

SOME NARCOTIZING, KILLING, FIXING, AND PRESERVING
REAGENTS AND THEIR USES, COMPILED FROM BOTH LO BIANCO
AND WAGSTAFFE-FIDLER

(A) LIST OF REAGENTS (*INDICATES CONSIDERABLE
TO MUCH USE OF THE SUBSTANCE)

ACIDS

Acetic, glacial, 55% concentrated
Acetic, Osmic
*Chromic .25, .5, 1, 5%
Chromic & Osmic .25, 1%
Chrom-acetic #1 = 1% chromic 100 cc & concentrated
acetic 5 cc
*Chrom-acetic #2 = 1% chromic 10 cc & concentrated
acetic 10 cc
Chrom-formalin 4%
Chrom-osmic 1%
Chrom-picric solution
Chrom-alcohol
Hydrochloric-alcohol
Nitric
Osmic 1%
Picric, saturated
Pyroligneous, concentrated
Sulphuric

Albumen
Alcohols - 35, 50, 70, 95%
Iodized 35, 70%
Alcoholized seawater 3, 10%
B-Eucaine hydrochloride
Benzamine hydrochloride 2% aqueous solution
Bouins
Cellosolve, pure (ethylene glycol mono-ethyl ether)
Chloral hydrate .2, 5%; .1% in seawater
Chloroform
*Cocaine
Copper sulphate
*Corrosive sublimate
Corrosive sublimate - 90% alc.
*Corrosive sublimate - acetic acid
Corrosive sublimate - Chromic acid
Corrosive sublimate - osmic acid 1%
*Corrosive sublimate - copper sulphate
Corrosive sublimate - seawater
*Distilled water

Ether - seawater
 Ethyl acetate fumes
 Ethyl bromide
 Euparal
 Fleming's fluid (1% chromic acid 25 cc 1%
 osmic acid 10 cc glacial acetic 5 cc,
 distilled water, 60 cc)
 Formalin 4, 6, 10%
 Formol alcohol
 Gilson's fluid
 Glycerin
 Glycerin 1 part, 70% alc. 2 parts, seawater 2 parts
 *Kleinenberg's solution (picric acid, saturated 100 cc,
 sulphuric acid, concentrated 2 cc, filter and add
 3 times the volume of distilled water)
 Magnesium chloride
 Magnesium sulphate crystals
 Menthol crystals
 Muller's solution (Potassium bichromate 2 g, sodium
 sulphate 1 g, distilled water 100 cc)
 Perenyi's solution (40 cc of 10% nitric acid, 30 cc
 of 0.5% chromic acid, 30 cc of 90% alc.)
 Potassium bichromate - osmic acid
 Prussian blue
 Schaudinn's fluid
 *Seawater
 Sodium chloride solution
 Sodium hydroxide 10%
 Stovaine
 Tobacco smoke

(B) SOME NARCOTIZING REAGENTS AND THEIR USES

Most invertebrates are highly contractile and to be preserved in an extended condition must be narcotized slowly in water, either until dead or until they may be killed or fixed without contracting. The following reagents and methods are recommended.

ALCOHOL. This is advised for the larger invertebrates. A 50 or 70% solution is added drop by drop, to the surface of the water. Lo Bianco's mixture containing 40 parts of 70% alcohol, 20 parts of glycerin, and 40 parts of water is also excellent and is poured onto the surface of the water and allowed to diffuse slowly.

B-EUCAINE HYDROCHLORIDE. This is used as a 1% solution gradually introduced to the water containing the animals. It is especially valuable for Flosculariae, Vorticellidae, Rotatoria and many larval forms and dissolves in seawater to approximately 0.5%. In the British Collector's Handbook, the following is recommended;

Eucaine 1 g
Alcohol 90% 10 ml
Distilled water 10 ml

to be introduced slowly over a period of time. It is primarily for larger forms.

CARBONIC ACID GAS. This has been used for Coelenterata, Echinodermata, and Hirudinea. The gas is introduced by squirting the contents of a soda-water bottle into a considerable volume of water containing the animals so that the water is saturated by the gas.

CHLORAL HYDRATE. Very good results have been obtained with this chemical with Actinae, Annelida, Mollusca, freshwater Polyzoa and many larval forms. The crystals should be dropped directly into the water containing the animals, or the animals placed directly in a 5-10% solution. For more delicate work, the solution should be introduced very gradually over a period of several hours. It is used for killing animals in rock crevices, incrustations of calcareous algae, and colonies of serpulas and madrepores. The animals should remain in the solution for 4-6 hours.

ADVANTAGE: If animals do not narcotize as desired, they may be replaced in clean seawater, and will recover.

CHLOROFORM. This is useful for highly contractile animals and should be employed by squirting small quantities of it through a fine syringe every five or ten minutes onto the surface of the water containing the animals. It may also be placed in a separate container and enclosed under a belljar together with the animals to be treated. For some forms one or two drops should be added every 5 or 10 minutes to the vessel containing them.

CHLOROTONE (Acetone Chloroform). This is recommended as a 1% solution for small Polyzoa, used in the manner described for chloroform and added slowly.

COCAINE. 2 g of powder in 100 cc of 5% alcohol is a very good narcotic.

ETHER. Oestergren (Zeit. Wiss., Mik., xix, 1903, p. 300) recommends a saturated solution (7-8%) in either seawater or fresh water, used either concentrated or diluted to approximately 1%.

