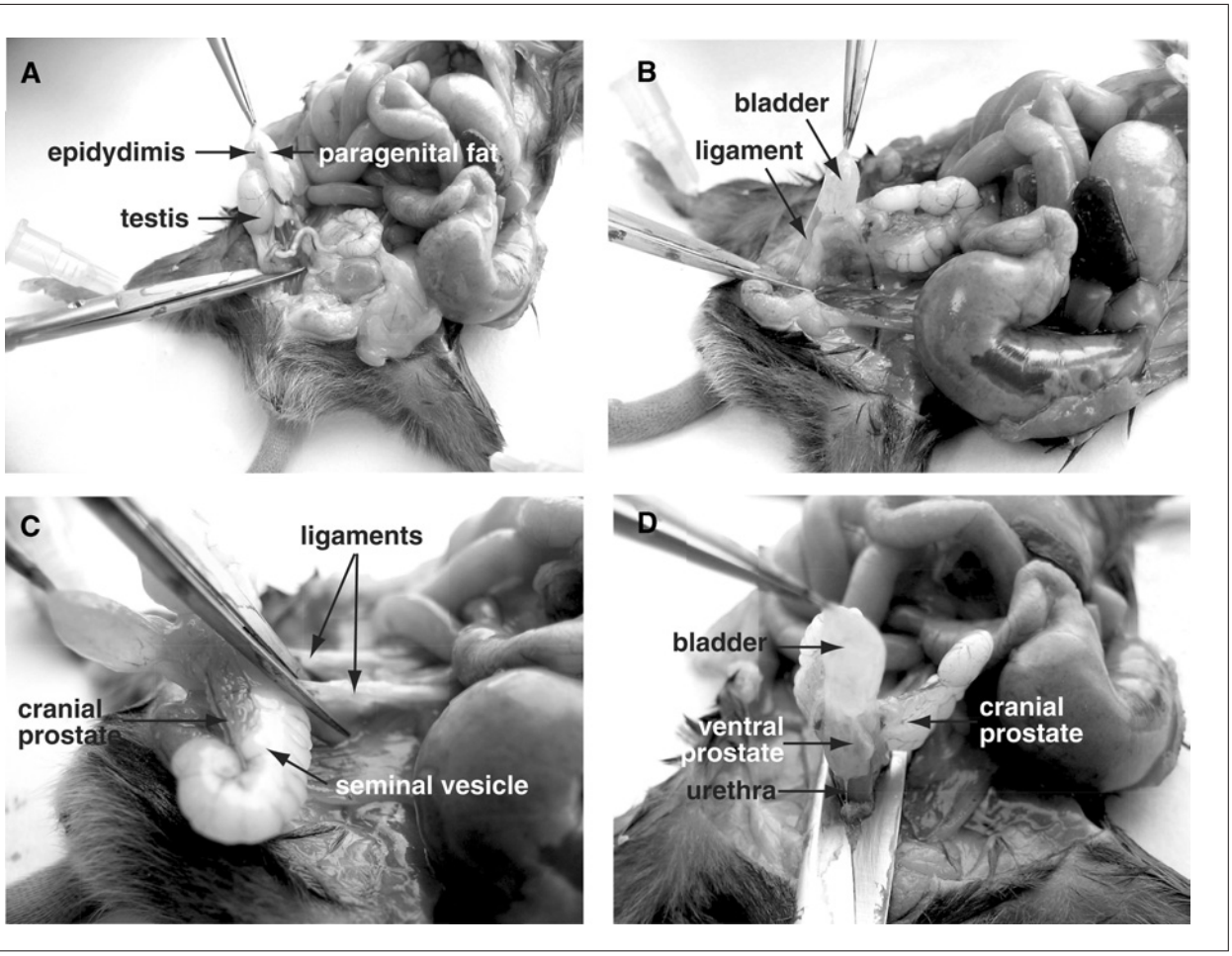

Figure 29A.4.8 Dissection of testis (**A**), urinary bladder, seminal vesicle, and prostate (**B,C,D**).

16a. Cut the thin ligament connecting the tail of the epididymis to the body wall. Place the right testis and epididymis into cassette no. 6A and transfer to formalin. Place the left testis and epididymis into cassette no. 6B and transfer to a container with ~20 volumes of Bouin's fixative solution. Discard the remaining portion of the vas deferens.

