Mouse Surgery Protocols

(edited 1/11/96)

I. Preparation of anesthetics:

A. 2,2,2-tribromoethanol (Avertin™) --- From Papaioannou and Fox (1993); ref#2008

1) Solvents for 2,2,2-tribromoethanol:

a) Add 2.5 g tribromoethanol (Aldrich) to 5 ml 2-methyl-2-butanol (tertiary amyl alcohol; Aldrich) and dissolve by heating to 50° C with stirring or shaking. Add 200 ml distilled water and continue to stir until butanol is totally dispersed.

b) Alternatively, add 1 g tribromoethanol to 50 ml distilled water. Stir for >1 h in foil-covered vessel. 1000mg / 50ml

2) Filter-sterilize, then store as 50-100 ml aliquots in dark containers at 4° C.

3) Warm to 37° C and shake well before using.

4) Use 0.1 to 0.2 ml/10 g body weight administered i.p. – 4 mg / 10g

5) NOTES AND CAUTIONS: Avertin is stable for at least 2 years when stored properly. Initial pH of the solution will depend on the pH of the water used to prepare it. Decomposition can result from improper storage. Provided the pH of the original solution was >5, this can be tested by adding 1 drop of Congo Red (0.1%w/v) to 5 ml of anesthetic. Purple color developing at pH <5 indicates decomposition to dibromoacetic aldehyde and hydrobromic acid. If this occurs, these products are toxic and the anesthetic should be discarded.

B. Ketamin-Zylazine --- From Margaret Delano (Animal Care Office, U. Mass.)

1) Prepare stock solutions by adding 1 ml of a 100 mg/ml solution of ketamine, 0.5 ml of a 20 mg/ml solution of xylazine and 8.5 ml saline yielding a final volume of 10 ml.

2) Administer 10 µl/g of body weight for mice 23-32 g.

C. Analgesics for surgery — present federal standards suggest that optimal post-operative care should include analgesics to minimize pain. Bupivacaine can be used as a local anesthesia for surgeries that do not invade the body cavity (*e.g.* mammary transplants). Buprenorphine is to be used as analgesic for major surgeries in which the body cavity is invaded (*e.g.* ovariectomy, pituitary transplants, etc.) For references see:(Flecknell *et al.*, 1991). **Monitoring** for distress includes assessing the attitude, appearance of the fur, membrane color, ability to ambulate for 24 h after surgery. Visual assessment of water and food intake are also included. Body weight measurements can be taken before and after surgery as a quantitative measure.

1) Bupivacaine — for use as a local anesthetic around sites of surgical incisions of the skin.

a) Product name: Sensorcaine-MPF (Astra, Inc.) (Bupivacaine Hcl, methyl paraben-free, in sterile isotonic solution) Supplied as 0.25% (2.5 mg/ml) solution.

b) Dose for mice: 5 mg/kg

c) ml/10g BW 0.02 ml

d) Administration: Administer with a fine gauge needle (27 or 30 gauge) on 1 ml syringe. Administer subcutaneously. Inject equal volumes of total 6-8 sites in an elipse 0.5-1 cm from the planned incision site. Allow 3-5 minutes to take effect. One dosing is sufficient for 6-8 h duration.

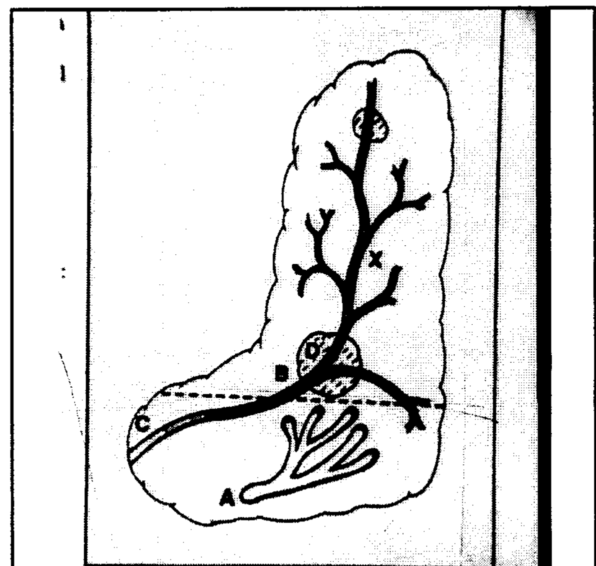
2) Buprenorphine — for use as analgesic for mice
   a) Product name: Buprenorphine (source?); supplied as 0.3 mg/ml solution
   b) Dose for mice: 0.05-0.1 mg/kg
   c) Preparation: 1 ml plus 9 ml sterile diluent
   d) Typical dosing volumes:

Body Wt (g)	Dose (mg)	Volume if undiluted (ml)	Volume if diluted (ml)	
15	.00075	.0025	.025	
20	.001	.003	.03	
25	.0013	.004	.04	
30	.0015	.005	.05	

   e) Administration: Administer pre-operatively or peri-operatively subcutaneously. The duration of action is 8-12 h. Doses can be repeated one time if needed.

3) Acetaminophen — analgesic for mice
   a) Product name: Acetaminophen, pediatric form 32 mg/ml
   b) Dose for mice: 110-305 mg/kg (suggested 1.5 mg/10 g BW)
   c) Preparation: 3 ml/ 97 ml water; assumes water consumption of 1.5 ml/10 g BW/ 24 h
   d) Administration: Provide diluted in water for 24 h post-surgery

II. Surgical Procedures
  A. Transplantation of mammary epithelium into cleared fat pads
    1) Prepare the following equipment:
      a) Cautery and spare batteries
      b) Surgical tools: DeWecker iris scissors (7mm, sharp-sharp); 2 curved iris forceps; tissue forceps; needle-point forceps; hemostat; sterile sutures
      c) Ear tags
      d) Auto-wound clips and remover tool
      e) Avertin on ice
      f) 25 gauge needles and tuberculin syringes
      g) Gloves
      h) Dissecting scope and illuminator
      i) 70% ethanol
      j) Materials for samples: microscope slides, liquid N_2, 10% NBF, cryotubes
      k) Petri dish and 1X PBS for



tissue transplants
l) Dissecting boards

2) Anesthetize mice <12 g body weight (3-4 wk old) with 175-250 μl of Avertin.
3) Make an inverted Y-shaped incission along the ventral throacic-inguinal region to expose the mammary fat pads.
4) Use the forceps handle to blunt-dissect the skin from the peritoneum. Use pins to hold the internal organs away from the mammary fat pad.
5) Cauterize the nipple and mammary artery running between the #4 and #5 fat pads and separate the two fat pads.
6) The mammary fat pad containing the parenchyma is removed using DeWecker iris scissors. The proximal lymph node in the fat pad provides a convenient landmark for the farthest limits of dissection.
7) Once 2-4 mice recipients are prepared, the transplant tissue can be obtained from donor mice. The tissues should be cut into 1 mm cubes.
8) Implant the tissue or cultured cells (10 μl of cells at a concentration of 5 x 10^7/ml) into each fat pad.
9) Close the skin with four wound clips.

B. Ovariectomy
1) Shave the back of the mouse.
2) Make a single mid-line incision along the back.
3) Lay the mouse on its side and locate one ovary. The ovary is beneath a deposit of white fat that is quite apparent in contrast to the surrounding dark red organs. Make an incision through the peritonium and pull the ovary out using the sharp jewlers forceps (#6). Note that the kidney can be easily damage because it is attached to the ovary by loose tissues.
4) Use the Serrifin clamp to hold the ovary. Place a ligature at the base of the ovary, but try to remain above the Fallopian tubes.
5) Remove the ovary, then close the peritoneum with one or two stitches.
6) Repeat the procedure on the contralateral side.
7) Close the skin with 2-9 mm wound clips.

