NECROPSY PROCEDURE FOR THE LABORATORY MOUSE

On-line Reference: **Comparative Anatomy and Histology.** You will be asked to log-in via your Onyen and password.

1. **Prepare all instruments and supplies**. Recommended supplies include:

cork board & pushpins for pinning out the mouse

paper towels or wipe-all to cover the board

squeeze bottle of 70% ethanol to wet fur and clean instruments

gauze pads

small dissecting or iris scissors

small thumb forceps (rat-toothed and serrated tips)

mosquito forceps (hemostat)

1 ml & 3 ml syringes; selection of 18, 22 & 25 gauge needles

specimen jars with fixative (or saline for practice)

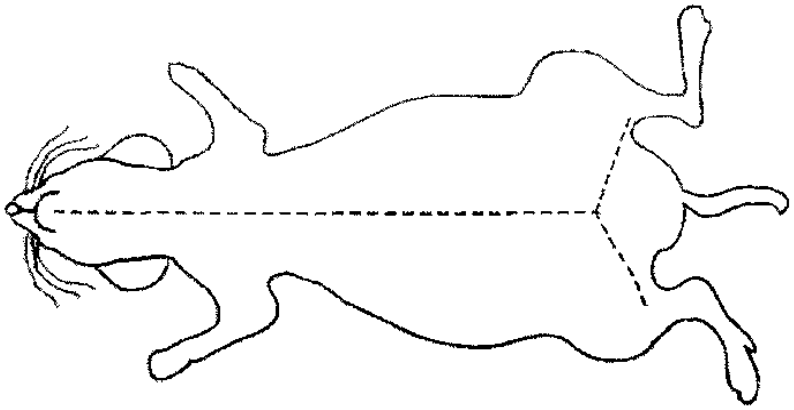
weighing balance(s) that read up to 50 g (whole mouse) and down to 3 decimal places (organs).