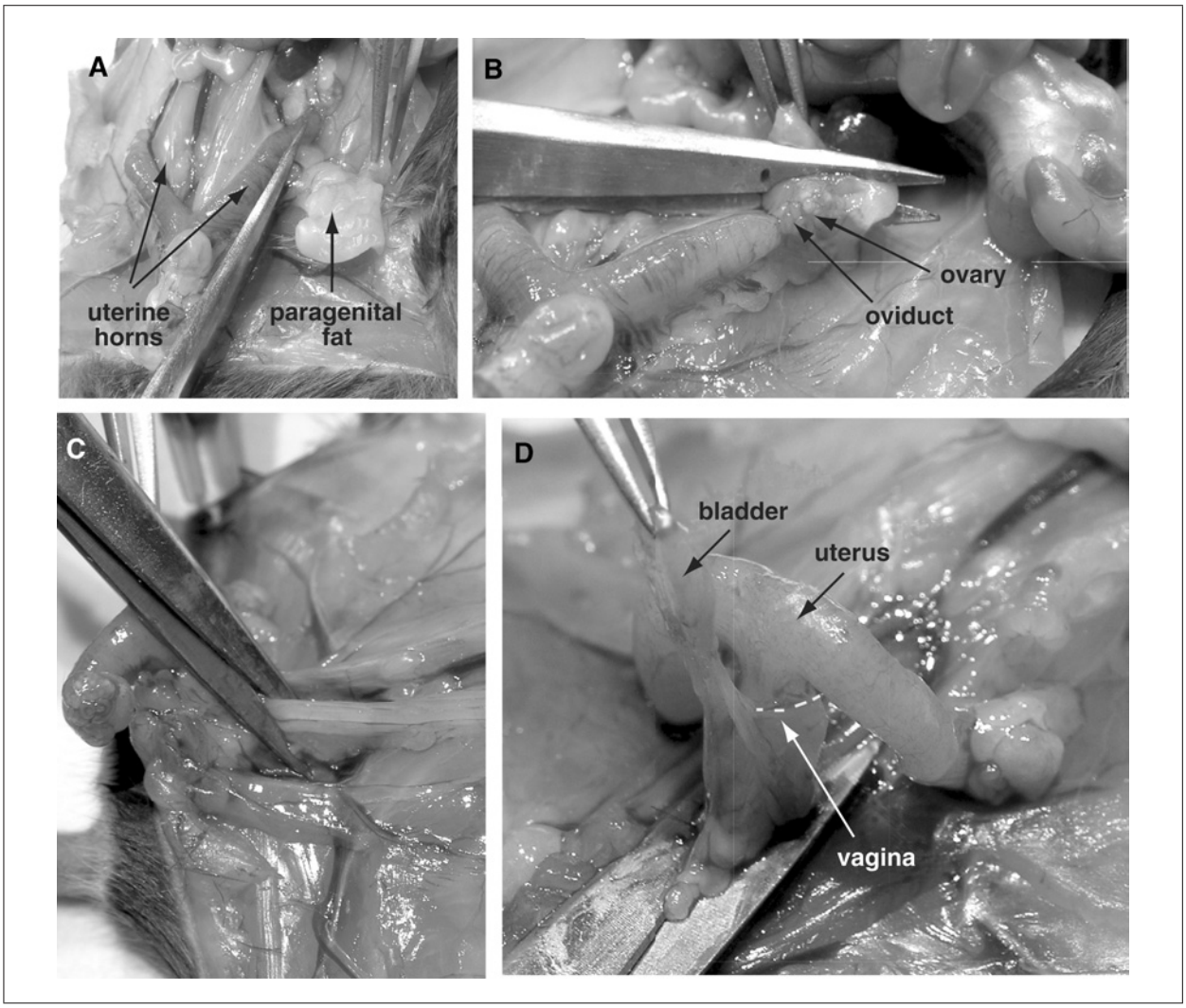

Figure 29A.4.9 Dissection of paragenital fat in the female (**A**), ovaries and oviduct (**B**), and bladder, vagina, and uterus (**C,D**).

17a. Using forceps, grasp the highest point of the urinary bladder, lift it up, and cut the ligament connecting the urinary bladder to the ventral body wall (Fig. 29A.4.8B).

18a. Recline both seminal vesicles towards the tail to uncover the ligaments connecting the prostate and dorsal body wall. Cut these ligaments (Fig. 29A.4.8C), and then make a transverse cut through the pelvic portion of the urethra (Fig. 29A.4.8D).

19a. Place the group composed of the bladder, seminal vesicle, and prostate into the 50-ml tube containing formalin. Allow to fix for 24 hr. Proceed to step 20.

Dissecting a female

15b. Dissect and weigh the paragenital fat (Fig. 29A.4.9A), and place it into the 50-ml plastic tube containing formalin.

The paragenital fat will be placed into cassette no. 11, together with brown adipose tissue, skeletal muscle, and tongue.

16b. Cut the ligaments connecting the ovaries to the dorsal abdominal wall (Fig. 29A.4.9B).

17b. Using forceps, grasp the highest point of the urinary bladder (Fig. 29A.4.9C). Pull the bladder upwards, cut the ligaments connecting the uterus body and vagina to

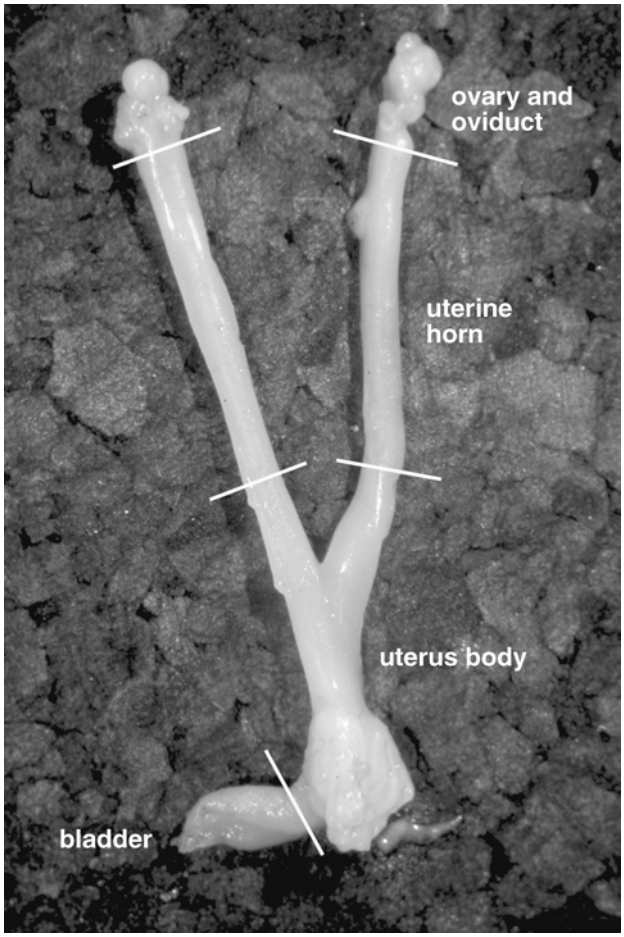

Figure 29A.4.10 Dissected ovaries, oviduct, bladder, vagina, and uterus.

the dorsal abdominal wall (Fig. 29A.4.9D), and make a transverse cut through the proximal vagina.

18b. Remove the genital tract and ovaries en bloc. Separate the urinary bladder. Make a transverse cut at the junction between the uterus and oviduct, and a second one at the junction between the uterus body and uterine horns (Fig. 29A.4.10).

19b. Place ovaries with attached oviducts into cassette no. 6, and place the vagina, uterus, both uterine horns, and the urinary bladder into cassette no. 7. Close the lids and transfer to formalin. Proceed to step 20.

20. Remove the adrenal gland (Fig. 29A.4.11A) and the mesenteric lymph nodes (Fig. 29A.4.11B) and place them into a biopsy capsule placed in a Petri dish with 1× PBS.

Later in the dissection procedure, this capsule will also contain the thoracic aorta.

Dissect neck and thorax

21. Cut the diaphragm along its insertions on the rib cage, cut the pleura connecting the lung to the diaphragm, and then section the distal portion of the thoracic esophagus. Remove the aorta (Fig. 29A.4.12) by making a transverse cut through its distal thoracic part and dissecting it away from the vertebral column. Take a 1-cm segment.

22. Place the aorta in the biopsy capsule containing the adrenals and lymph node. Close the capsule, place it into cassette no. 8, and transfer to formalin.

