Journal of Biological Curation

Title: Zoological Preservation and Conservation Techniques

Author(s): Harris, R. H.

Source: Harris, R. H. (1990). Zoological Preservation and Conservation Techniques. *Journal of Biological Curation*, *Volume 1 Number 2*, 4 - 24.

URL: http://www.natsca.org/article/1039

Zoological Preservation and Conservation Techniques

R.H. Harris

Hazards Warning

Please note that some of the chemicals used in the formulae here presented, such as xylene and formaldehyde, are known carcinogens; inhibisol can often be used as a substitute for organic solvents. Several other substances, such as glacial acetic acid, mercuric chloride, and phenol, can also be very hazardous. All the formulae should only be mixed using protective clothing (including eye protection), and the work should be done within a suitable fume cupboard. The manufacturer's hazard information and instructions for use should always be consulted before handling or mixing any chemicals.

Editor's Preface

Editing a manuscript without being able to consult the author is difficult, but fortunately the draft left by Reg Harris was practically completed. Apart from some minor corrections of spelling, etc., the main work has been rationalising the layout, setting headings into a consistent hierarchy, re-drawing the figures and moving the references from the end of each section to a unified citation list at the end. Otherwise Reg's work is published here as he left it, as a tribute to a pioneer in his field. The BCG Committee decided that it was not appropriate to attempt to bring the list of references up-to-date, so post-1987 work is not cited.

Author's Introduction

There has always been a tendency to regard the practice of techniques in zoology as a throwback to the past with an eloquence of bygone knowledge and small hope for the future. Zoology has become a mosaic of specialities and with the increase of research in many spheres it is more than ever essential that well founded technical research is needed to ensure that the specimens of today will be available to workers in time to come. Without adequately prepared material, taxonomic and systematic research will become virtually impossible. It is curious to note that the great efforts to accumulate and install collections have not been supplemented, in many cases, by adequate means of conservation and preservation. It is to be hoped that this small volume will play its part in ensuring that the techniques for the care and conservation of specimens will not disappear.

R. H. Harris

Zoological Preservation and Conservation Techniques I. Fluid Preservation

R. H. Harris

History

Preservation and Fixation

Alcohol

Formaldehyde

Formaldehyde assay

Formaldehyde releasers

Post fixation reagents

Keeping solutions

Colour preservation

Anti-oxidants

Respiratory pigment preservation

Metallic salts

Neutralising agents

Fixative solutions

Fixation: Preliminary treatment of specimens before preservation

Preservation: Treatment after fixation

Mounting of fluid preserved specimens for museum and study purposes

Mounting fluid preserved specimens in glass museum jars

Cements for glass museum jars

Specimen attachment

Repair and conservation of fluid preserved specimens

Mounting fluid preserved specimens in plastic jars

Tissue reconstitution

History

One of the most significant advances in the preservation of biological tissues was the introduction of fluid preservation in the mid-17th century. In 1660 Elias Ashmole showed Charles II two abortions that had been preserved in fluid in a glass jar and these aroused considerable interest at court (Gunther, 1927). Later in 1662 Robert Boyle recorded the use of spirits of wine for preserving tissues. It seems very likely that the two puppy dogs shown by Mr Croune at a Royal Society meeting on June 4th 1662 were preserved in spirits of wine following Boyle's information (Birch, 1746). In 1670 Swammerdam mentioned spirits of wine in his museum catalogue and in 1681 Grew mentioned liquid preparations in the Royal Society catalogue of the year. In 1710 Ruysch mentioned the use of liquid preparation in his catalogue and the Hunter collections in 1768 contained a large number of fluid preserved specimens (Cole, 1944). In 1786 Seba records the use of "kilduivel" or killing devil, a popular name for spirits of wine (Engel, 1937).

