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PREPARATION TECHNIQUES FOR BIOLOGICAL MATERIAL

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(In this shortened version of the paper presented at the first meeting of the Biological Curators Group Reg Harris highlights some preservation methods which are still in an experimental stage).

In preservation the emphasis is on good and even penetration of fixatives (moving aside organs if necessary) and similar routines are carried out with plants, remembering that penetration of the cellulose walls is assisted by the use of acetic acid. Fixation 'renders tissues stainable for histology' and 'renders tissue suitable for colour preservation' - preservation may not do this.

Preservatives are of three main types Dry, Fluid and Embalming.

Dry Preservation

Most dry preservation techniques are well established so this is just a summary of current methods -

HEAT Using Sand Baths (very useful for plants and some invertebrate animals).

AIR DRYING After heating, or injection with a suitable fixation agent e.g. Formol acetic acid (injected into crustacea and echinoderms this gives good colour and general preservation).

FREEZE DRYING Involves the sublimation of vapour from ice crystals in frozen, generally relatively impermeable, tissue (for entire animals and plants, fur, skin, etc.,).

VACUUM DEHYDRATION Dry frozen tissue over a dessicant under a medium vacuum, a method intended for permeable tissues (insects, fungi, etc.,).

Fluid Preservation

Traditionally alcohol (industrial methylated spirit) and formaldehyde are used. Other alcohols which may be found are isopropyl alcohol (American material is often in this) and tertiary butyl alcohol (which is a better preserver for both animals and plants to retain colour).

It is important to know the strength of preservatives being used. For alcohol, alcoholometers are available for direct readings of percentages. With formaldehyde (where a 2% solution acts as a cell macerator so that cells, although well-preserved, are disassociated) knowledge of concentration is more critical.

Steedman's method for estimating strength of formaldehyde solutions uses the principle of quantitative liberation of sodium hydroxide when formaldehyde reacts with sodium sulphite and water. [1]

The change of pH may be followed by using thymolphtalein as an indicator and the amount of sodium hydroxide liberated is estimated by titration. From this the amount of formaldehyde present in the fluid can be calculated as follows -

1. Place 50 ml of a 30% sodium sulphite solution in distilled water in a clean beaker.

2. Add 4 drops of thymolphtalein.

3. Add a few drops of normal sodium hydroxide solution until the solution gives a faint blue colour.

4. Add normal sulphuric acid in drops until the colour just disappears.

5. Weigh out 3 gms of the formaldehyde sample. Add to the sodium sulphite solution. Blue colour appears.

6. Titrate with normal sulphuric acid or with normal HCl until the blue colour <u>just</u> disappears.

7. The percentage of formaldehyde is estimated as follows -

$$\% \text{ formaldehyde} = \frac{\text{Acid titre X Normality X 3.003}}{\text{Weight of sample}}$$

Example - if acid titre was 5 ml

$$\frac{5 \times 1 \times 3.003}{3} = 5\% \text{ formaldehyde.}$$

A number of other techniques have been developed in recent years although some are not fully tried and tested.

FORMALDEHYDE RELEASERS 'Dowicil',[2] obtainable as a yellow powder is made up in 10% aqueous solutions. This liberates formaldehyde in the presence of protein and is useful for expedition and general collecting trips. There are doubts about the state of cells and histology but entire samples seem to preserve reasonably well and further fixation can be carried out if necessary. (Still very much in the experimental stage).

'KEEPING SOLUTIONS' There are reagents in which samples may be kept after collection for further histochemistry or other preservation after some time. 'Chinosol' is used by UNESCO workers for entire animal and plant preservation at 15 to 20% aqueous solutions. They say it preserves natural colour much longer than alcohol or formaldehyde. We in the UK use the solution strictly as a 'keeper' and transfer to a fixation or preservation agent as soon as possible. Our[3] 'Chinosol' is 8-hydroxy quinoline sulphate, now called 'Seraquin', and is a benzoate and less corrosive than the sulphate (which corrodes metal etc.,).