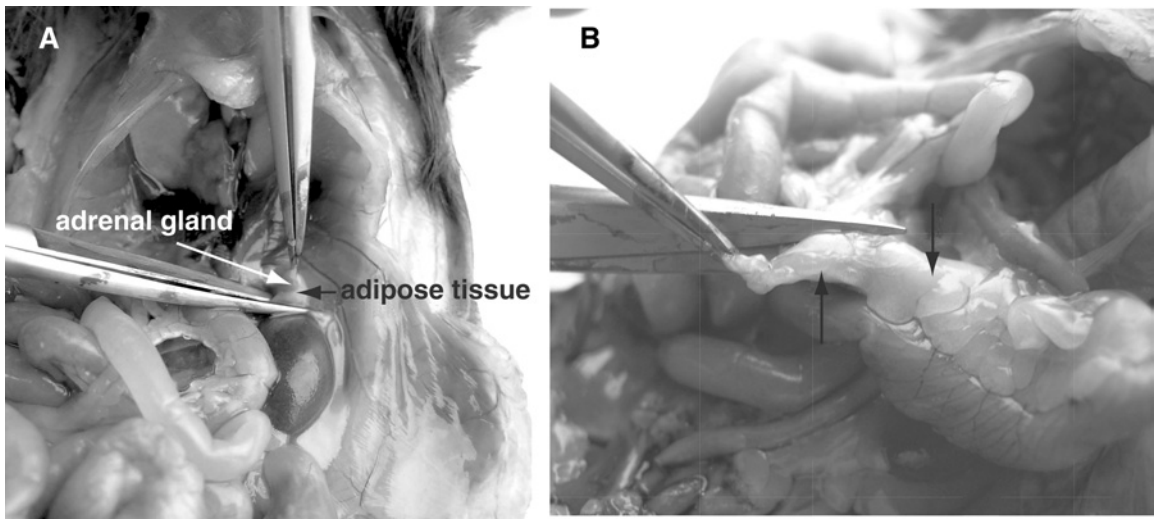

Figure 29A.4.11 Dissection of adrenal gland (**A**) and mesenteric lymph nodes (**B**).

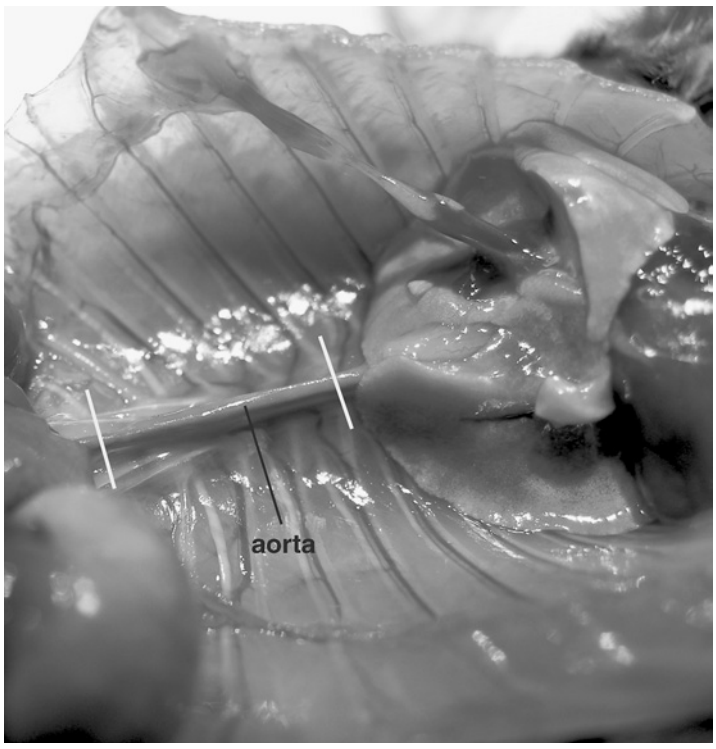

Figure 29A.4.12 Dissection of aorta.

23. Remove and discard the neck muscles covering the trachea and the thyroid (Fig. 29A.4.13).

24. Open the rib cage by cutting through the dorsal third of the right ribs, then recline the rib cage and sternum towards the left, and pin it onto the cork board (Fig. 29A.4.14A).

25. Slowly inject 2 ml formalin into the lungs by introducing a 25-G needle between the tracheal rings and compressing the trachea with forceps for 30 sec to maintain the fixative in the lungs (Fig. 29A.4.14B).

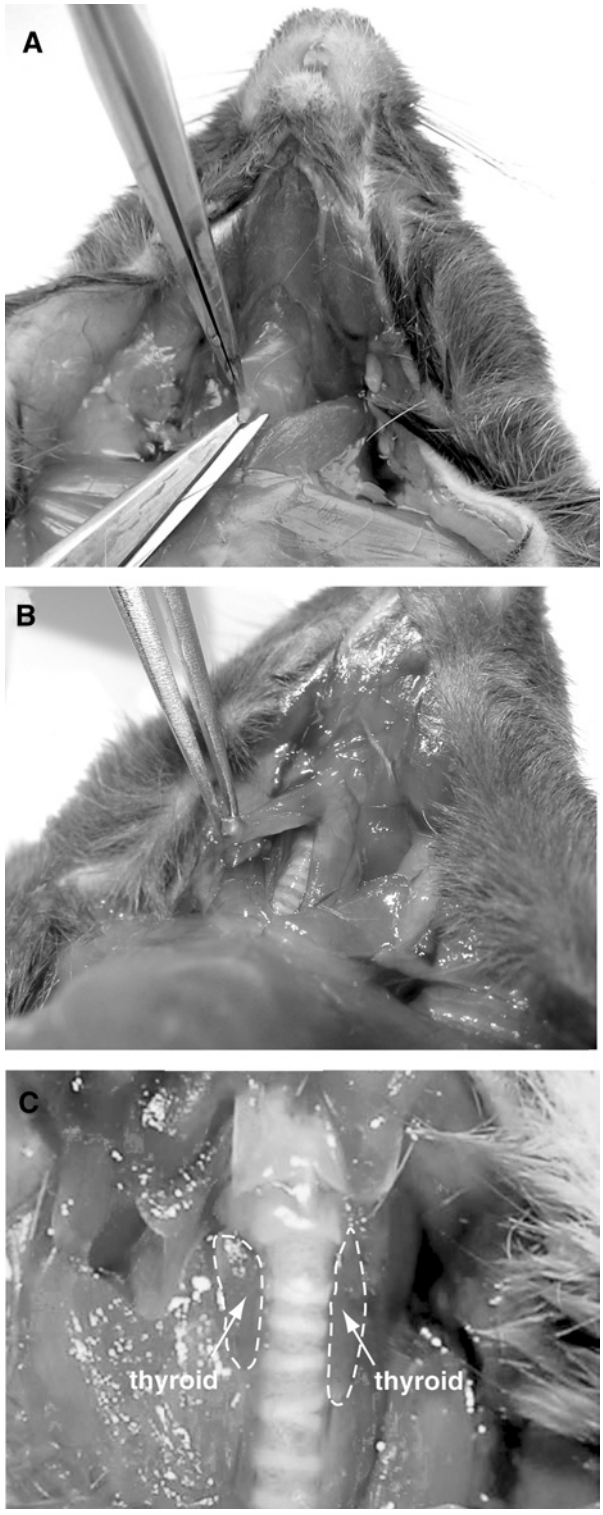

Figure 29A.4.13 Removal of neck muscles (**A,B**) and view of exposed thyroid and trachea (**C**).

26. Remove the superior (cervical) part of the trachea, with the thyroid glands and esophagus attached (Fig. 29A.4.14C), by cutting transversally at the level of the inferior pole of the thyroid glands. Place the group into cassette no. 9 together with the thymus. Close the cassette and transfer to formalin.

