

## Preservation of Animal Tissues, with a Note on Staining Solutions

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### SUMMARY

Methods are given by which animal material, fixed by any desired method, may be preserved against attack by fungi or bacteria. The reagents employed are glycol monophenyl ethers ('Phenoxetol'), and esters of *p*-hydroxybenzoic acid ('Nipa' esters). These reagents, dissolved in water, produce colourless and almost odourless solutions, in which a variety of animals have been preserved for many months without showing any traces of deterioration or decomposition.

**P**RESERVATION of animal tissues is usually undertaken with one of three ends in view: for dissection, for museum display, or for histology. Fixation of tissues differs from preservation in that the former is designed to render most or some special elements insoluble and proof against further change. The aim of preservation is to maintain this condition.

Some reagents such as 75% methylated spirits in water, and 10% formalin may serve both functions, but as preserving fluids they have the disadvantage that they are volatile, and formaldehyde is pungent and unpleasant to use. An ideal preserving fluid should have the following properties. It should

1. maintain the fixed tissues in good condition, and
2. be pleasant to handle,
3. odourless,
4. non-volatile,
5. transparent,
6. colourless,
7. non-poisonous,
8. bactericidal and fungicidal,
9. low in price, and
10. maintain the natural colour of the specimen.

Ethylene glycol monophenyl ether and *p*-hydroxybenzoic acid, with their derivatives, possess most of these requirements. They are manufactured by Nipa Laboratories, Treforest, Pontypridd, Glam., the former compounds being referred to as 'phenoxetols', the latter as 'Nipa' esters. In their pure forms these compounds may be considered poisonous, but in the dilutions suggested here they may be used freely and without special precautions. The bacteriostatic and bactericidal value of ethylene glycol monophenyl ether was reported by Berry (1944). The narcotic properties of phenoxetol (Gough and others, 1944), and of propylene phenoxetol (Owen, 1955) have been mentioned, also the preserving properties of propylene phenoxetol used with gelatine solutions (Gough and Wentworth, 1949). The capacity of propylene phenoxetol to

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restore the natural colour to blood-pigments in pathological specimens was commented on by Gough and Wentworth (1949), and the value of the esters of *p*-hydroxybenzoic acid as preservatives in ointments, &c., is generally recognized (Boehm, 1933).

Preliminary experiments on the preservation of animal tissues by propylene phenoxetol and Nipa ester combination No. 82121 have been carried out in this Department. It is regretted that none of the animals has been preserved for longer than 6 months, but the method is considered so promising that an early account was considered desirable. With propylene phenoxetol as a preserving fluid the procedure is as follows:

1. Fix the specimen for the minimum length of time (12 hours for an opened frog; 48 hours for an opened dogfish) in 10% formalin or 70% alcohol. Frogs and similar vertebrates should be fixed in a position suitable for subsequent dissection. Improved colour-retention of the specimens may be obtained by fixing in the following solution (Gough and Wentworth, 1949):

Formaldehyde, 40%	500 ml
Sodium acetate	500 g
Water	5,000 ml

2. Wash in running water 12 to 24 hours.
3. Transfer the specimen to 1% propylene phenoxetol in water.

The procedure with 'Nipa' ester combination No. 82121 is essentially the same. Transfer the specimens after fixation and washing to 0.2% Nipa ester in water. As the rate of solution of both propylene phenoxetol and Nipa ester is rather slow, it is essential to add them slowly to water and to stir vigorously at the same time, or alternatively to keep stock solutions of 20 ml of propylene phenoxetol or 20 g of Nipa ester in 80 ml of industrial methylated spirits. The required volume of stock solution is then added to water with stirring. The alcohol aids the dispersion and solution of the preservatives. If only a small volume of preserving fluid is required, propylene phenoxetol or Nipa esters may be added to warm or boiling water and the solution allowed to cool, but for large containers such as dogfish tanks the alcoholic solution method is to be preferred.

The animals which have been preserved in the above solutions are as follows:

<i>Animal</i>	<i>Fixative</i>	<i>Preservative</i>
<i>Acanthias vulgaris</i>	Formalin	0.2% Nipa ester and 1% propylene phenoxetol
<i>Bufo bufo</i>	Formalin	1% propylene phenoxetol
<i>Rana temporaria</i>	Formalin	1% propylene phenoxetol
<i>Mus musculus</i>	Formalin	1% propylene phenoxetol
Sheep heart	Formalin	1% propylene phenoxetol
<i>Helix pomatia</i>	Formalin	1% propylene phenoxetol and 0.2% Nipa ester
Various lamellibranchs	Alcohol and formalin	1% propylene phenoxetol and 0.2% Nipa ester

After 6 months all the specimens were of excellent texture and appearance, with no trace of mould or bacteria—excellent for dissection or museum display. The frog, toad, and sheep heart retained some of the natural colour of the blood.

The following comparative prices per litre of diluted preserving fluids may be of interest.

10% formalin	3·8d.
10% perminal KB (Steedman, 1955)	4·0d.
75% methylated spirits	10·0d.
0·2% Nipa ester No. 82121	1·2d.
1% propylene phenoxetol in water	15·7d.

It should be emphasized strongly that these solutions are preserving solutions, to be used only after adequate fixation. Animals such as fresh dogfish placed directly in the above solutions will decompose.

#### THE PRESERVATION OF DYE SOLUTIONS

The aqueous solutions of many commonly used stains develop moulds and frequent filtration is necessary. Moreover, the intensity of the staining reaction decreases with time and it is advisable to use freshly made solutions. The addition of thymol merely reduces the rate of growth of the mould, or at higher concentrations it may produce an undesirable deposit. These disadvantages may be overcome by dissolving the stain either in 0·5% propylene phenoxetol in water or in 0·1% *p*-chlorophenoxetol (the *p*-chloro-phenylether of ethylene glycol) in water. The compounds should be added to warm water (50 to 60° C), and the stain added when the water has cooled to room temperature.

This method produces mould-free staining solutions of aqueous eosin, alcian blue, &c., which are as vigorous in their staining reactions after 6 months as they were when fresh.

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