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Title: The use of formaldehyde as a preservative

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Dick Hendry from Glasgow Museum outlined the preparation and mounting of the museum's St Kilda exhibition; how two houses were reconstructed using light-weight plastics, plaster and papier-mâché for the exhibition and how children and adults could extract island artefacts and natural objects from a beachcombing 'lucky-dip' and then identify them by comparison to a range of objects on display in an adjoining room. The home of the St Kildan wren, mouse and Soay sheep had much to offer visitors, especially to those who thought that St Kilda was a Pacific Island!

After an excellent lunch in a nearby pub there was a chance to look around the museum or view the Hanwell environmental monitoring system and see how the museum's galleries suffered from low RH (30-45%) - obviously a good case for displaying freeze-dried specimens!

Julian Carter filled the post-prandial slot and most ably prevented us from drifting off by showing the importance of using the correct buffer for formaldehyde in fluid-preserved collections. Although he does not advise the use of formaldehyde as a fixative, especially due to its DNA-masking property, he showed how certain buffers will halt or slow down carbonium ion production, essential for continuing fixation and preservation of tissues. Using elasmobranch intestinal tracts, notoriously difficult for long-term preservation due to their lipid content, he showed the effects of adding sodium acetate or using buffered formol-saline (whose pH dropped from 9.0 to 4.5!). He advised the use of sodium hydrogen phosphate mix, or better, sodium β-glycero-phosphate as the best buffers for formalin.

The final two talks were palaeontological and it was refreshing to have such a wide range of disciplines. The first, by Gordon Turner-Walker of Norwich Museum concerned the discovery and subsequent removal of most of an elephant skeleton from the 600K year old deposits that form the cliffs at West Runton. The bones and skull were covered with a wet tissue compress and embedded in a plaster jacket with aluminium foil. This technique preserves the bone well enough for SEM examination but is unadvisable for long-term storage since the sealed in moisture gives rise to pyrite decay.

Lorraine Cornish of the Natural History Museum completed the day's talks explaining the techniques for casting fossil material and the dilemma that many museums face when required to make casts for display or when offered sums of money for making casts of *Aepyornis* eggs, skeleton of *Hypsilophodon* (tree dwelling dinosaur) or *Archaeopteryx* but which takes conservators away from their real work. She then explained the important steps of making a 'master-cast' of important but fragile specimens, how the flash line should be preserved to facilitate future mould making. She also gave much useful information in the form of tips: cracks and holes in bones should be filled with soluble plasticene (Rixon, 1976 see below), the use of Teepol W as a separator and methylene chloride as a solvent for removing old varnish. She also outlined a new technique whereby a laser can cut out the shape of a skull, including its internal morphology, using a stereo-lithograph software system.

Formula for water-soluble putty (Rixon, 1976):

polyethylene glycol	70g
glycerol	23g
water	15ml

Mix cold, then warm slightly and stir into a smooth paste, allow to cool and then thicken slowly with 29g of precipitated chalk for every 100g of the mixture.

Rixon, A.E. (1976): *Fossil Animal Remains* Athlone Press, University of London.

The groups first AGM followed and apart from Paul Brown no-one volunteered for any of the committee posts. Paul replaces Clare Valentine who resigned as a committee member, James Dickinson was also dropped from the committee as his post of meetings co-ordinator had been subsumed by those whose own venue for meetings automatically volunteered then for this duty. The meeting formally thanked Clare and James for their past services on the committee. The effect of the long day had taken its toll on many and although the formal business of the AGM was discussed and concluded, it seemed to be less well ordered than usual and I hope that members and intending members were not put off. The conference was quite well attended despite late publicity and the standard of the day's talks was high showing that, as a group, we have much to offer. The group has been asked to mediate at a conservation workshop on the Thursday evening of the Cambridge Conference in August.