index cards cut in small strips

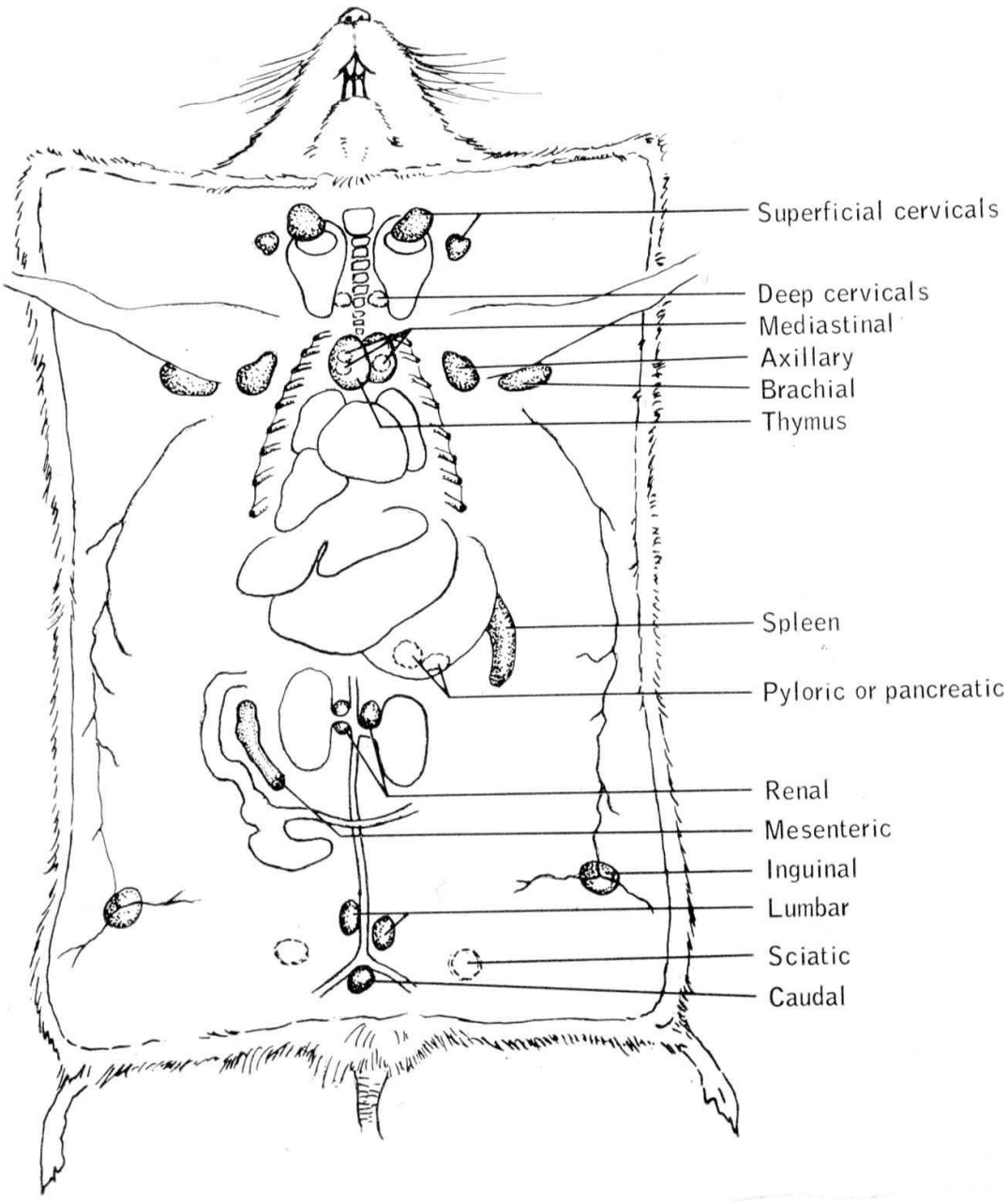
recording sheet/program for findings

2. **Examine the external surfaces and orifices of the mouse**. List and gross pathology and take samples where needed for histologic preparation. Note any general body characteristics (i.e. emaciated, obese, decomposed, etc.). Weigh intact mouse and record whole body weight.

3. **Pin the mouse to the cork board**, ventral surface up and wet the fur with 70% ethanol. Make a midline incision **through the skin only**, from the external genitalia to the tip of the chin. Then incise the skin along the hindlimbs. Using closed scissors, separate the skin from the musculature and pin it out. The skin over the forelimbs and neck can be pulled away without further incisions.

