Protocol for Whole Animal/ Isolated Organ Vascular Perfusion Fixation (of rats/mice)

Advantages:

• Fixation begins immediately after arrest of systemic circulation. This minimizes the alteration of cell structure resulting from post-mortem effects.

• Under in situ conditions, vascular perfusion results in a uniform and rapid dissemination of fixative into all parts of the tissue via the vascular bed, resulting in an increased depth and rate of actual fixation.

• The manipulation of tissues after the arrest of the systemic circulation but prior to fixation is minimized resulting in many fewer artifacts.

• Many organs/ tissues may be effectively fixed at one time, thus maximizing the use of each animal.

• In the case of immunocytochemical procedures employing relatively mild fixation conditions, fewer autolytic artifacts result; redistribution or translocation of cellular components is minimized; and greater immunocytochemical activity is retained.

Pre-perfusion:

1. The rat/mouse is retrieved from the animal quarters and brought upstairs to the surgery room in a cage. The person removing the animal should sign and date the animal inventory card (yellow card) in the out column.

2. The rat/mouse is weighed and injected with a mixture consisting of ketaset(75 mg/ml) + xylazine(5 mg/ml). The recommended injectable dose of this anesthetic is 1 ml/gm of body weight, IM. Allow 10 to 15 minutes for anesthesia to occur, indicated by the loss of sensory/ reflex response, i.e. non- response to tail pinching, or paw pinching. (Note: Metofane, as an inhaled anesthetic, may be used instead of the ketaset/ xylazine mixture. In this case, assistance of another person is advised.)

3. Once anesthetized, and during the surgical procedures for whole animal or isolated organ perfusion, care should be taken to prevent heat loss in the anesthetized animal. They are quite prone to hypothermia. (use of a lamp is recommended)

4. People should consult Michael for the perfusion method for their particular needs. In general, an isolated organ perfusion will yield the best results, but concern should be taken to ensure maximum, most efficient use of the rat/mouse. For example, in the case of an isolated perfusion of the heart/lungs, the rest of the animal might still be suitable for biochemistry.
5. In most instances, the rat will expire during the perfusion. However, in cases where the animal survives or lingers, cardiac puncture is the appropriate means of sacrifice.

6. Upon completion of the procedure the carcass is wrapped in a surgical pad/ or benchcoat, placed in a plastic bag, and returned to the animal quarters in the cage. The carcass is placed in the freezer; and check that the animal inventory card (yellow card) is signed off.

**Recommended routes for vascular perfusion:**

- Whole animal- descending aorta or vena cava.
- Central nervous system/ pituitary- aorta, via the left ventricle.
- Kidney- descending aorta, proximal to its distal bifurcation.
- Liver- portal vein

**Perfusion Protocol**

**Perfusion pressure, in most instances, should be maintained between 60 and 100 mm Hg. Use a sphygmomanometer or gravity feed apparatus... we have both!!!**

1. Generally, it is optimal to aerate/oxygenate the flush and fixation prior to beginning. This can be maintained during the perfusion.

2. Flush the animal/organ first with 1XPBS containing 1% sodium nitrite, pH 7.4 for 30 seconds.

3. Follow up flush with perfusion of fixative for 5 minutes. Fixative contains:
   - 3% formaldehyde (freshly prepared from paraformaldehyde);
   - 1.5% glutaraldehyde
   - 2.5% sucrose
   contained in 100mM cacodylate, pH 7.4

   200mls of fixative should be plenty for a mouse; 500 mls for a rat.

4. After 5 minutes of continuous perfusion, organs can be harvested; appropriately dissected in fixative/buffer (100mM cacodylate, 2.5% sucrose, pH 7.4); and tissue pieces allowed to fix an additional 1 hour.

5. Wash in 0.1M Cac/2.5% sucrose pH 7.4 3X 15' EA.
6. Post-fix with Palade's OsO4 for one hour on ice, light tight, under hood.
5 ml Palade's 1% OsO4 = 1 ml Acetate-veronal stock
   + 1.25 ml 4% OsO4
   + 1 ml 0.1N HCl
   + 1.75 ml ddH2O

Acetate-veronal stock = 1.15 g NaAcetate Anhydrous
   (J.T. Baker 1-3470)
   + 2.943 g NaBarbituate (Veronal)
   labelled Barbital--(Sigma B-0500)
to 100 ml with ddH2O

7. Rinse 1X with Kellenberger, then incubate for 1 to 2 hrs. at RT. (or preferably overnight)

10 ml Kellenberger = 2 ml Acetate Veronal Stock
   + 2.8 ml 0.1N HCl
   + 5.1 ml ddH2O
   + 0.05 g Uranyl Acetate
   Check pH with paper before adding UA.
   (Should be ~6)

8. One quick rinse in ddH2O, then one quick rinse in 50% ethanol.

9. Dehydrate with graded series of cold (4°C) ethanol (70, 95, 100); then three 10' washes in fresh 100% ETOH at rm. temp; and then finally two 5' exchanges with propylene oxide (PO).
14. Place in 50% PO/50% Epon (can be old) overnight, uncovered under vacuum (or hood).

10. Replace with fresh, 100% epon, and leave under vacuum for 2-6 hours.

11. Embed in fresh, 100% Epon. Put typed or pencil-written label in dummy capsules with wooden stick, at least two capsules per sample. Pour tissue out of tube into mincing dish. Place tissue in flat mold with small amount of Epon (to avoid curling) with a wooden stick and place in 60°C oven overnight.