Simon Moore

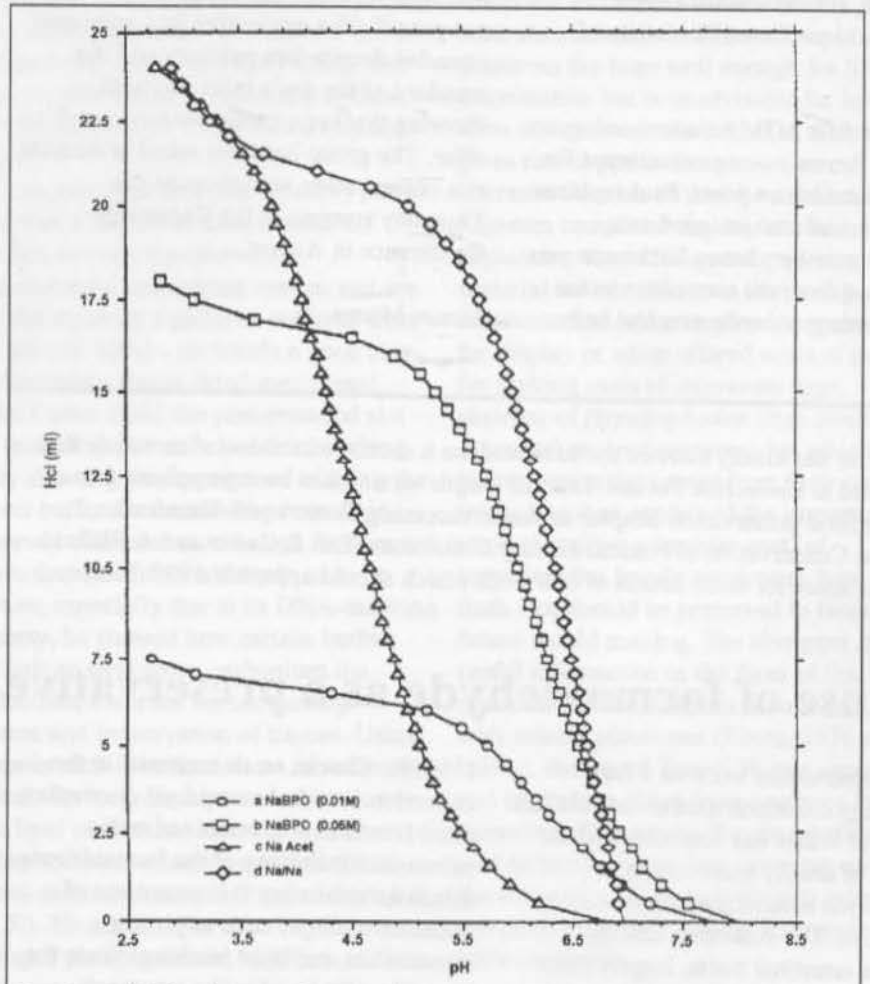
Julian Carter has kindly allowed me to reproduce a shortened version of an article that he submitted to *Collection Forum*. The full length version has been paraphrased as a part of the fluid preservation chapter in the forthcoming Butterworth-Heinemann publication Conservation of Natural History Collections (Eds D Carter and A Walker). Watch this space for more details of this work which should appear in 1997 (Simon Moore).

The use of formaldehyde as a preservative

Recent conservation work on a fish parasitology collection held at the National Museum of Wales has demonstrated the problems of acidity occurring in formaldehyde solutions when used as a preservative. The collection mainly comprises intestinal tracts, largely from elasmobranchs, which have remained in their original 4% formaldehyde fixative solution since the material was collected in 1990/91. Checks on the material in the collection revealed an acid pH developing, often less than 4.0, combined with a substantial yellowing of the formaldehyde solutions indicating the occurrence of chemical changes such as protein dissociation and lipid leaching. Since the parasites contained in the intestinal material have been found best preserved in formaldehyde for subsequent work by light

or electron microscopy it was decided to replace the formaldehyde with a suitably buffered 4% formaldehyde solution. Initially 15% wv sodium acetate was used with 4% formaldehyde made up in a saline solution. However subsequent checks revealed that pH levels were returning to near their original values within a few months.

Four suitable buffering agents were tested in both deionised and artificial seawater: 3.5:6.5 mix of sodium dihydrogen phosphate and di-sodium hydrogen phosphate, 4% wv sodium acetate, 2.5% wv sodium B-glycerophosphate (0.01M) and 15% wv B-glycerophosphate (0.05M). The pH of each was measured before and after immersing a fish intestine. Thereafter



Graph Acid titration results - pH Curves for Buffered Formaldehyde Solutions When Titrated Against 01.M HCl

readings were checked daily for one week and then on a weekly basis. After these studies the effective range of each buffering salt used was measured by acid titration (see graph). The steeper the curve the smaller the change in pH. so the sodium B-phosphate is effective in the range of 5.5 to 6.5, with the 0.05M considerably more effective than the 0.01M, while the sodium acetate is effective at a lower pH, 4.0 to 4.5.

The study showed that the original buffers used did not maintain a suitable pH level for two reasons: the buffering effect of the saline solution is only temporary, and the sodium acetate will not maintain a high enough pH. In conclusion the most effective buffer was the 0.05M sodium B-glycerophosphate when used with formaldehyde in de-ionised water solutions. The sodium hydrogen phosphate mix was also considered to be effective but only in deionised water since a precipitation reaction occurred when used with saline. Buffered formaldehyde solutions are recommended to be made up with deionised water only.

The reasons for buffering formaldehyde

Formaldehyde solutions are buffered to prevent the formation of a reactive molecule known as a carbonium ion. The carbonium ion is capable of electrophilic attack on protein molecules by reacting with many of the functional groups which causes a crosslinking of the proteins. This leads to the formation of insoluble macromolecular complexes that prevent subsequent protein loss from the tissues. One these reactions occurs with the amine groups of amino acids in tissues producing fatty acids which is why biological specimens have the effect of lowering the pH of formaldehyde based fixatives.

To achieve the best level of preservation it is advisable to fix in unbuffered 4% to 10% formaldehyde for a short time and then to transfer the fixed specimen to a buffered formaldehyde preservative.

This will greatly reduce any extra post-fixation side reactions occurring during specimen storage both by decreasing the active fixation property in reducing the number of fixative-active carbonium ions, and maintaining a near to neutral pH. Low pH gives rise to protein embrittlement and dissociation, decalcification of bone leading to vertebrate specimens becoming undesirably and unnaturally flexible. High pH leads to the possible gelatinising of the proteins.

Further reading:

Zoology fixation and preservation. Steedman, H.F., The Unesco Press, 1976

Zoological preservation and conservation techniques. Harris, R.H., Journal of Biological Curation 1(2), 1990.

Conservation of natural history specimens: Spirit collections. Horie, C.V. (Ed.) The Manchester Museum and Department of Environmental Biology, The University of Manchester, 1989

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