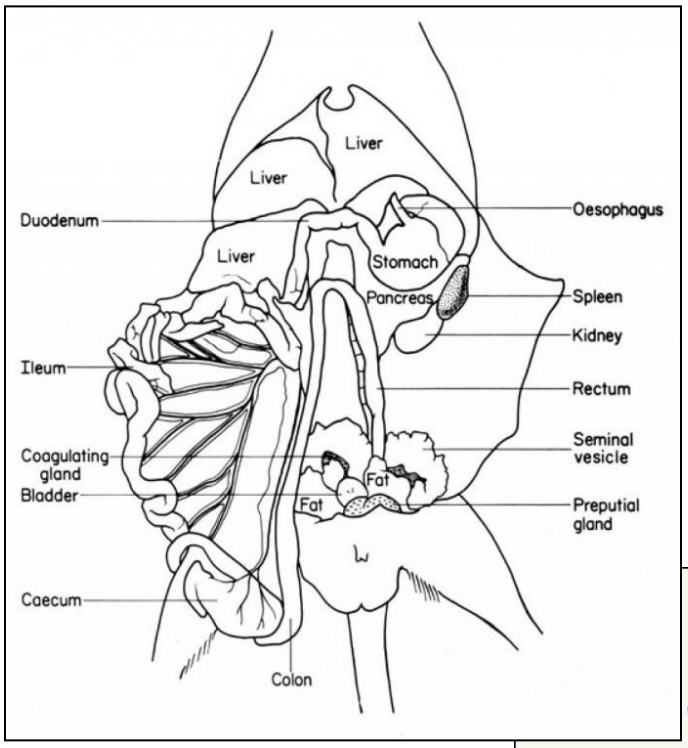


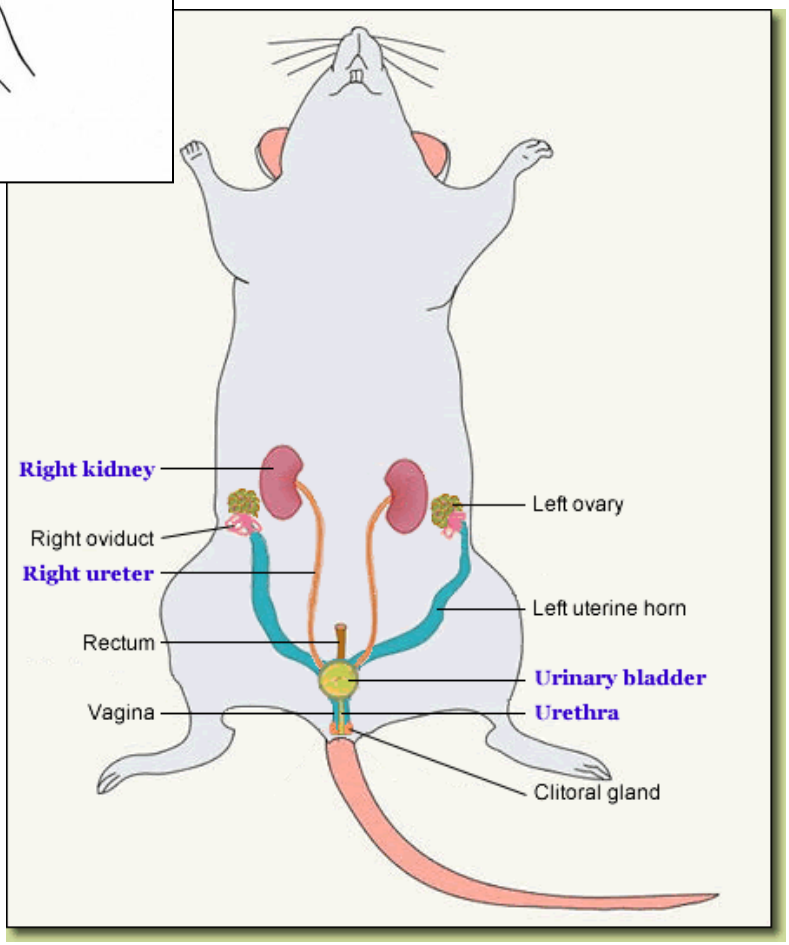
4. **Examine and remove for fixation the following lymph nodes (LNs):** 2 inguinal nodes with skin and cervical nodes with salivary glands. HINT: place the skin/inguinal nodes and salivary glands/LNs "sticky-side" down on a piece of index card and place in the fixative. This will keep the specimens from curling up as they fix and give you better histology preparations. Note the other LNs below and see if you can find some of them.



5. **Examine the abdominal cavity**. Incise the abdominal musculature (being careful not to perforate the intestines) from the genital area anteriorly along the left side of the mouse (on your

right) to the thoracic wall, then towards the right side along the line of the diaphragm. Flip musculature to your left to expose the abdominal organs. Without moving the viscera, examine for pathological placement of the organs.



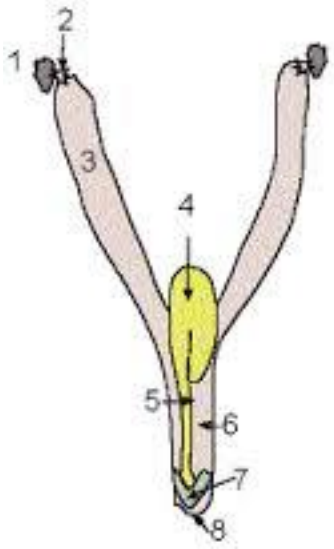


6. **Removal of the abdominal viscera**.

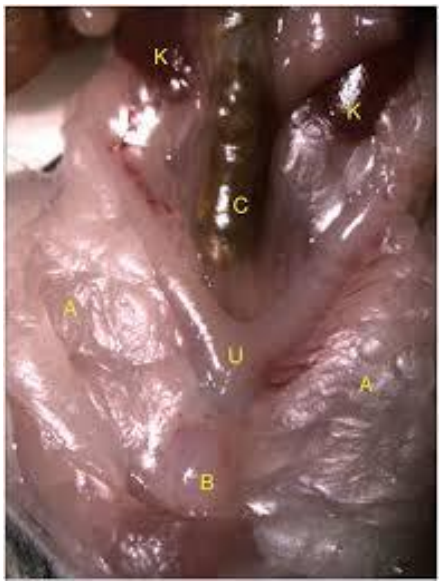
a) Remove the entire spleen with attached pancreas - if the spleen is large, fix a central segment with pancreas; if spleen is small, halve it; if very small, fix entire organ.

