

THE TECHNIQUE OF INTRATHORACIC INJECTION:

M. EICHNER

The injection medium consists of 1.04 g RPMI (Serva) diluted in 80 ml of sterile filtrated, demineralized water complemented with 20 ml of heat inactivated fetal calf serum, 0.2 g CaCO₃, 200 µl penstrep^R and 100 µl amphotericin B^R, adjusted to pH 7.3 - 7.5 by using NaOH and HCl. It has to be centrifuged for five minutes at 1500 U/min and can be stored for some days in the refridgerator.

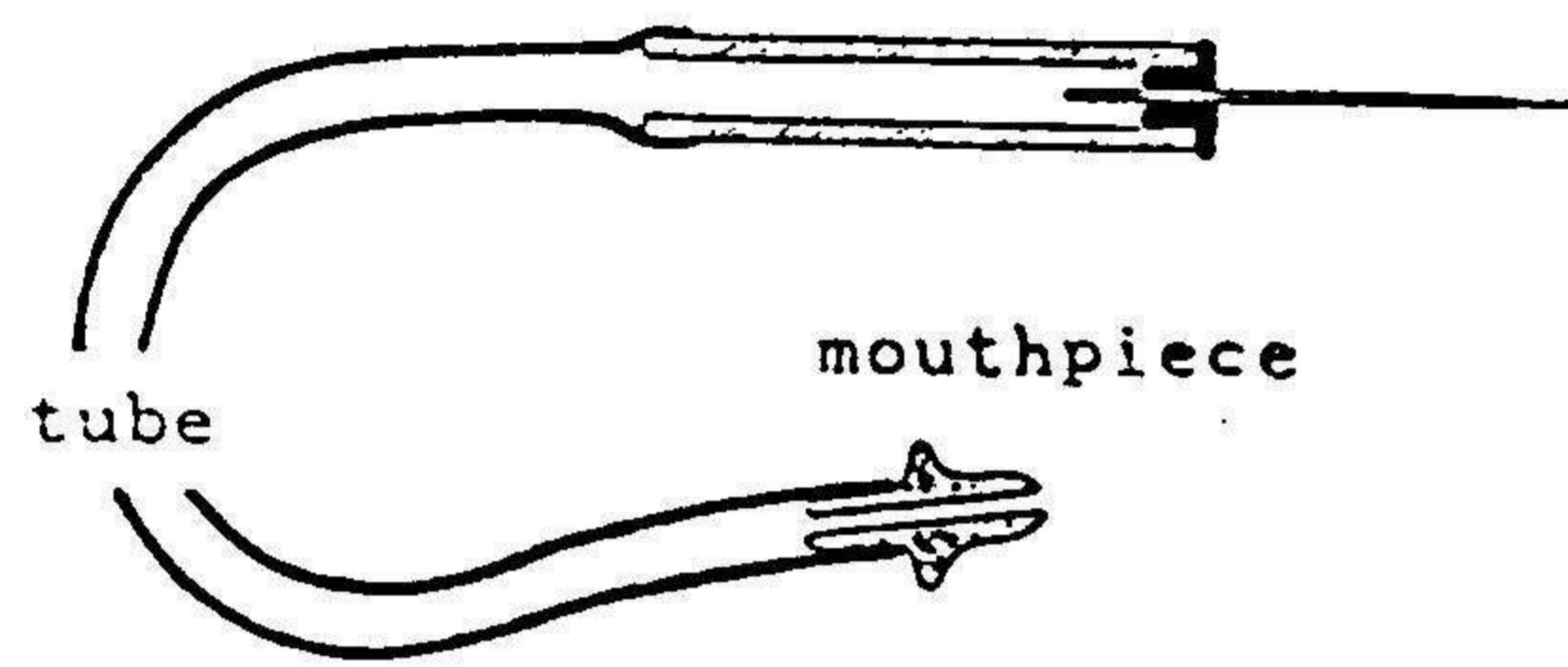


Figure 1: Sucking tube

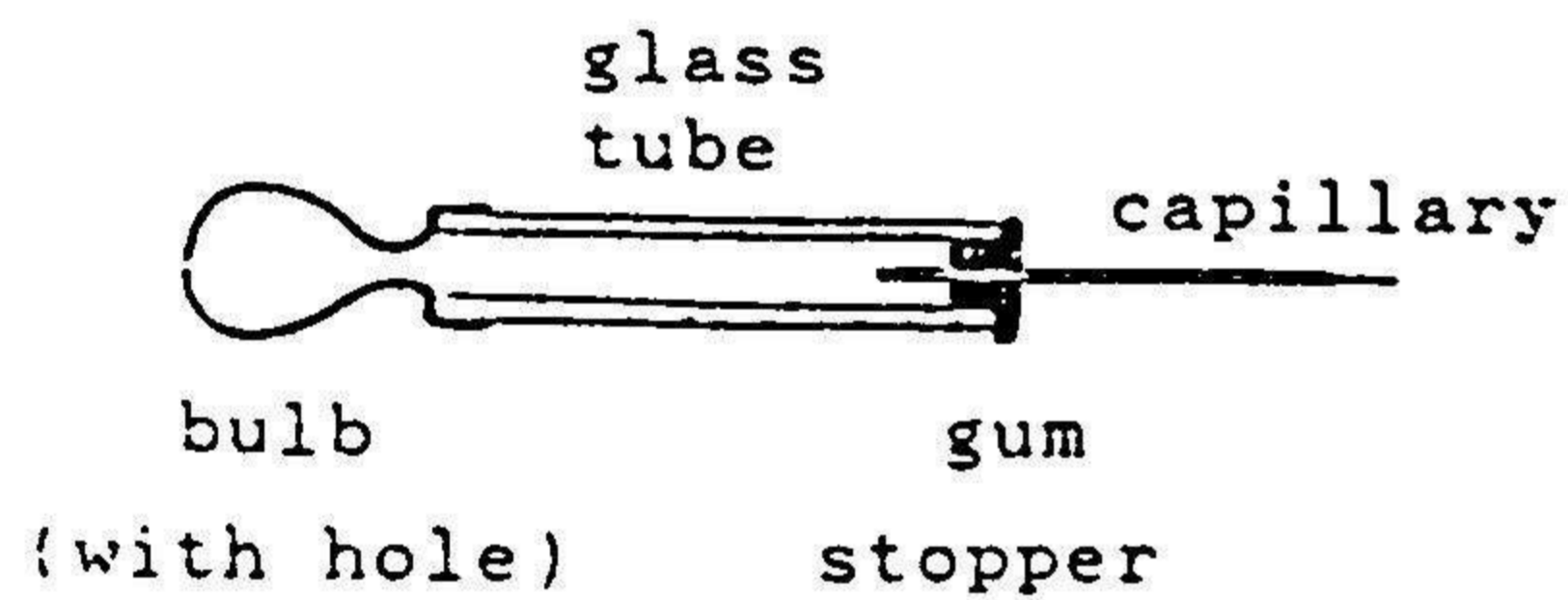


Figure 2: Syringe

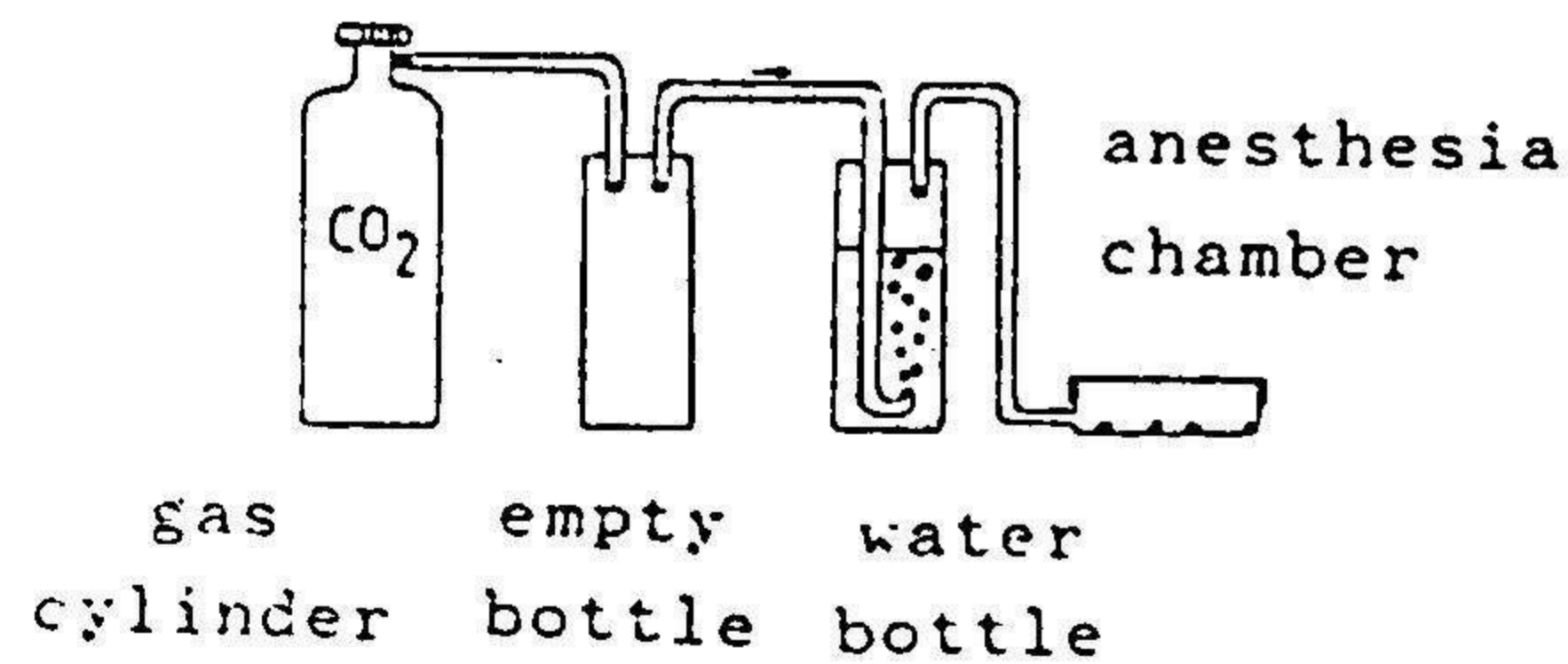


Figure 3: Anesthesia chamber

For the production of injection needles (unheparinized) haematocrit capillaries are used. It is possible to heat them with a Bunsen burner and to pull them by hand. An easier and better mean is to use an electrical pipette puller. The needles used for injection should have a tip diameter of about 30 µm.

Skin snips are incubated in at least 100 µl of medium each. Evading microfilariae are sucked by mouth into the injection needle (fig.1; the ammount of injection medium must not exceed 1.5 µl). Then the needle is fitted into the syringe (fig. 2).

Simulium pupae are collected from their breeding sites, transported to the laboratory and placed in a hatching box. Hatched flies are transferred by a sucking tube into the anesthesia chamber (fig. 3). After a few minutes a fly is anesthized and can be taken out with a fine forceps. It is placed on the operational table and one wing is fixed with a slide to avoid an escape of the fly (fig 4). The injection needle is inserted into the pleural membrane (which is the only soft part of the thorax where an injection is possible at all; fig 5). Injection is done by closing the hole in the bulb of the syringe with a finger and pressing it gently to force the injection medium into the fly's thorax.