27. Remove the heart, weigh it, and place it into cassette no. 10.

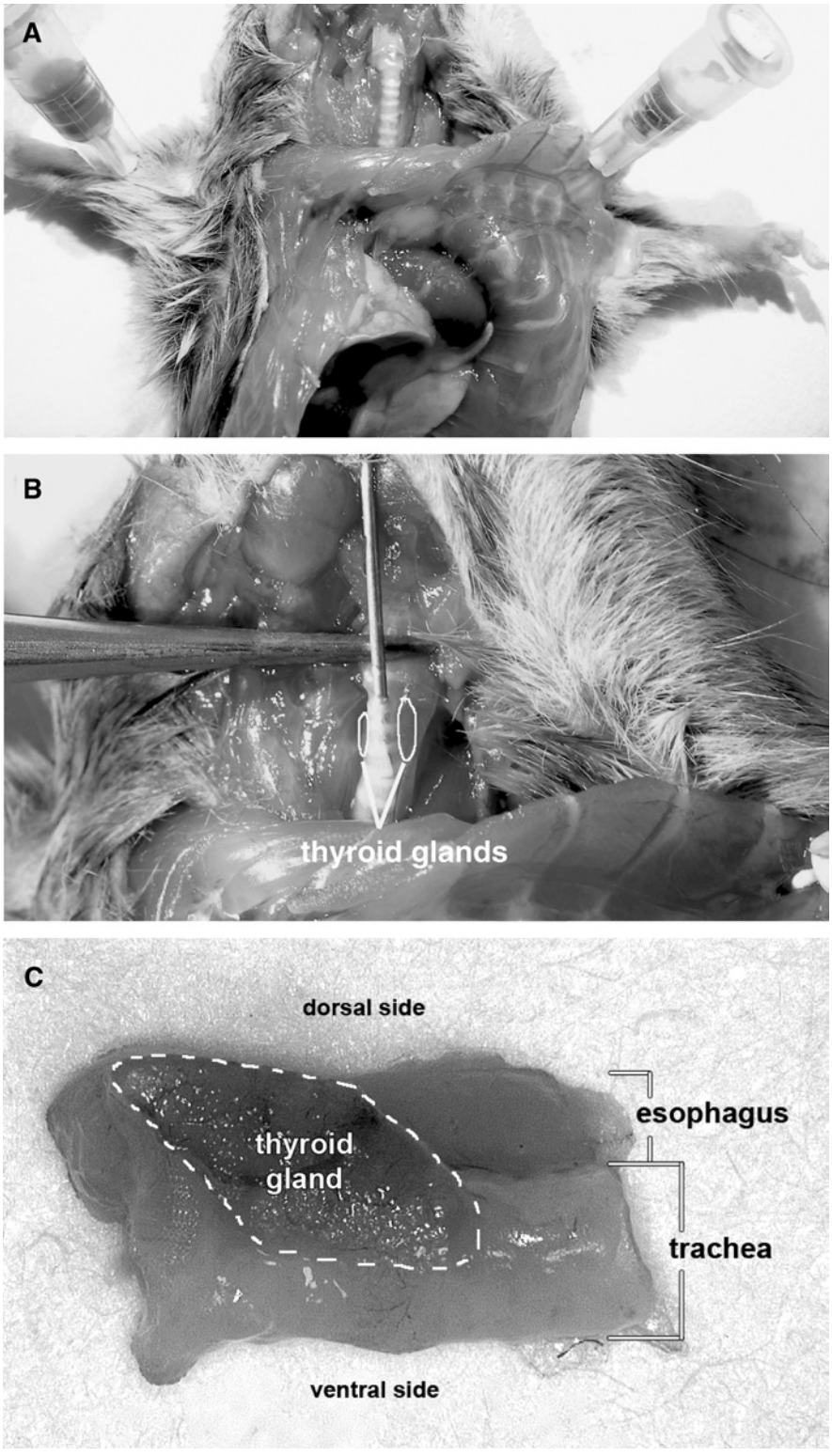

Figure 29A.4.14 (**A,B**) Injection of fixative through trachea for fixation of lungs. (**C**) Lateral view of trachea with esophagus and thyroid.

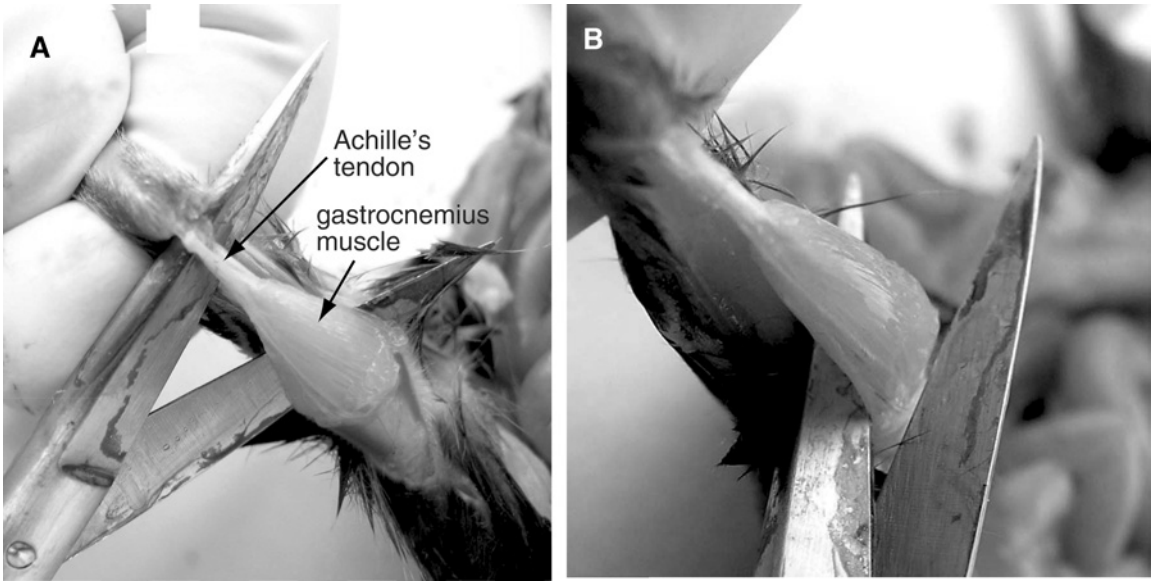

Figure 29A.4.15 Dissection of gastrocnemius and soleus muscles.

28. Dissect the remaining cervical trachea and esophagus from their adherences to the cervical spine. Extend the dissection into the thorax and remove the lungs en block. Put the entire block into cassette no.10 with the heart, and place the cassette into formalin.

Dissect muscles, skin, fat, and bones

29. Remove the skin from the hindlimb. Insert the scissors under the Achille's tendon and spread the blades (Fig. 29A.4.15A) to separate the gastrocnemius and soleus muscles from the deep muscular layer. Cut the muscles at the level of the knee joint (Fig. 29A.4.15B) and place them into cassette no. 11.