b) Fill the 1 cc syringe with ~ 1 ml of fixative. Insert the needle through the wall of the stomach in the lower, more opaque portion. Slightly inflate the stomach with about ½ of the fixative. Find the blind-ended sac in the intestines (cecum) and slightly inflated it with the fixative. Cut the stomach free at the esophagus. Holding the esophageal stump with your forceps, gently retract the gut out of the abdomen while cutting the mesenteric attachments to the body wall. Cut the rectum (containing fecal pellets) just anterior to the pelvic bones, then remove the entire intestinal mass and place it aside for later examination.

c) **FEMALE** mouse: Identify the Y-shaped uterus (U). Cut away the uterine mesentery and fat pads (A) that lie along the outer borders of the uterine horns. Cut through the vagina just distant to the urinary bladder (B). Holding the vaginal stump in your forceps, lift the uterus out and cut the ovaries free from their peritoneal attachments just behind the kidneys (K). Place the entire uterus with ovaries and bladder attached on an index card, then put the card and tissues in fixative.

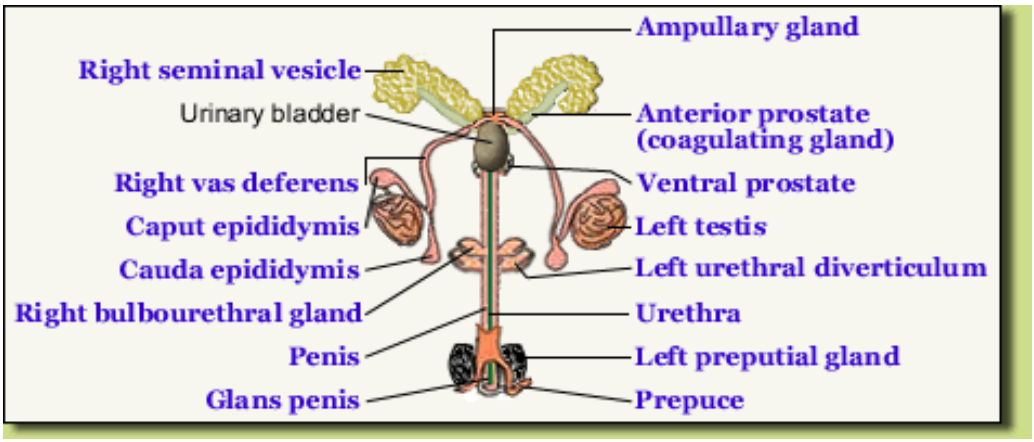


1. Ovary
2. Fallopian Tube
3. Uterus
4. Urinary Bladder
5. Urethra
6. Vagina
7. Clitoral Glands
8. Vulva



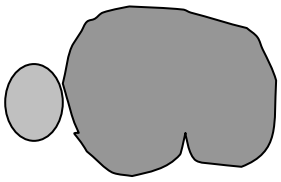
d) **MALE** mouse: Find the large fat pads on within the lower abdominal cavity. Retract these and pull the testes from the scrotum back into the abdomen. Cut away the fat pads being careful to leave the epididymides with the testicles. Remove the testes with attached epididymides. Then cut the urethra and separately fix the bladder with entire mass of secondary sex organs (seminal vesicles, prostate, etc.) on an index card.

It's a boy!!!

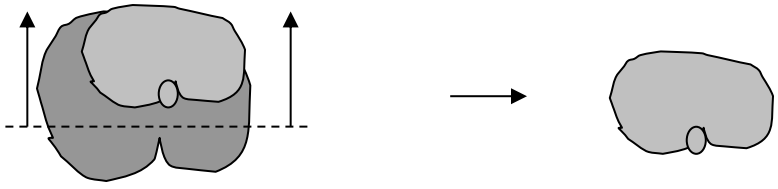


***NOTE:** Optimal fixative for evaluation of testes is **Bouin's solution**, not 10% normal buffered formalin (NBF). You would place the mouse testes intact in this fixative overnight, then rinse in 70% ethanol until no more yellow color leaches from the fixed testes. Submit the testes to the histology lab in 70% ethanol.

d) Remove the left kidney with adrenal attached and place in fixative.



e) Grasp the diaphragm where it attaches to the back of the liver. Gently retract the liver and cut it free from its attachments to the diaphragm. Examine the entire liver for lesions and fix any pathological tissues. If no pathology is noted, grasp the central lobe at the base of the gall bladder and make a transverse cut across just proximal to the forceps. Fix the central lobe and gall bladder.