C. Pitutary transplantation to the kidney capsule
1) Removal of pituitaries
a) Prepare sterile PBS on ice for holding the pituitaries.
b) Cervically dislocate the mice by pinching the neck. This is needed to prevent damage to the brain surrounding the pituitary.
c) Decapitate the mouse.
d) Expose the pituitary. This is done by first pulling the skin over the cranium and cutting the muscle overlaying the bone to gain a clear view of the skull. Cut along the sutures on the sides of the skull and remove the bone. Remove the cerebrum and cerebellum with forceps from an anterior to posterior direction to avoid damage to the pituitary.
e) Outline the perimiter of the pituitary cavity with sharp, half-curved forceps to break tissue attachments. Lift the pituitary gently with minimal squeezing.

Both lobes should be apparent under the dissecting scope.

f) Place the pituitaries in 1X PBS for up to 2 hours.

2) Implantation into recipients

a) Equipment
   (i) PBS
   (ii) Suture
   (iii) Sterile cotton swabs
   (iv) Trocar
   (v) Neosporin opthalmic solution

b) Make a mid-sagital incision on either side of the mouse. However, the spleen is on the left side.

c) Make an incision throught the peritoneum over the kidney.

d) Before exteriorizing the kidney, prepare the pituitary implant. With the bevel of the trocar in the proper orientation, draw some saline into the trocar, place the pituitary in the trocar, and draw it completely into the trocar. Dip a cotton swab into PBS.

e) Use a cotton swab to push the kidny out.

f) Hold the kidney between the thumb and index finger.

g) Nick the kidney capsule with sharp forceps. Insert the trocar into the hole in the capsule and well under the capsule. Deposit the pituitary and withdraw carefully the trocar. (If the capsule tears, it will be necessary to try the contralateral kidney or use a different mouse.)

h) Close the peritoneum with one stitch and add a drop of neosporin solution.

i) Close the skin with one wound clip.

D. Growth factor implants into mammary fat pad

E. Vasectomizing mice (as described in detail in "Manipulating the Mouse Embryo") The male mouse is anesthetized with Avertin. The abdomen is shaved and wiped with 70% ethanol. Using sterile instruments, the abdominal skin is cut at a point level with the top of the legs. A similar incision is made in the body wall. Using blunt forceps, the fat pad is pulled from the incision with the attachee testis, epididymus and vas deferens. With a sharp forceps, a hole is poked in the membrane beneath the vas deferens and a loop of silk suture is pulled through. The suture is cut to give two pieces of suture under the vas deferens. Both pieces are tied and a section of the vas deferens between is removed. The procedure is repeated on the other contralateral vas deferens. The testes are placed back into the body cavity and the body wall is closed with two to three stitches. The skil is closed with wound clips. The mice are placed under a heat lamp until recovery which is usually within 20 minutes

F. Embryo transfers (as described in detail in "Manipulating the Mouse Embryo") The recipient is anesthetized with Avertin. The lower back is shaved. After wiping the mouse's back with 70% ethanol, a small transverse (<1 cm) incision is made with a sterile dissecting scissors about 1 cm to the left of the spinal cord at the level of the last rib. The skin is slid around until the incision is over the ovary or fat pad. The body wall is picked up and a small incision is made just over the ovary. The incision is stretched

with the scissors to stop any bleeding. The fat pad and ovary are exteriorized and a serafine clamp is attached to the fat pad and laid down over the middle of the back. The infundibulum is located with the aid of a dissecting microscope. A hole is torn in the ovarian bursa and the embryo transfer pipet is inserted into the infundibulum with the aid of forceps. After embryo delivery, the serafine clamp is removed, the ovary is placed back into the body cavity, and the body wall is closed with 1-2 stitches. The skin is closed with wound clips. Mice are placed under a heat lamp until recovery which is usually within 20 minutes.

III. Freezing tissues in liquid nitrogen

A. Cryopreservation of HAN and tumors

1) Mince the tissue into fine pieces.
2) Place in 1 ml of freezing medium (DMEM/F12 + 10% fetal bovine serum + 7% DMSO).
3) Place tubes in cell freezer, then transfer to liquid nitrogen the following day.