30. Spread out the jaws with the tip of the scissors, cut the tongue, and place it in cassette no. 11.

31. Turn the mouse over on its ventral side. Make a longitudinal incision in the skin between the two scapulae (Fig. 29A.4.16A). Spread out the edges of the incision, remove the interscapular brown fat (Fig. 29A.4.16B-C), and place it into cassette no. 11.

32. Take a piece of white adipose tissue from the tissue stored in the 50-ml plastic tube (step 15a or 15b) and place it into cassette no. 11. Close the lid and immerse the cassette into formalin.

33. Dissect and discard the hindlimb muscles to uncover the knee joint. Separate the knee joint by sectioning the bones above and below the knee (Fig. 29A.4.17). Place it into cassette no. 14 in a fresh container with formalin (different from the one used for the previous organs).

Dissect head

34. Make a midline incision on the dorsal side of the head skin from the neck to the snout. Cut off the head.

35. (Optional) Place entire head in fixative for 48 hr.

The following operations can be performed on fresh tissue or after a 48-hr fixation of the whole head.

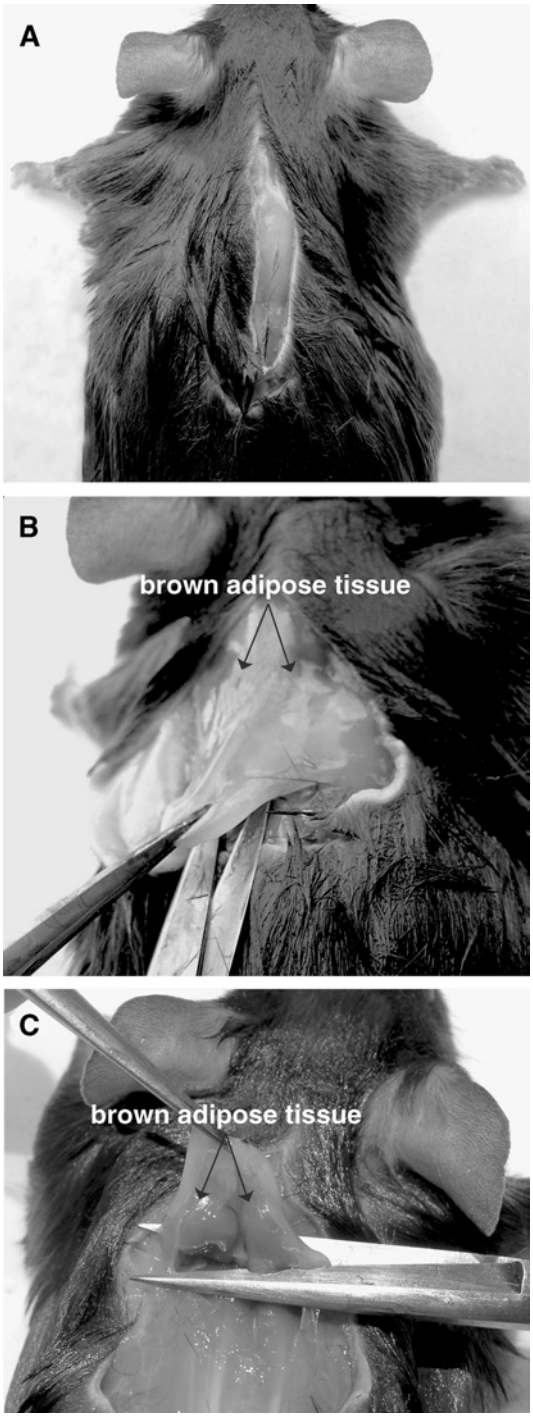

Figure 29A.4.16 Dissection of brown adipose tissue.

36. Insert the scissors into the foramen magnum and make a midline incision through the occipital bone (Fig. 29A.4.18).

37. Place the head on the cork board and firmly insert the tips of the scissors at the junction of the frontal and nasal bones, on the midline (Fig. 29A.4.19A). Open the scissors.

    This action will break the dome of the skull along the medial suture (Fig. 29A.4.19B-C).

38. With a pair of forceps, detach the two halves of the dome and expose the brain (Fig. 29A.4.19D).

Figure 29A.4.17 Dissection of knee joint.

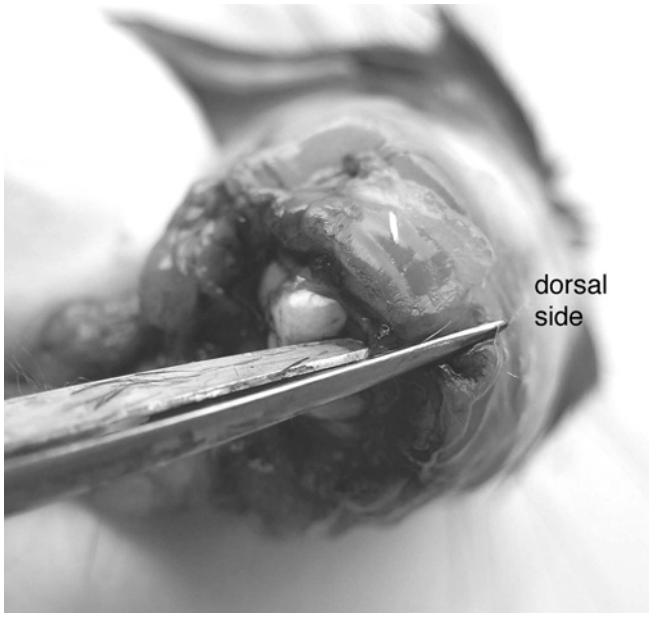

Figure 29A.4.18 Incision through occipital bone for dissection of brain.

