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Fluid Preservation
Report from the One Day Seminar

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A brief history of fluid preservation, with some basic facts about it, including labels and inks.

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Evidence of fluid preservation can be traced back to early civilisations – even by the time of the Elder Pliny, a spider pickled in a glass of wine was supposed to induce a death-like sleep!

Apart from pickling spiders, toads and people throughout its long history, alcohol (as Spirits of Wine) was recorded by Boyle as being used as a fluid preservative in 1662, along with spirits of sal ammoniac and brine. By the time of Ruysch in his *Thesaurus Animalium* of 1710, all sorts of amazing and slightly gruesome objects were being preserved in alcohol and the jars prettified with Florentine Paper. Levi Vincent's *Elenchus Tabularum* of 1719 shows a museum gallery with rows of pig's bladder-sealed jars containing fantastic creatures, as they would then have appeared.

The Russian chemist Butlerov isolated formaldehyde in the mid 19th century but it was not until 1893 that its antiseptic effect was realised on animal tissues. During the early to mid 20th century a rapidly-growing plethora of compounded specialised fixatives and preservatives appeared for preserving cell contents to specialised tissue by-products such as amyloid, and colour preservation techniques were put forward in the mid 20s by Kaiserling and later by Wentworth. By the mid 50s Owen and Steedman were investigating the use of the embalming agent phenoxetol as a pre-

serving agent; this work being further advanced by Steedman in 1976 with his work on marine zooplankton preservation.

Since then we have had time to take stock of the effects, both good and bad, of these embalming preservatives but generally, and with the discovery that DNA is best fixed by alcohol, we seem to have come full circle resulting in many collections now being transferred back into that fluid.

Despite all the information on the subject, many still don't understand fully the terms fixation and preservation. Fixation is the initial 'dunking', if you like, of freshly-dead tissue into a fixing agent or fixative to prevent autolysis and other undesirable chemical changes from occurring by rendering chemical stability to the fixed tissue/s. Denser or larger tissues will require injection with fixative so that they are also perfused, from the inside out, as well as *vice-versa* particularly if the fixation penetration rate is slow (as with formalin). Fixatives act by either coagulating protein or by creating a more stable cross-linkage and should be osmotically balanced with the tissues they are fixing to prevent osmotic shock, which causes tissue rupture, shrinkage and distortion.

Most tissues are roughly, osmotically similar to water, therefore an aqueous fixative is best. Samples that are to be DNA extracted, however, are fixed in absolute alcohol. The osmotic shock may distort the tissues but not the DNA. Conversely, formaldehyde will chemically alter DNA structure but will not cause osmotic shock to fresh gross tissue samples.

So far I have only mentioned formalin and alcohol as fixing agents. Bear in mind that formalin is roughly a 40% solution of formaldehyde gas in water so that a 10% solution of formalin, the normal fixing strength, contains 4% formaldehyde. There are other fixatives including osmium tetroxide buffered with sodium cacodylate, which is excellent for cell contents and is used for transmission electron microscopy but can also be used as a gross fixative for such small organisms as hydromedusae that are then transferred to an alcohol preservative. The internal organs are stained black by the fixative but are in a much better state of preservation than those treated with more conventional fixatives. 2 examples showing hydromedusae fixed in the late 1880s were compared with similar specimens from the same period and showed how much better looking were those

fixed by the osmium tetroxide technique. Osmium, however, tends to make Health & Safety Officers rather twitchy due to its high toxicity and it is very COSHH regulated!
Preservatives are agents that continue the work of the fixative but without altering the state of the fixed tissue. Phenoxetol and propylene glycol preservatives have, over time, been found to swell some tissues so that Steedman's PFP (post fixation preservative) and 1% aqueous phenoxetol can cause swelling and have been found to be ineffective as preservatives for densely-muscled animals (such as larger fish). The preservative has difficulty in maintaining the depth of penetration that was originally achieved by the fixative. This naturally indicates to achieving as near perfection as possible with fixation before transferring to a preservative.

Bearing this in mind there has appeared on the market a preservative known as Opresol which is a mixture of 2-phenoxyethanol and diethylene glycol – a similar preservative to Steedman's PFP. It seems to work very well on invertebrates, especially small crustaceans and small vertebrates. Specimens were shown at the seminar preserved since 1986 and still in a good state. The advantages of these preservatives are that they are relatively non-toxic, compared to many others, and don't upset the mucous membranes, they are also non-flammable and slow to evaporate (if at all!), although they do have a tendency to seep by capillary action, leaving jars clumsily-handled jars with a sticky surface which can affect external labels causing them to flake and disintegrate in time. Inks can also become faded to illegibility.

Labels, however, should NEVER be attached externally to such jars as not only will they become damaged in time - fading, mildew, crumbling (especially if the paper is acidic), eroded by handling or they will fall off as the adhesive dries out. They also tend to hide a multitude of sins inside the jar, such as lipid leaching, fluid contamination, falling fluid levels! Labels must be placed inside jars. When getting such labels printed you must order them well in advance since they must be left to dry out for at least 6 months or else the ink will bleed into the fluid and turn it (and the specimen) blue! At the Hampshire CC Museums Service I use Goatskin Parchment (Arjo Wiggins) since it is the most durable of immersion papers. There are still labels in the Arachnid section collection at the Natural History Museum written by my own fair hand in Indian Ink back in 1968

which are still perfect. Many conference posters, papers and talks have centred around using computer printer-generated labels and since the days of 'alphabet soup' in the bottom of jars, and other such disasters, there have been developments and improvements in this field. I still handwrite my labels in Indian Ink until these newer techniques have proved their test of time.

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Histological Effects of fixation and long-term preservation. Are preservatives beneficial or not?

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Having gained a better understanding about the workings of fixatives on tissues, we are faced with the dilemma of using preservatives. Some tissues will start to deteriorate over the long term if stored in preservative - can lead to swelling, fragility or loss of fixative state due to poor penetration if specimen is densely muscled; or if stored permanently in fixative. This is particularly exacerbated if the chemical nature of the storage solu-