IV. Hormone treatment of mice

A. Estrus cycle and hormone profiles

1) Vaginal smears (method and stages from Boot, Kwa and Ropcke, 1981 in Mammary Tumors in the Mouse, Hilgers & Sluyser, Eds.; see ref. #2679) — A cotton swab is used to collect cells from the vagina daily. The cells are swabbed onto a slide, air-dried, then stained according to the usual H&E protocol. It should be noted that vaginal stimulation can induce pseudopregnancy, but mice are less susceptible to this than rats.

a) Diestrus: Smear contains predominantly leukocytes, some epithelial cells with stained nuclei.

b) Proestrus

(i) Stage 1: Leukocytes dominate, but partly degenerated; gragmentation and cytolysis are observed. The vaginal contents are scanty, as a result the smears are thinly spread.,

(ii) Stage 2: Leukocytes still constitute ~90% of cells. Among the epithelial cells, some appear to have no stainable nuclei indicative of keratinization.

(iii) Stage 3: Proportion of leukocytes decline rapidly such that leukocytes should be less than 60% and the epithelial cells comprising about 40%. About 1/4th of the epithelial cells should have no stained nuclei. The vaginal content is increasing leading to thicker smears.

(iv) Stage 4: Total disappearance of the leukocytes, while ~50% of the epithelial cells are keratinized. The keratinized cells may still be quite small. The smears continue to thicken.

c) Estrus

(i) Stage 1:Thick smears in which the keratinized cells predominate. Stained nuclei should be present in <10% of cells. This stage corresponds to ovulation.

(ii) Stage 2: The smears contain only keratinized cells. They have a large, flattened form characteristic of full estrus.

d) Metestrus

(i) Stage 1: The same as above, but leukocytes reappear (<10% of cells). They may have a degenerated appearance.

(ii) Stage 2: The leukocytes now number up to 40% of cells.
(iii) Stage 3: Leukocytes incease to ~90% of cells.
(iv) Stage 4: The smears are now very thick with vaginal contents becoming "pasty" or "milky". Leukocytes dominate and stain clearly showing their characteristic clumping in large masses around the epithelial cells. The epithelial cells are still of the large keratinized type with a few smallers cells with stained nuclei be present as well.
(v) Stage 5: Thick smears composed of largely leukocytes, characteristically surrounding the epithelial cells. About half of the epithelial cells have stained nuclei as the keratinized layer of the epithelium dissolves rapidly.
(vi) Stage 6: A transition to diestrus; this stage is still set apart because of the thick smear and the typical arrangement of the leukocytes around epithelial cells that now all have stained nuclei.

2) Hormonal profiles during estrous cycle in mice
3) Hormonal profiles during pregnancy in mice — See McComack and Greenwald, 1974 (Ref #2675). Compare with humans reported by Tulchinsky et al., 1972 (Ref # 2676)
4) Hormonal levels during pseudopregnancy — See Christov et al., 1993; Ref#1869).

B. Growth of the mouse mammary gland — Methods for estimating gland development and responses to treatments is reviewed by Ceriani and Hilgers, 1981. (In Mammary Tumors in the Mouse, Hilgers & Sluyser, Ed.; Ref # jj2680)
1) DNA assay
2) Morphometric methods
3) Effects of hypophyseal transplants

C. Superovulation --- See Fowler & Edwards, 1957 (jj2765)
1) Prepare hormone solutions and store as 1 ml aliquots at -70° C.
   a) PMSG (Sigma # G4877)
      (i) Dissolve in 5 ml DPBS, then add to 35 ml DPBS to yield a final concentration of 5 IU/200 µl.
   b) hCG (Sigma # CG-5)
      (i) Dissolve in 10 ml DPBS, then add to 190 ml DPBS to yield a final concentration of 5 IU/200 µl
2) Inject 200 µl i.p. of PMSG.
3) Inject 200 µl i.p. of hCG 48 h later.
4) Cycle changes based on data from rats. For hormone profiles of normal rat see p 620 in *"The Physiology of Reproduction, Volume II, Second Edition"* by Knobil and Neil, editors. In the section of this text entitled "Endocrinology of Reproduction" they review the superovulation procedures and effects. PMSG has both FSH and LH activities whereas hCG is primarily LH-like. Therefore, superovulation will cause multiple follicles to develop, ovulate, then form corpus lutea. Therefore, there will be an exaggeration of the hormonal profile during estrus and subsequent phases. The luteal period will have extraordinary levels of progesterone due to multiple C.L.'s. For experimental data refer to data from C. Snelham (CS-I-p51). In this experiment samples were taken at the following times: 18 h post-hCG for estrus; 36 h post-hCG for early metestrus; 60 h post-hCG for late metestrus; and 100 h for diestrus.
   a) Estrus: Mice will ovulate at 12 post-injection. (12-36 h post-hCG) High FSH levels, but levels of Progesterone, Estradiol, Prolactin and LH decline to