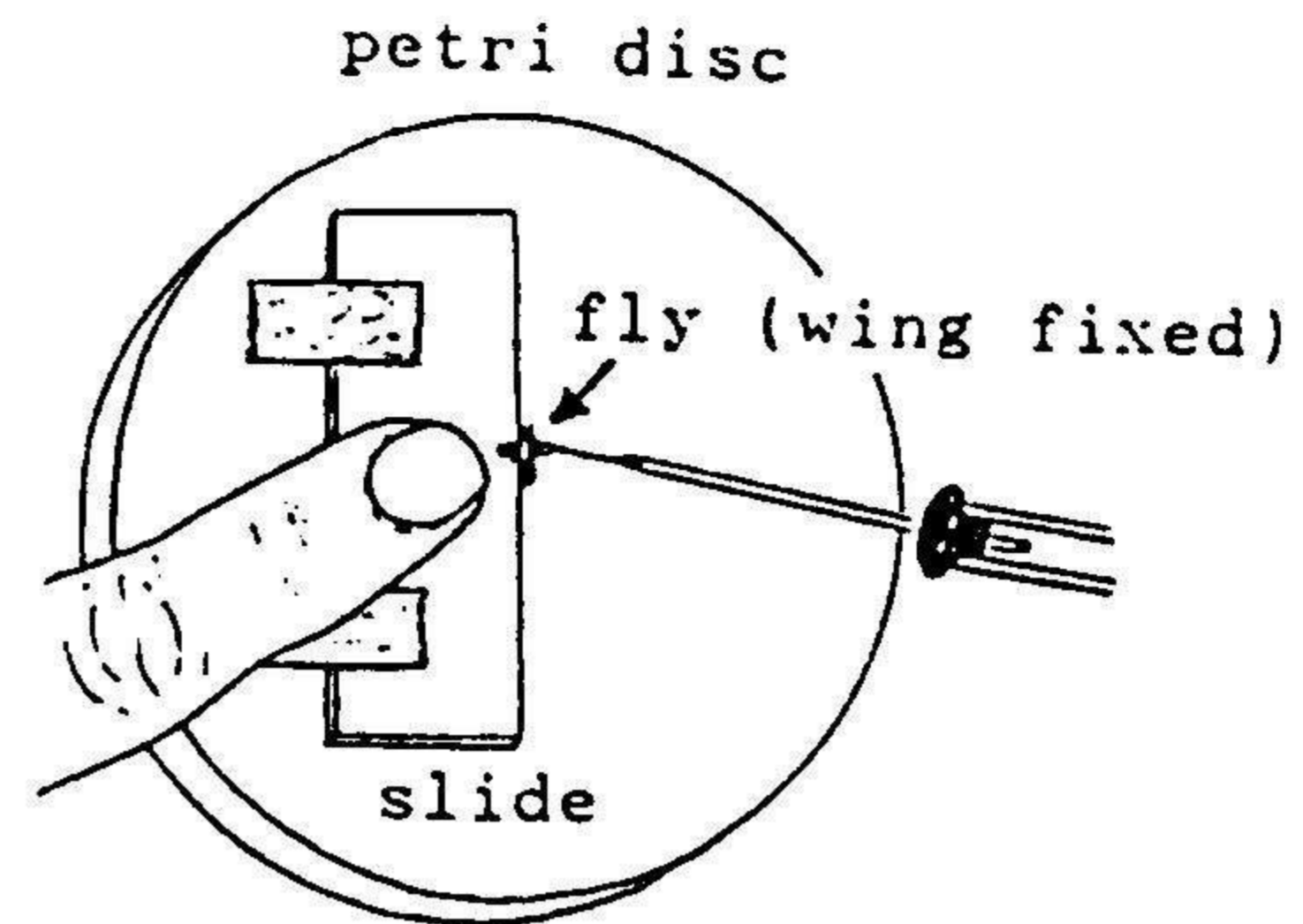


Figure 4: Operational table

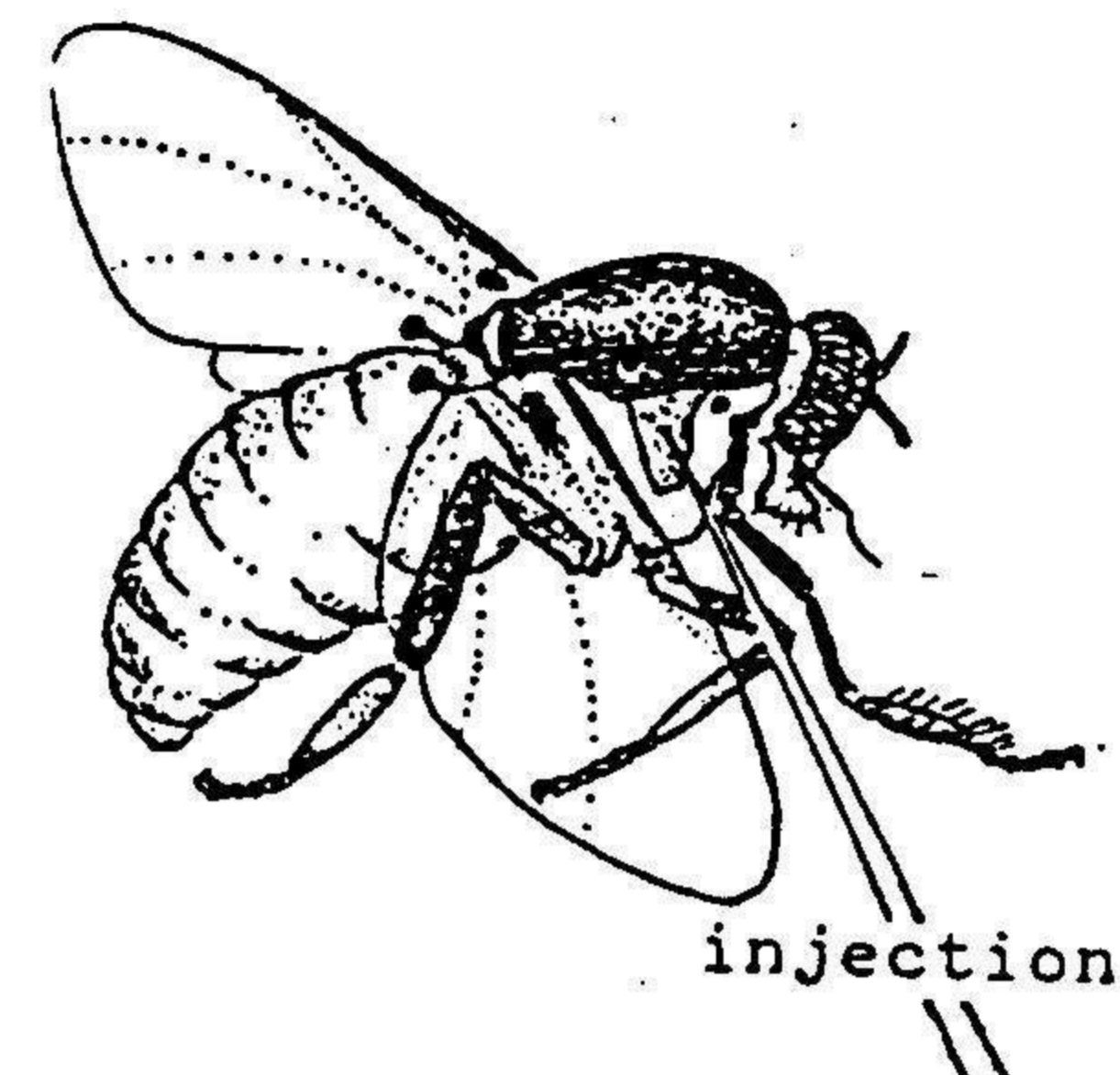


Figure 5: Pleural membrane

After Injection the fly's wing is released and the fly (which is still pinned up by the capillary) is transferred cautiously to a plastic tube.

To keep a high percentage of flies alive it is necessary to use very fine needles and least contaminated medium. Every kind of "stress" before and after operation increases the mortality enourmously. Therefore we advice to use a simple maintaining system like the one shown in figure 6, which keeps the flies in a dark chamber at constant temperature with circulated air of maximum humidity. Fungi were often observed at the tarsi and mouth parts of dead flies. Because of this it seems to be necessary to do some experiments with different antibiotics as additives in the sugar solution as well as in the injection medium.