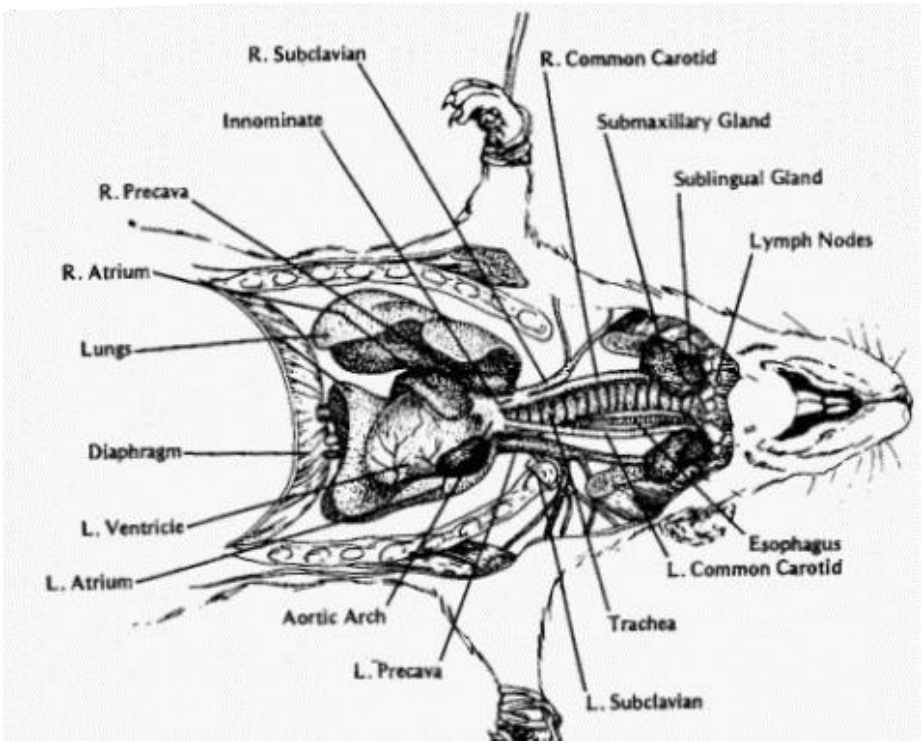
f) Remove and fix the right kidney with adrenal attached.

7. **Removal of thoracic viscera**.

a) Using your "bone scissors", cut through the ventral rib cage from the diaphragm through the 1st rib, 5 mm to the left and right of the sternum. To get that first rib without cutting the underlying trachea, slip the closed tips of the scissors under the sternum and out the thoracic inlet and open them slightly to make a space. Then remove the scissors and cut the last rib on each side Remove the rib stubs from sternum and fix the sternum.

b) Fill a 3ml syringe with at least 1 ml of fixative. Insert the 18-22 gauge needle into the trachea and hold it in place with forceps. Use the green capped 18 gauge needle for adult mice and the blue capped 22 gauge needle for juvenile or other small mice. Slide the bevel of the needle into the trachea between the rings of cartilage until the bevel is just covered. Inflate the lungs with ~ 1 ml fixative.

c) Grasp the trachea with your thumb forceps and pinch it as you remove the needle. Cut the trachea/esophagus above the larynx or cut through the mandibular symphysis with "bone" scissors and grasp the tongue. Using the grasped trachea or tongue, lift them up and cut midline attachments of the trachea and esophagus as you pull the organs towards the chest cavity. Continue to retract and cut heart/lung block free of their midline attachments. Place the entire "pluck" in fixative.