baseline within 5 h post-ovulation).

b) Diestrus I: 48 h post-hCG (37-60 h post-hCG) Rising levels of Estradiol and Progesterone; basal for rest. Luteal phase is apparent(?)

c) Diestrus II: 72 h post-hCG (61-84 h post-hCG) Progesterone levels drop within 5 h following luteolysis. Estradiol levels continue to increase.

d) Proestrus: 96 h post-hCG (85-108 h post-hCG). Estradiol continues to increase. Prolactin also increasese during mid-proestrus, but appears to precede the Progesterone release that coincides with the LH surge.

D. Estrogen + Progesterone Priming (Vonderhaar procedure)

E. Hormonal treatments for altering mammary development

1) Ductal development (Nandi, 1960; see Ref#jj0632)

a) 1 ug estradiol for 30 days was sufficient to induce ductal elongation in hypophysectomized and ovariectomized.

b) 2.5 ug estradiol + 1 mg progesterone for 30 days was sufficient to induce ductal branching.

c) These hormones are soluble in ethanol at approximately 1 mg/ml. However, mice can tolerate no more than approximately 50 ul of ethanol. Therefore, most investigators appear to make aqueous suspensions in PBS. A volume of 100-200 ul was used for sub-cutaneous injections into the doral region over the front shoulders.

2) Long-term treatments to mimic prophylactic effects of pregnancy

a) Preparations for daily injections

(i) Dissolve estrogen in a stock solution of 2 mg/ml of 100% ethanol.

(ii) Dissolve progesterone in a stock solution of 1 mg/ml in sesame oil. This is accomplished by incubating the mixture overnight at 60 C in a glass scintillation vial with a magnetic stirrer and covered with foil. Be sure to add the oil, start stirring, then add the progesterone SLOWLY.

(iii) These are combined to yield final doses of 1 ug E and 1 mg P in 100 ul volume.

(iv) Notes from Lakshmi Sivaraman: Regarding the EP injections, my lab mates working with the PRKO and PRAKO mice still inject the animals with 1ugE/1mgP (made up in sesame oil) and look for gene expression changes as early as 4h-6h. At least I am sure they do RPAs. So long or short term the steroids are made up in sesame oil, not in PBS.

b) Preparation for beeswax implants for use in rats (Sivaraman et al., 1998; see Ref # jj2448)

(i) Dissolve estrogen in a stock solution of 2 mg/ml of 100% ethanol.

(ii) Dissolve progesterone in a stock solution of 1 mg/ml in sesame oil. This is accomplished by incubating the mixture overnight at 60 C in a glass scintillation vial with a magnetic stirrer and covered with foil. Be sure to add the oil, start stirring, then add the progesterone SLOWLY.

(iii) Appropriate volumes are mixed with beeswax to yield pellets containing 20 ug E + 20 mg P. One pellet is implanted s.c. into rats to mimic mammary gland development observed in pregnancy.