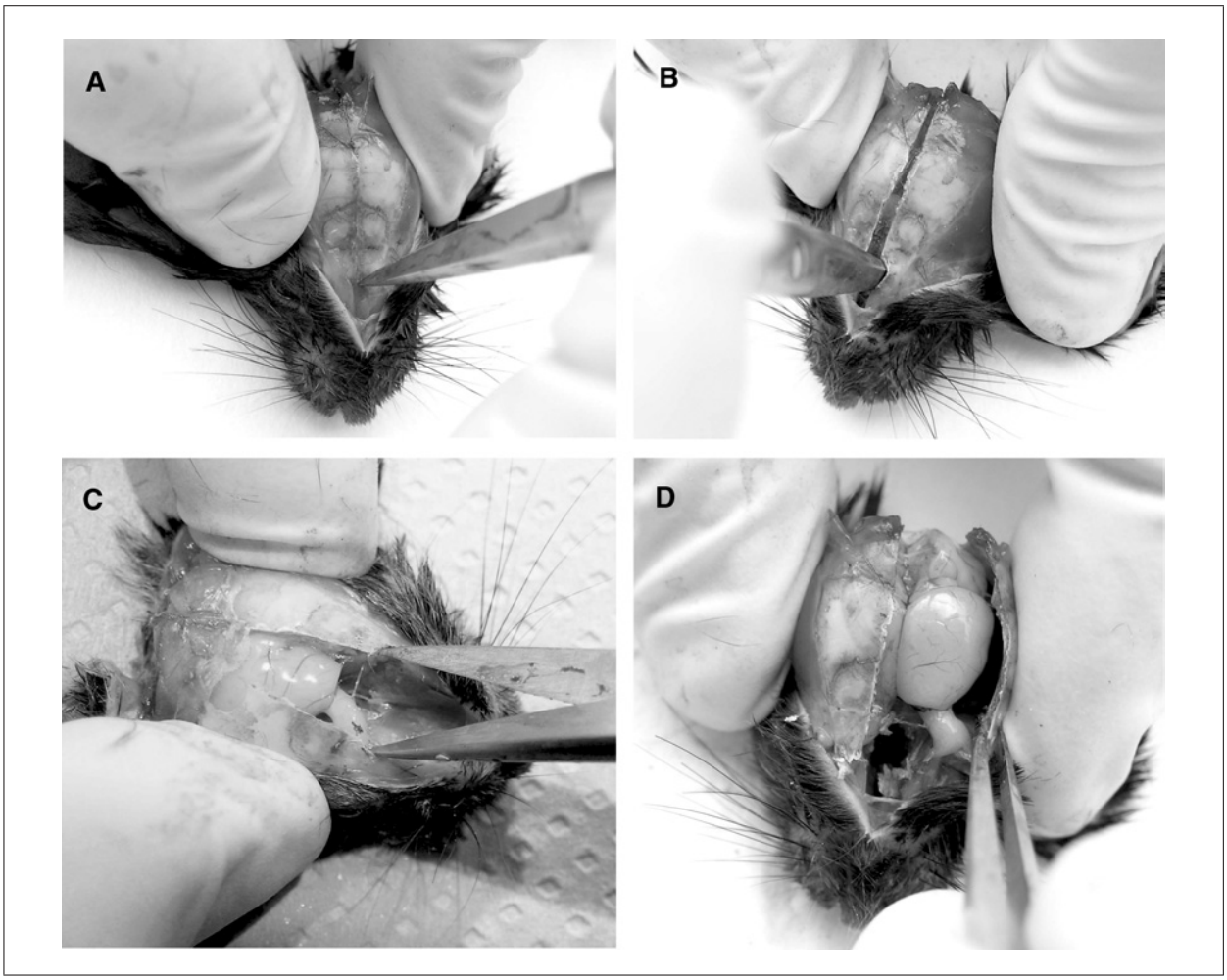

Figure 29A.4.19 Removal of brain. Placement for insertion of scissor tips (**A**), splitting of the skull along medial suture (**B,C**), and removal of skull halves (**D**).

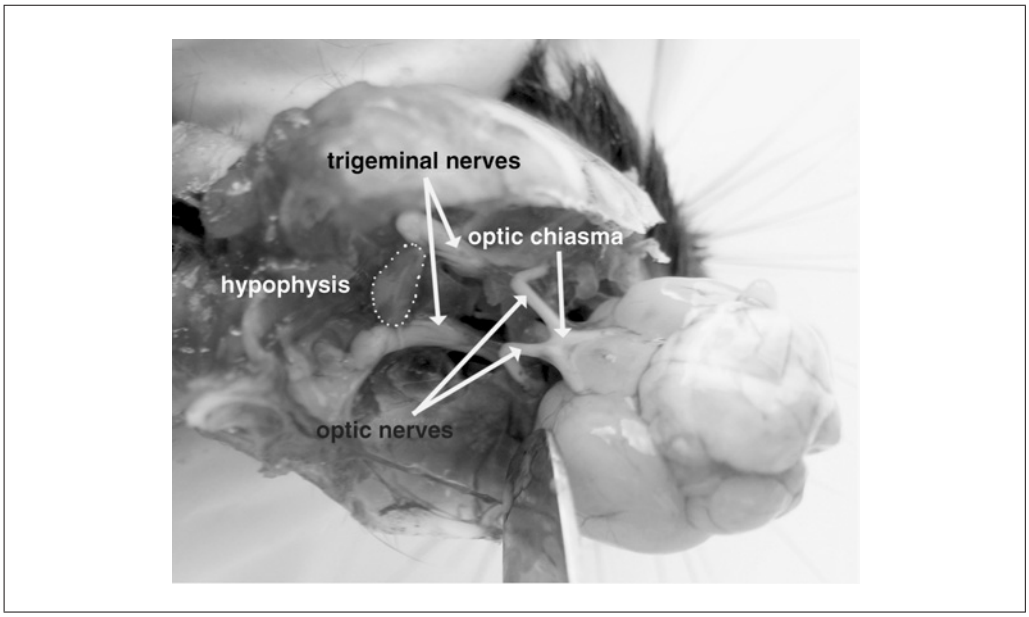

Figure 29A.4.20 Brain reflected towards anterior, showing optic chiasm and hypophysis underneath.

39. Using a spatula, remove the brain, taking care not to damage the hypophysis located underneath (Fig. 29A.4.20).

40. Place the brain in a Petri dish containing 1 ml of 1× PBS. Place the brain, ventral surface up, in the mouse coronal brain matrix (Fig. 29A.4.21A).

    The ventral surface of the brain must be parallel to the top surface of the mold.

41. Use the 4th, 6th, and 10th channels, starting from the anterior part of the mold, to generate the brain slices (Fig. 29A.4.21B). Insert razor blades in the first and last channels, and then insert a razor blade in the middle channel. Remove the three razor blades at the same time, extracting the brain slices from the mold. Place the brain slices in cassette no. 15 and transfer to formalin.

    The manipulations performed to remove the brain from the skull have broken the skull base. This renders eye removal easy as, in most cases, the orbit is already open.

42. Cut the membrane at the edges of the orbit all around the eyeball. Manipulate the eye using the optic nerve or the skin. Do not pull on these tissues, as there is a risk to detach the retina.