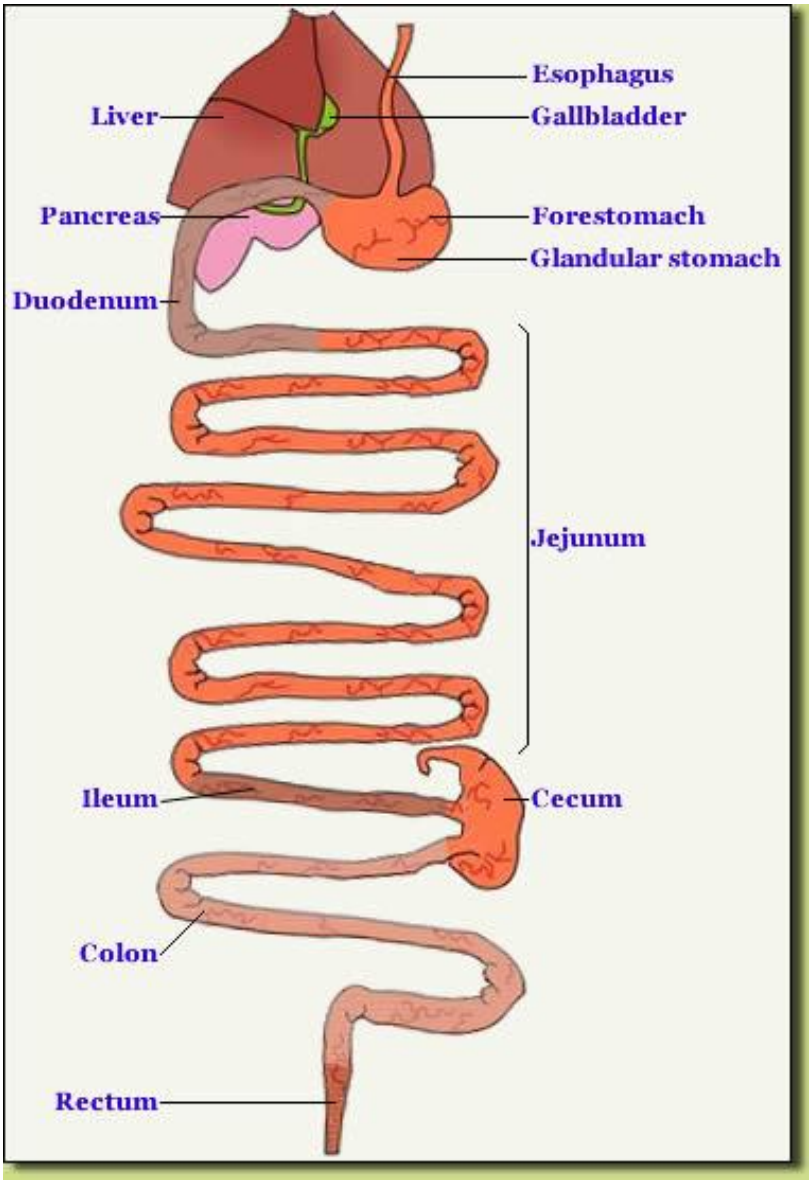


8. **Examination of intestinal tract**.

a) Sever the duodenum and place the entire stomach and duodenum in fixative.

b) Find the cecum and lay it to one side. The ileum enters the cecum and the colon exits the cecum adjacent to one another. The mesenteric lymph node lies in the mesentery between the ileum and colon. Cut the ileum and colon and fix the ileum, cecum, mesenteric LN, and colon in a block. If the cecum is very large, cut off the blind tip and fix it. Then remove the midsection of the cecum and discard. You can also inject fixative into the cecum as you did with the stomach for better fixation.

c) Fix section(s) of jejunum with Peyer's patches.