F. Glucocorticoid treatment to inhibit involution
   1) Previous doses reported
      a) Lund et al., 1996 (#jj2194) --- 0.5 mg/g BW/day; made as a suspension in PBS to provide a longer-lasting treatment (personal communication); delivered by daily injection s.c.
      b) Li et al., 1997 (#jj2238) — 7.5 mg/mouse/day; delivered by daily injection s.c.
      c) Feng et al., 1995 (#jj2282) — used hydrocortisone or progesterone to inhibit involution; delivered as pellets.
   2) Solubility characteristics of hydrocortisone 21-acetate (Sigma #)
      a) DMSO: 400 mg/ml; used 18.75 ul to deliver 7.5 mg/day

V. Irradiation of mice

A. Gamma-irradiation will be administered at the dose rate of 400 Rads/minute using a ^{137}Cs source located in Room 112 of Paige Laboratory. Total whole body doses will not exceed 500 Rad. This will be accomplished by placing single mice in a cardboard box (10 cm x 5 cm x 4 cm) that provides ample ventilation. The box is placed into the irradiator for <75 seconds to achieve the desired dose. Mice will be sacrificed within 24 hours and tissues used for biochemical and histological analyses.

B. The doses are sublethal and result in no acute or chronic affects on the mice (Storer, J.B. 1975. *Acute Responses to Ionizing Radiation.* In: Biology of the Laboratory Mouse, 2nd Edition, pp429-433.) Any discomfort should be minimal as the animals will be maintained <48 hours following exposure to radiation.

VI. DMBA treatment

A. Dimethylbenzanthracene (DMBA, Calbiochem) will be dissolved in cottonseed oil to yield a 0.25% (w/v) solution. A dose of 0.2 ml will be administered by gastric intubation once each week for 3 consecutive weeks starting when the mice are 8 weeks old. DMBA is made up fresh and just before use. The carcinogen in solution is light sensitive so the flask containing the solution is wrapped in aluminum foil. We ensure safe handling by using carcinogen dedicated balance, flask and spatula. Wear gloves and administer carcinogen to mice in a hood and use disposable diapers or coverings on any surface area where carcinogen is placed or used. The room should be low light or reflected light. The flask, spatula and feeding needle are rinsed in acetone and the solution put in a dedicated bottle exposed to light. Eventually the filled bottle is collected by environmental safety and the material appropriately disposed. The half life of DMBA in oil solution is approximately 30 minutes when exposed to normal ambient laboratory lighting conditions. The bottle containing the powdered carcinogen is kept in a locked cabinet. The cages containing the mice receiving carcinogen are handled specially for four weeks following carcinogen feeding. All feces, cage shavings and uneaten food are collected and incinerated at >1800 degrees. The carcinogen shows up in feces and urine for up to 10 days after treatment, so we build in a safety factor of an additional 20 days.

VII. Embryo transfer and screening transgenic mice

A. Oocytes for pronuclear injection are collected from females that have been hormonally stimulated to cause superovulation. The eggs are flushed from the oviducts following euthanasia of the donor. After pronuclear injection of the DNA, the embryos are

surgically injected into the oviducts of recipients females.

B. Transgenic mice will be screened by removal of approximately 1 cm of the tail tip from which DNA is then extracted. This procedure inflicts minimal discomfort, and therefore, requires no anesthetic. Usually bleeding is not a problem, however, the vessels can be electrically cauterized if needed.

VIII. Biological Characteristics and Data:

A. Mice, like most species have a circadian rhythm. Investigators should be aware that this may affect biological data and should standardize the time of day that samples/measurements are taken to avoid this affect. The standard light/dark cycle at the University of Massachusetts is ???.The adult mouse weighs approximately 40 grams and this small size and resulting large surface area/body weight ratio makes them susceptible to changes in environmental conditions. The core body temperature is easily affected by small changes in temperature which may modify the physiologic responses of the animal. The acute hearing of mice makes them highly sensitive to ultrasounds and high pitched noises inducing a stress response that has been empirically related to annibalism of pups by their dams. The well developed sense of smell is used to detect pheromones used in social interactions. The poor vision of mice makes them unable to detect color and red light is often used to observe animals during the dark cycle.

B. Basic Biological Data

Adult body weight: male
20-40gm
Adult body weight: female
20-40gm
Body surface area
10.5(wt. in grams)
Life Span
1.5-3 years
Food consumption
15 gm/100 gm/day
Water consumption
15 ml/100 gm/day
Breeding onset:male
50 days
Breeding onset:female
50-60 days
Gestation Period
19-21 days
Body Temperature
36-37 C

36.5-38.0 C

Heart rate
500-600 beats per minute

325-780 beats per minute