43. Place the eye and the corresponding Harderian gland in cassette no. 13**.** Close the lid and transfer to formalin.

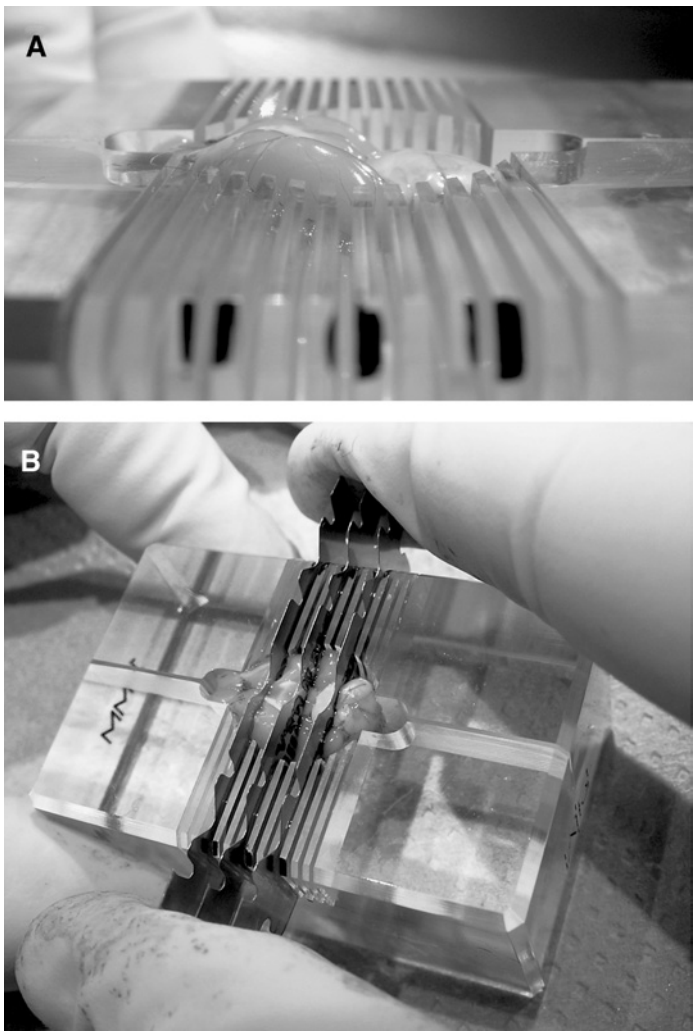

Figure 29A.4.21 Placement of brain in brain matrix (**A**) and position of razor blades for making sections (**B**).

44. Place what remains of the skull into the 50-ml plastic tube containing formalin and allow to fix for 24 hr.

Process carcass

45. Transfer the mouse carcass to a large container with formalin. Allow to fix for 24 hr.

Process tissues after 24-hr fixation

46. Remove the intestines. Separate the stomach by making a transverse section at its junction with the proximal duodenum, and place the stomach in a Petri dish containing 70% ethanol. Cut with scissors along the lesser and greater curvatures and wash out the contents. Place both halves in cassette no. 2 and place the cassette back into formalin.

47. Take a 0.5-mm segment of the proximal duodenum, a 0.5-mm segment of the distal ileum closest to the caecum, and the first 0.5-mm segment of the proximal colon. Place the three segments in cassette no. 3 and place the cassette back into formalin.

48. Collect 2 × 4–mm of pieces of skin from the following locations, place into cassette no. 12, and transfer to formalin:

    a. Back skin: Remove a rectangular piece of dorsal skin, parallel with the longitudinal axis of the body.
    b. Tail skin: Cut a 1-cm segment of the proximal tail, incise it longitudinally (Fig. 29A.4.22A), and detach the skin from the vertebra using a scalpel (Fig. 29A.4.22B).
    c. Footpad skin: Remove the skin from one of the hindlimb footpads.
    d. Snout skin: Remove the vibrissae by cutting them with scissors and take a rectangular piece of snout skin.

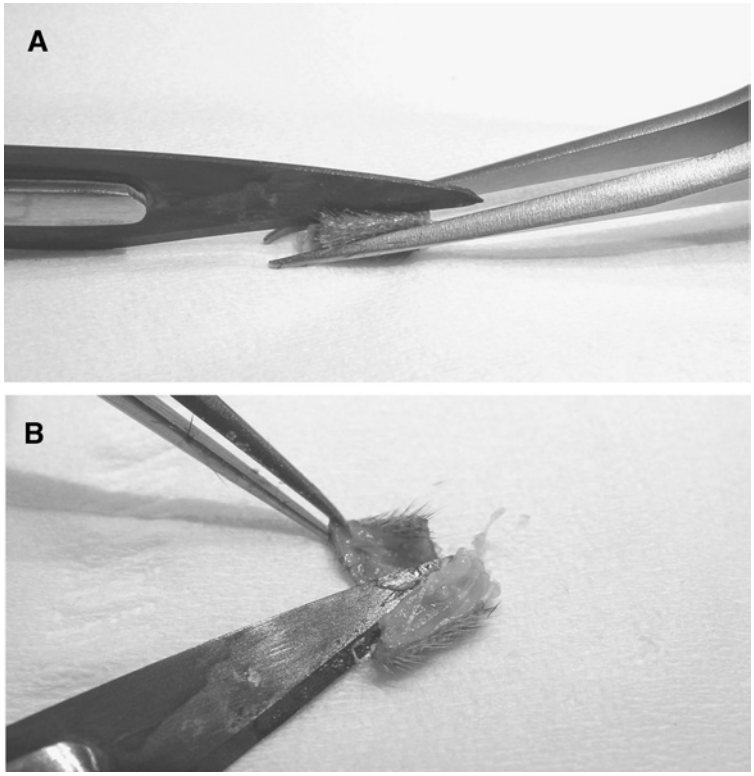

Figure 29A.4.22 Dissection of tail skin. Longitudinal cut (**A**) and removal of spine (**B**).

49. Remove the sample of male reproductive organs (prostate, seminal vesicles, bladder) from the 50-ml tube of fixative (step 19a), trim (see the appendix of this unit), place in cassette no. 7, and transfer to formalin.

50. Remove the skull from fixative (step 44), remove the hypophysis, place in a biopsy capsule in cassette no. 16, and transfer to formalin.

51. Store the remainder of the carcass, together with the remaining organs, in formalin at room temperature for further use.

    Good histology results are obtained even after one year of storage in formalin.

TISSUE COLLECTION FOR MOLECULAR BIOLOGY APPLICATIONS

BASIC PROTOCOL 2

Molecular analyses require preservation of tissue components at the RNA and protein levels. To comply with this requirement, rapid tissue collection and snap freezing is critical. For this purpose, the chronological order of tissue collection is dictated by the interests of the investigator. As a general rule, start with tissues containing high enzyme levels (e.g., pancreas, intestines, adrenals) or, if these tissues are not required, with tissues that are highly sensitive to hypoxia (e.g., brain, adrenal glands).

NOTE: For organ localization and sampling, see Basic Protocol 1.

Materials

Mice
Liquid nitrogen
70% ethanol

Dissection instruments (e.g., forceps, scissors)
2-ml sterile vials (e.g., Eppendorf)
Large styrofoam box with lid to hold the liquid nitrogen

1. After excising desired organs from mice, rapidly place the tissue in a sterile 2-ml vial.

   An amount of 100 mg tissue is usually sufficient. For small organs (e.g., ovary, adrenal glands), collect organs bilaterally.

