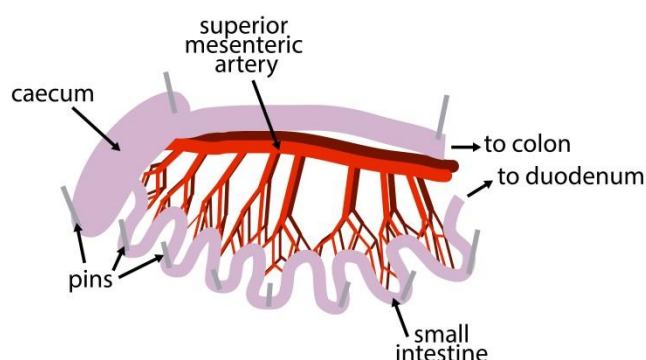


## Preparation of *en face* arterial endothelium

Intact preparations of *en face* endothelial cells were prepared as described briefly here:

1. An intact mesenteric bed (inc. intestines, caecum, large intestine & colon and associated fat/blood vessels) from male rats was excised and submerged in MOPS buffer.
2. This can then be sorted and pinned out with 200  $\mu\text{m}$  diameter pins, in a glass petri dish coated with Sylgard and filled with fresh MOPS buffer.

**\*\*\*NOTE\*\*\*** By placing the caecum upper left most and the colon in the upper right, the arterial vessels remain uppermost with the more fragile veins underneath. Excess colonic tissue or intestinal tissue from where it was attached to the stomach can be trimmed now.

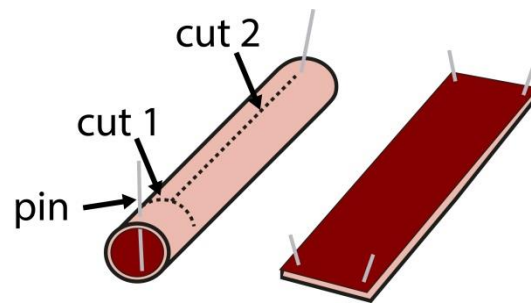


3. The fat that surrounds the mesenteric arteries can now be cleaned away by gently using forceps and microscissors. Pull the fat gently away and snip the connective tissue holding it onto the vessel taking care not to touch, poke or nick the artery.
4. When sufficient fat has been cleared, the downstream end of the artery can be pinched shut with forceps and cut.

**\*\*\*NOTE\*\*\*** At this point it is important not to let the vessel 'snap' back, by holding the vessel with forceps and slowly releasing the tension, this can be avoided.

5. Finally to excise the artery from the mesenteric bed the same cut is made at the upstream end of the artery. The excised vessel can then be transferred to a Sylgard block or Sylgard coated flow chamber filled with MOPS.
6. Once in the imaging vessel, 50  $\mu\text{m}$  wire cut to make fine pins can be used to carefully pin either end of the excised vessel, keeping just enough tension on the artery so that it isn't flapping around. A transverse cut is then made through the top half of the artery (below - cut 1) close to one end, facilitating the entry of micro-scissors into the lumen. The tips of a pair of micro-scissors can then be inserted into the lumen and a cut made through the top wall of the artery along its full length (cut 2).

**\*\*\*NOTE\*\*\*** It is very easy to try and put too much of the scissor blade into the lumen. This will damage the bottom face of endothelial cells, and should be avoided at all costs.



7. Once the longitudinal cut has been made, the corners of the artery can be gently pulled flat and pinned. The excess tissue at the ends can now be trimmed leaving a flat rectangle of intact endothelial cells.
8. The *en face* preparation is ready for loading.