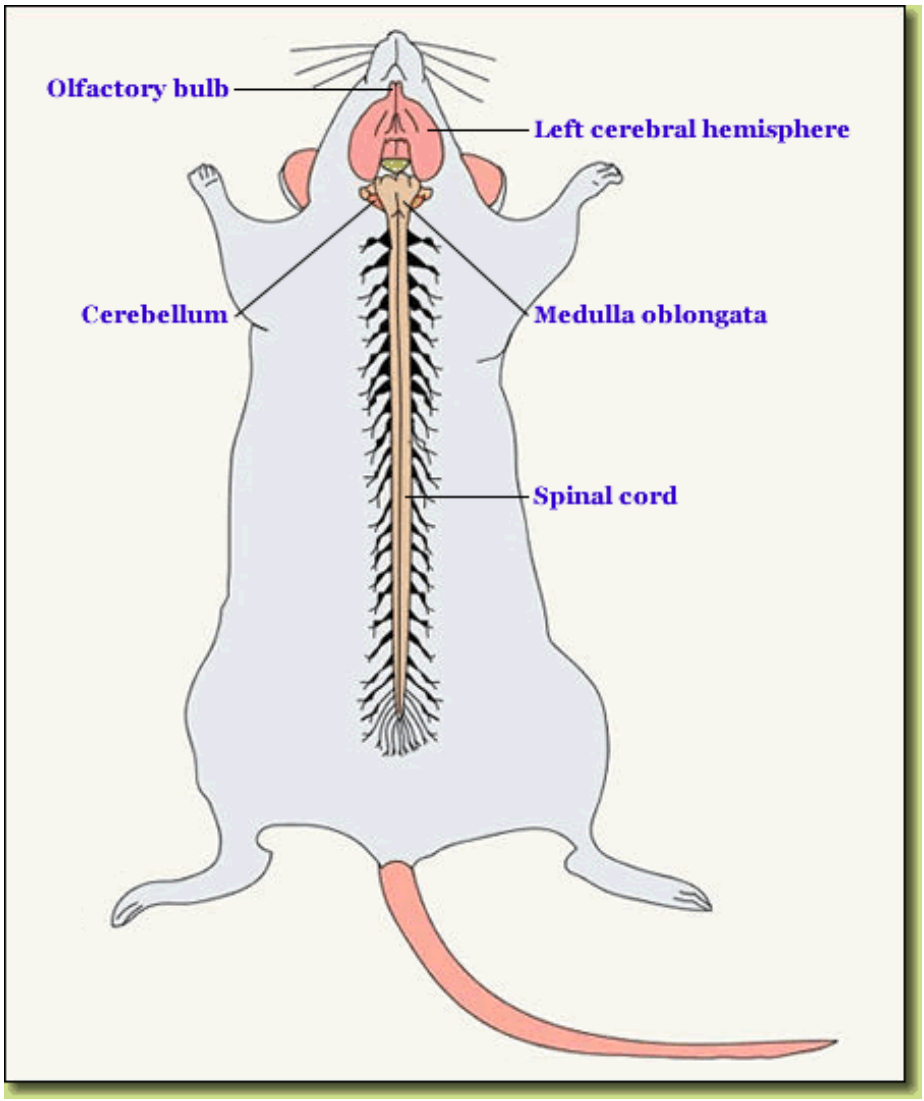
9. **Head, skeleton, nerves**.

a) Extrude the eyes, pluck them out and fix. Alternatively, you can leave the eyes within the sockets and fix them with the skull. Cut the skin at the back of the neck and free the skin on left, right, and dorsal part of skull to eyes. Cut the skin across the dorsal part of nose and remove it. Slide scissor blades under neck muscles and sever them at their attachments to the skull. Sever the neck at 1-2nd cervical vertebra to free the head.

b) Place the head right side up on a towel. With pointed scissors, cut the skull just posterior to the eyes, then around the side of the skull on each side just above the middle ear through to the Foramen Magnum. Remove the skull cap (calvarium).

c) Observe the cranial contents and note any pathology. Place the entire head with the exposed brain in place in the fixative.

d) Remove a femur and place it in fixative. If warranted, take sections of sciatic nerve with surrounding thigh muscles, intact vertebrae with spinal cord, etc.



9. **General Rules.**

a) Measure the size of tumors.

b) Any large mass (over 5 mm) may be fixed *in toto* but optimally should be bread-sliced to get optimal fixation of the specimen.

This is a suggested standard procedure for obtaining tissues for regular light microscopic analysis. The procedure should be modified, as needed, to obtain optimal specimens for particular needs.

Examples for special handling:

a. Samples for frozen sections or nucleic acid analysis should be taken **immediately** to prevent degradation.

b. Tissues that autolyze rapidly (brain, eyes, testes, intestines) should be **sampled and fixed first** if these tissues are the main object of study.

c. Samples for electron microscopy should be taken and fixed immediately, **maximum sample width of 1 mm**

d. Clinical pathology specimens like heart blood for hematology, swabs for bacterial cultures, or organs for tissue culture require sterile technique and immediate handling.