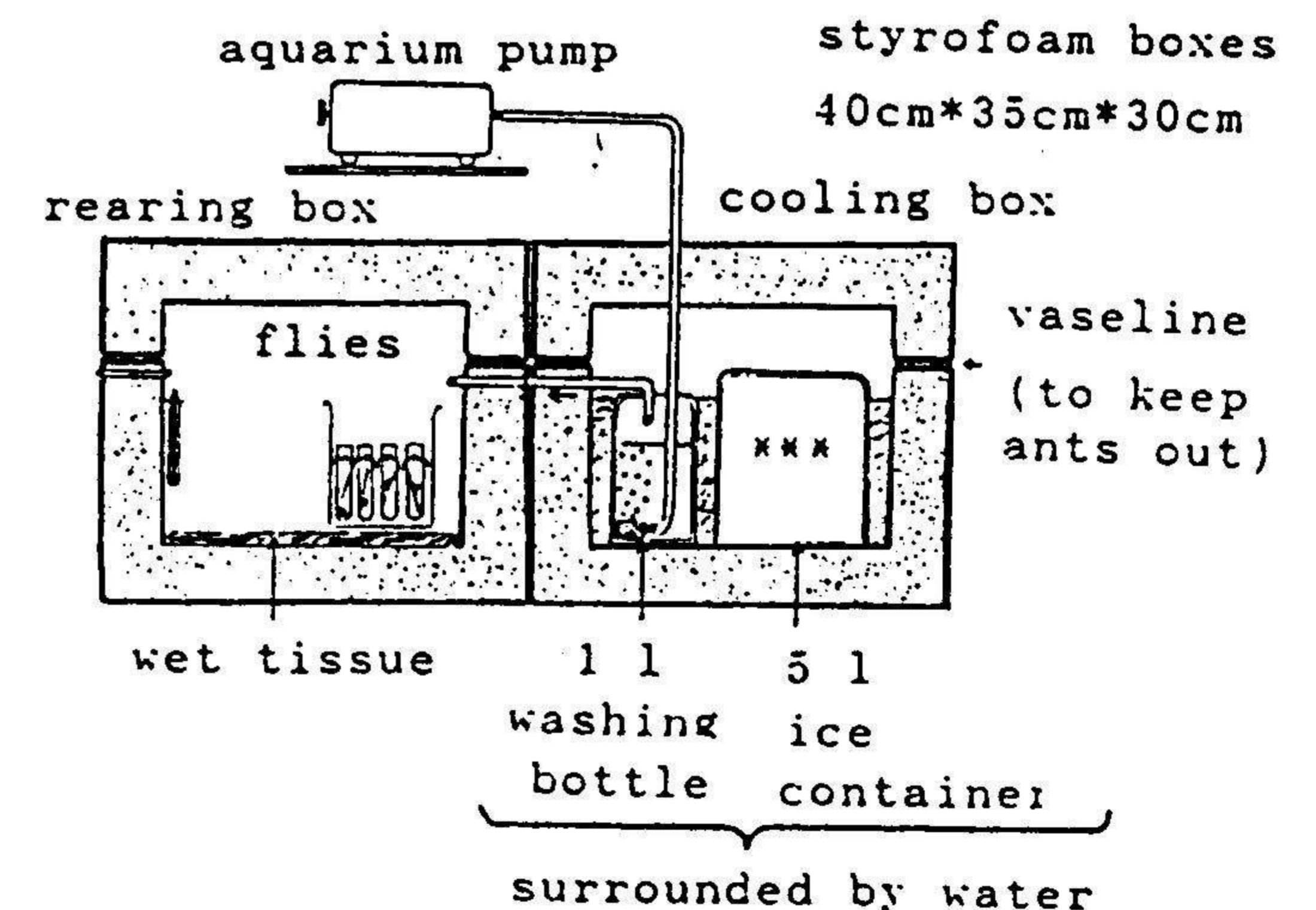


Figure 6: Rearing apparatus