

APPENDIX C:

Narcotizing Methods

Small Invertebrates, Larvae, and the Like

1. Place the organisms in a 0.1% solution of Chloretone (chlorobutanol 1,1,1-trichloro-2-methyl-2-propanol, Sigma Chemical Co. product no. T5138) and allow them to remain there until quiet. If kept moist, the animals will revive when returned to water.
2. Warm water (37° to 38° C), if properly regulated, will relax worms, clams, and so on, without stopping the circulation. This is an excellent way to open or peg freshwater clams. 48° C is lethal.
3. Freshwater worms and the like may be narcotized by placing them in a watch glass or Petri dish with water to barely cover them and blowing ether or chloroform vapor over them. (Instructions for ether-vapor tube are given later.)
4. Annelids, cnidarians, molluscs, echinoderms, and the like may be slowly narcotized by being placed in a very small amount of the water in which they were collected. Allow them to settle and expand and then gradually add any one of the following: magnesium sulfate crystals (Epsom salt); menthol crystals; saturated solution of magnesium sulfate or menthol; or 70% to 95% alcohol. Add the liquid drop by drop. It may take several hours to render some forms insensitive.
5. Earthworms may be placed in 7% ethanol until relaxed, or immersed in 0.09% tricaine methanesulfonate (MS222) for 1½ to 2 hours.
6. Echinoderms can be relaxed in 7% MgCl₂ made up in tapwater. It will take about 40 minutes. Anesthetized animals can be restored to activity by bathing in running seawater. Perhaps the best way to relax sea cucumbers is to seal them in a jar filled completely with seawater (no air) and allow the water to become oxygen free; this technique is especially useful for relaxing before preservation (see Appendix 2).
7. Urochordates can be placed in 1% chloroform in seawater and allowed to sit until relaxed. Or, place the animals in a small amount of seawater, sprinkle menthol crystals over the water, and leave for 1 to 4 hours.
8. For rotifers, phenylephrine (Neo-Synephrine) is suggested. It is obtained from the Sigma Chemical Co. (product no. P6126) or at drugstores as the hydrochloride in a 1% solution and is used as a 0.1% to 0.5% solution. It works best in slightly acidic waters.
9. For protozoans, a 1% nickel sulfate solution paralyzes cilia; 1% potassium (or sodium) iodide solution is said to prevent myoneme constriction in *Stentor* (and presumably other ciliates); and tobacco smoke in a test tube inverted over a drop of culture (or hanging drop inverted over the test tube) is useful. Methylcellulose constricts movement but does not narcotize.
10. Other suggested narcotizing agents:
 - a. Carbon dioxide added as ordinary charged water (soda water) to fluid containing animals (particularly cnidarians and echinoderms) or as CO₂ gas bubbled directly into the container.
 - b. Chloral hydrate, 10% solution, added drop by drop until organisms are fully extended.
 - c. Clove oil, a few drops scattered on surface of water. This is very effective for crustaceans.
 - d. Magnesium chloride, 2.5% solution of the hexahydrate in tap water.
 - e. Magnesium sulfate, saturated solution, added very gradually until the organism is relaxed (for marine organisms).
 - f. Tricaine methanesulfonate (MS222) (0.33 g/L in the ambient medium) for cold-blooded animals.
 - g. Eucaïne; prepare a solution of B-eucaïne hydrochloride (1 g), 90% alcohol (10 ml), and distilled water (10 ml), and add drop by drop to culture of microorganisms.

Vertebrates

Frogs: Two methods are suggested for anesthetizing frogs.

1. Immerse the frogs in 2% MS222.
2. Inject 1 ml of 5% urethane per 30 g of animal weight into the dorsal lymph sac of each frog.