2. Close the vial and place it in a container filled with liquid nitrogen.

3. Clean the dissection instruments with 70% ethanol.

4. Store the samples indefinitely at −80°C until processing.

   From tissue collection until processing, the samples should always be stored or transported at temperatures below −80°C (e.g., on dry ice) to avoid thawing.

COMMENTARY

Background Information

This unit describes tissue collection for a systematic histological screen; for a complete commentary on this topic, the reader is referred to *UNIT 29B.4*. The necropsy protocol provided here is designed for systematic investigation of mutant/treated mice. The advantage of this working method is the comprehensive view of the macroscopic and microscopic aspects it provides of almost all tissues and organs of the mouse. Depending on the interests of the investigator, this protocol can be adapted for a smaller number of tissues and organs. Specific experimental considerations might, for instance, call for partial approaches directed toward more specific analysis of individual tissues; approaches requiring more specialized dissection or sampling procedures; or combinations of histological and molecular approaches (for example, fixing and freezing separate parts of a single tissue).

Critical Parameters

For good morphological preservation, it is critical to accomplish the full dissection within 45 min following the death of the mouse. When mRNA extraction is intended, tissues should be sampled within <5 min. Tissue

manipulation is an important parameter for good morphology preservation. Manipulate tissues using non-informative regions of the organ (e.g., ligaments, capsule) or adjacent tissues (e.g., diaphragm for the liver). Avoid unnecessary tissue manipulation. To minimize tissue damage by crushing, try to finish the dissection of a given organ without having to release it and grasp it again. It is worth noting that expert assistance for pathologic and/or histologic analysis can often be obtained from veterinary staff in the animal facility or from colleagues with specific expertise.

As in any phenotypic analysis, it is critical that cohorts be carefully planned for a histological analysis, and that mutant animals be compared with strictly comparable normal animals (i.e., of the same gender, age, strain, and so on). For an in-depth discussion of these issues, see *UNITS 29A.2 & 29B.4*.

Key References

Brayton, C., Justice, M., and Montgomery, C.A. 2001. Evaluating mutant mice: Anatomic pathology. *Vet. Pathol.* 38:1-19.

Hebel, R. and Stromberg, M.W. 1986. Anatomy and Embryology of the Laboratory Rat. BioMed Verlag, Worthsee, Germany.

Popesko, P., Raijtova, V., and Horak, J. 1992. A Colour Atlas of Anatomy of Small Laboratory Animals. Wolfe Publishing, London.

Smith, R.S., John, S.W.M, Nishina, P.M., and Sundberg, J.P. 2002. Systematic Evaluation of the Mouse Eye: Anatomy, Pathology, and Biomethods. CRC Press, Boca Raton, Florida.

Internet Resources

http://www.eumorphia.org/
The EUMORPHIA Web site, which provides information about understanding human disease through mouse genetics.

http://main.uab.edu/sites/ComparativePathology/links/
The University of Alabama at Birmingham: Comparative Pathology Laboratory Web site.

http://eulep.anat.cam.ac.uk/
Pathbase Web site.

APPENDIX: TRIMMING ORGANS AND DEFINING PLANES OF SECTION

Skin and subcutis, musculoskeletal system

Mammary gland: Depending no orientation is required. Embed and section to obtain the largest cut surface.

Knee joint: place the bones along axes parallel to the bottom of the embedding mold to generate histological longitudinal sections.

Muscle: the longest axis lies parallel to the bottom of the embedding mold, to generate longitudinal sections. The tendon insertion helps to orient.

Preputial glands: embed and section to obtain the largest cut surface.

Skin: embed vertically, the cut surface being parallel to the bottom of the mold, to generate transverse sections through the whole thickness of epidermis, dermis, and hypodermis.

Abdominal organs

Adrenal glands: embed to obtain the largest cut surface. Trim the paraffin block to obtain histological sections showing both cortex and medulla.

Digestive tract: orient the two halves of the stomach such as its cut surface is in contact with the embedding mold, to generate longitudinal histological sections through the cardial, keratinized, glandular, and pyloric regions of the stomach.

The three segments of intestine are embedded in a vertical position, to generate transverse histological sections.

Fat (brown and white): no orientation required. Embed and section to generate the largest cut surface.

Kidney: place the cut surface in contact with and parallel to the bottom of the embedding mold to generate longitudinal histological sections through the renal pelvis.

Liver is embedded to obtain the largest cut surface. Three sections are taken, at 200 μm apart from one another.

Lymph nodes are embedded and sectioned to obtain the largest cut surface.

Ovary: embed and section to obtain the largest cut surface. The paraffin block must be trimmed to generate histological sections through the cortex and the medulla.

Pancreas: embed and section to obtain the largest cut surface.

Prostates, seminal vesicles, urethra, and bladder: the group formed by seminal vesicles, dorsal, ventral, and cranial prostates, bladder and pelvic urethra is taken out of the fixative and first sectioned with a razor blade according to a sagital median plane through urethra and urinary bladder. The seminal vesicles with cranial prostates attached to them are then separated by cutting at ~3 mm from the midline. The distal parts of seminal vesicles

that have no cranial prostate attached to them are discarded. The two halves with bladder, ventral and dorsal prostates and the two pieces of seminal vesicles and cranial prostates are placed in an embedding cassette. The four organ pieces are positioned with their cut surfaces in contact with and parallel to the bottom of the embedding mold.

Spleen: generate longitudinal sections through the organ.

Testis with epididymis is placed in the embedding mold with its longest axis lying horizontally, to generate longitudinal histological sections.

Uterus: one uterine horn is embedded in paraffin with the long axis in vertical position, to generate transverse histological sections. The second horn is embedded with the long axis lying horizontally, to generate longitudinal histological sections. The group formed by the uterus body and vagina is embedded with its long axis lying horizontally.

Cervical and thoracic organs

Heart is embedded in the vertical position, with the apex towards the mold bottom. Three transversal sections are taken at 3 mm from the apex.

Lungs are embedded and sectioned to obtain the largest cut surface.

Thymus is embedded and sectioned to obtain the largest cut surface.

Trachea, esophagus, and thyroid glands: the group formed by these organs has a pyramidal shape. It is embedded in paraffin with its base towards the bottom of the mold, to generate transverse histological sections of the trachea, esophagus, and thyroid glands on the same sections.

Aorta is embedded in a vertical position to generate transverse histological sections.

Salivary glands are embedded and sectioned to obtain the largest cut surface.

Head

Eye with Harderian glands are laid on the bottom of the embedding mold in their anatomical position, the cornea on the left side, the optic nerve on the right. The paraffin block is trimmed to generate histological sections through the middle of the of the eye ball.

Tongue is embedded in the vertical position to obtain transverse histological sections.

Brain: the brain slices are embedded with the anterior surface towards the bottom of the embedding mold.

Hypophysis is embedded with the flat surface towards the bottom of the embedding mold.