POST FIXATION REAGENTS To be used after fixation or when a transfer from alcohol to a water based formula for fire risk etc., is necessary. Best of the general PFR's is Phenoxetol, used now for over twenty years. Provided that it is made up correctly

(it is a very viscous fluid in the neat state) phenoxetol forms a very useful and trouble free method of maintaining fluid collections, no more evil smelling dogfish tanks etc.,! Steedman has produced a formula[4] which uses propylene glycol as a humectant and also forms an easy solvent for the phenoxetol. This was the best of over 200 formulae tried out.

FLUID COLOUR PRESERVATIVES The theory that preservation of the respiratory pigments, haemoglobin and chlorophyl, might bring about colour preservation has proved right.

For haemoglobin use Kaiserlings solution followed by fixation, development and final immersion.

Chlorophyl is maintained in plants by immersion in simmering solution of glacial acetic acid and copper acetate before fixation, drying or freeze drying

Formaldehyde also preserves colour provided oxidation is prevented. Yoshida, a Japanese worker, first used Vitamin C at a I% solution in formaldehyde to preserve colour in fish.[5] Pyridine [6], Butylated hydroxytoluene (BHT)[7] and nicotine (much too dangerous for general use) can also be used. Much research and care is needed in this investigation.

Corrosion and general embalming techniques

CORROSION involves the injection of a substance into vascular systems and then the surrounding tissue is dissolved away to reveal a 'tree' of vessels. This is used for brain, kidney, liver, entire systems of invertebrates etc., (Very good examples shown in the Royal College of Surgeons Museum in London[8]). It can also be used in botany for fern steles and, experimentally, for vascular systems of plants. The substance must resist the digesting acid; originally X-ray film mixed with acetone was used, now mixtures of celloidin and polyester resins are being tested.

EMBALMING The object is to allow the sample to remain in a relatively dry state for examination. The usual fluid which contains formaldehyde, phenol and glycerine, is injected through main blood vessels (vertebrates and invertebrates) followed by placing them in an atmosphere of the media injected.

The latest method is the use of 'chlorocresol'[9] to collect insects and other inverts and keep them in the atmosphere of the reagent until ready for further work.

References

1. Steedmans method of formaldehyde assay in UNESCO publication 'Monographs of Oceanographic Methodology No. 4 Fixation and Preservation of Marine Zooplankton' published February 1976.

2. 'Dowicil'
the London agent for the Dow Chemical Co (UK) Limited is
R.W. Greef,
31-45 Gresham Street,
London EC2 (01-606-8771).

3. 'Seraquin'
from Ward Blenkinsop & Co Limited,
Fulton House,
Empire Way,
Wembley,
Middx.

4. Propylene Glycol formula - in Unesco publication mentioned in (1) above.

5. Use of Vitamin C for colour preservation
in Yoshida, Y. 1962 'A way of making Fish specimens with their original body colours kept' Bull. Misaki. Mar. Biol. Inst. Kyoto Univ. No. 3 67-68.

6. Pyridine. The original paper by Romhany is in 'Makroscopische Preparaten' by T. Piechocki. Others are in Rumanian!

7. Butylated hydroxytoluene(Shell Chemical Co Limited)
reference - 'A method of preserving colour in biological specimens' Bioscience Vol. 15 No. 5 1965.

8. Corrosion techniques are mentioned in the book 'Anatomical Techniques' by Tompsett, Royal College of Surgeons (published by Livingstone).

9. 'Chlorocresol' is obtainable in the U.K. from
Koch-Light Labs,
Colnbrook SL3 OBZ,
Bucks.

If anyone wishes to try some of the new techniques Mr Harris is willing of offer advice and assistance to those who would like to contact him at the British Museum (Natural History).

If you write to any of the commercial firms mentioned it might be useful to mention the source of your information.