MAGNESIUM SULPHATE. Often used as a saturated solution into which the animals are plunged. More satisfactory results are obtained however if the crystals are added to the water containing the animals, or if it is introduced gradually over a period of some hours to the water in the form of a 20-30% solution. It is recommended for many marine forms, especially Actinae.

MENTHOL. One of the most useful narcotizing agents for many marine animals. It has been chiefly recommended for Mollusca, anemones (Zoantharia), sea cucumbers (Holothuroidea), and sea squirts (Tunicata). Animals are placed in a clean vessel containing clean seawater, and crystals are sprinkled on the surface.

ROUSSELET'S SOLUTION. A well known mixture widely used for narcotizing Rotifers and Polyzoa by many workers apparently with success. The original formula consisted of:

2% solution hydrochlorate of Cocaine	3 parts
Methylated spirit	1 part
Water	6 parts

Stovaine provides a satisfactory substitute for Cocaine. The mixture is added drop by drop to the water containing the animals.

STOVAINE (Amyl Chlorohydrin). This is a most useful and powerful narcotizing agent for small invertebrates. It now has largely replaced cocaine which is difficult to obtain. It is usually used as a 1% solution in distilled water which is gradually added, over a period of time to the water containing the expanded animals.

TOBACCO SMOKE. An effective narcotizing agent for many small organisms, such as Hydra and Infusoria. It should be slowly and carefully bubbled into water through a fine glass tube lying on the bottom of the container.

(C) SOME KILLING, FIXING, PRESERVING REAGENTS
AND THEIR USES

ACIDS

Acetic

- 1) Permeates and hardens tissues instantly.
- 2) Rapidly kills contractile forms (tissues soften if left in too long).
- 3) Objects remain relatively transparent in it.
- 4) Used with chromic acid it is effective for killing and hardening non-contractile forms.

Chromic

- 1) Next to alcohol, in an aqueous solution, it is the most useful reagent for killing and hardening soft, gelatinous forms.
- 2) Caution - Objects become fragile and too deeply tinged if left in it longer than necessary.
- 3) After treatment, wash animals in fresh water to avoid a precipitate when they are later placed in alcohol.
- 4) When mixing chromic with osmic, acetic, picric or corrosive sublimate, use FRESHWATER.
- 5) Solutions do not keep long.
- 6) Solutions that have turned green are not fit to use. (Solutions may be used if they are not too dilute when mixed with the water containing the specimen).

Hydrochloric - USE RARE - Mixed with 50% alcohol.

Lactic

- 1) A solution of 1-1000 seawater effective for larval and small gelatinous forms.

Osmic - USE RARE TODAY.

- 1) Hardens gelatinous forms and preserves transparency well, but its action is too great.
- 2) Caution - Animals should remain in it only until they are light brown color.
- 3) It is used in
 - a) Chrom-osmic mixture.
 - b) Potassium bichromate-osmic acid.
 - c) Flemming's solution.

Pyroligneus - USE RARE

Sulphuric - USE RARE. (Ingredient of Kleinenberg's solution).

Alcohol - Most indispensable liquid.

- 1) For preparation and preservation of transparent animals, filter and dilute with distilled water.
- 2) Caution -
 - a) if reused, acids and alkalies must be neutralized.
 - b) to avoid bubbles, mix previous to using.
- 3) Use 70% for preserving - 90% for special cases.
- 4) Soft and gelatinous animals must remain 2-6 hours in 35, 50, 60, 70% alcohol each.
- 5) Animals may be placed in alcohols or if too delicate siphon used alcohol and flood them with increasing strengths.
- 6) 70% used for permanent preservation.
- 7) Alcohol is effective for narcotizing and killing animals slowly or quickly.

Copper Sulphate

- 1) Use - killing larvae and delicate forms in a 5-10% solution of hot freshwater used alone or mixed with corrosive sublimate.
- 2) Caution - wash objects frequently with water or they will not remain clear. Treat with acid, if specimens become opaque.

Corrosive sublimate

- 1) Fixing agent. Permeates and hardens tissues quickly.
- 2) Use in concentrated solution in either fresh or seawater - hot or cold.
- 3) Caution -
 - a) do not use metal tools; they decompose and stain specimens,
 - b) make with hot water,
 - c) do not boil in open vessel - vapors are very poisonous,
 - d) keep hands out of solution especially if they are cut.

Formalin

- 1) Used to preserve animals temporarily only, because animals eventually decompose in it.

- 2) Can be used for animals that are
 - a) non-contractile,
 - b) have no lime, spicules, skeleton or shells.
- 3) Fish - use 5% injection through anus.
- 4) Place only a few animals in the same jar at the same time and have much fluid in proportion to animals.
- 5) Gelatinous animals - use 1-4%. Kill and harden at the same time with formalin plus 1% chromic acid in a ratio of 1:1.
- 6) Ascidians and Tunicates - use 2-6% formalin (the softer the animal the weaker the solution).
- 7) To make solutions, use fresh, or best, seawater.
- 8) Transfer animals directly from formalin to alcohol.
- 9) Effective for killing and hardening certain forms.
Advantage - Colors preserved longer in formalin than in alcohol.

Potassium bichromate

- 1) 5% solution used for slowly hardening gelatinous forms especially when it is not possible to use chromic acid.
- 2) Caution - A troublesome precipitate is formed when specimens are transferred to alcohol so its use is not recommended.
- 3) To bleach forms before placing them in alcohol, use a few drops of concentrated sulphuric acid.

Tincture of Iodine - USE RARE.

- 1) Used in a 35 or 70% alcoholic solution after animals have been killed in corrosive sublimate.