Until the introduction of formaldehyde in 1859 all fluid preserved solutions of any reasonable preservative value were based on alcohol. It was in this year that Buterlov discovered formaldehyde as an interesting substance but he had no real idea of its true value as a disinfectant or preservative. It was in 1867 that von Hofman demonstrated the use of the reagent, preparing it from methyl alcohol. It was the antiseptic properties of the formaldehyde that drew the attention of preservation workers. In 1893 Blum noticed the preservation qualities of formaldehyde, and that it hardened and caused very little shrinkage of tissue. He then transferred some of the tissue to alcohol and was probably the first worker to bring about a form of colour preservation in biological tissue. It must be remembered that the formaldehyde prepared here was produced by placing a hot platinum coil in a bath of methyl alcohol and was probably a chemical of high analytical purity with none of the oxidation effects so noticeable in modern reagents. Blum summed up the advantages of formaldehyde over alcohol by saying 'It seems to harden objects without shrinking them, that mucin secreting animals remain transparent in formalin and that formalin preserves colour better. Furthermore formalin solutions are considerably cheaper in price than alcohol'. Bluntson recommended at the end of 1893 that tissue should be fixed in 10% formaldehyde and then transferred to alcohol to bring back the colour. He commented 'the colour of the flesh and blood apparently lost during the formalin hardening, reappears as if fresh and the blood corpuscles show up under the microscope in their natural shape and colouring'. This two stage method forms the basis of modern colour preservation technique.

Preservation and Fixation

Fluid preservation is the prevention of cell breakdown by altering or eliminating the action of enzymes within the cells; the combination of enzymes usually present in cells is called kathepsin. To preserve a piece of tissue it is necessary to place it in a fluid in which it will neither shrink or

swell, nor dissolve or distort in any way, and it must also render the kathepsin inactive. A fixative is a solution which will preserve and render the tissue stainable for histological examination. Some fixatives are only used for very short times and are positively harmful to tissues left too long in them.

Some preservatives are not suitable for tissues intended for histological examination. It is essential to follow the correct procedure in the preparation of fixatives and preservatives. A solution that is too strong or too weak will often result in the breakdown of cells and tissues. In fact this phenomenon is utilised when cell studies require a complete dissociation of a tissue.

Further research in recent years using anti-oxidants together with formaldehyde solutions has brought success in a number of animal groups. Yoshida's (1962) use of vitamin C with formaldehyde for fish colour preservation is an example of this work. It seems very likely that alcohol and formalin based solutions will be used in preservation research for the foreseeable future.

However, with the Health and Safety problems aroused by the hazards of these two chemicals a search has been made for suitable substitutes or alternative reagents. This has initiated the introduction of post fixation reagents, formaldehyde releasing agents in the presence of protein and keeping reagents, which while not preserving, will keep tissues for a period to allow histochemistry or other biological analyses to be carried out. The search for the ideal fluid preservative continues.

There has been confusion over the various names formol, formalin and formaldehyde and no clear policy of how to use the terms. For the purposes of this thesis it will be stated that the product from methyl alcohol was first called formaldehyde and the other two terms are trade names from various German chemical manufacturers. 'Formaldehyde' is the name of a chemical compound ($HCHO$) which is gaseous at normal temperatures and pressures. 'Formalin' is the trivial name given to aqueous solutions (generally 40% weight/volume $HCHO/H_2O$) of the gas which usually contains methanol to inhibit polymerisation. 'Formol' is synonymous with 'formalin'.

Alcohol

Ethyl Alcohol (ethanol or absolute alcohol) - An expensive reagent used mainly for analysis and should not be diluted for preservation work.

Isopropyl Alcohol - A popular alcohol preservative in the USA and Europe. Can be diluted down to 50% and still give satisfactory results. Also used in place of ethyl alcohol in histology as it will not cause milkiness in xylene when used in dehydration technique. It can be used in all cases where absolute alcohol is required apart from pure chemical analysis.

Methylated Spirit - Ethyl and methyl alcohols to which mineral oils and colouring has been added. Useless for preservation. Goes milky on dilution with water.

Industrial Methylated Spirit - This is the alcohol used for biological preservation. To every ninety-five parts by volume of spirits are added five parts by volume of wood naphtha. Industrial methylated spirit is obtainable in a variety of strengths. The strength of alcohol is determined by excise officers according to a term known as Proof Spirit. This is a mixture of about equal parts of alcohol and water. The strengths of alcohol which are weaker than Proof spirit are measured on a scale of 100 degrees and those which are stronger are measured by a scale of about 75 degrees. Pure water is 100° under Proof, and pure spirit is 75.35° over Proof. The strengths available are usually 60, 66, 68 and 74° over proof. Proof alcohol is by definition around 57% at 60°F. Thus 66° over Proof is 95% and 74° over proof is around 98%. Much depends on the temperature and moisture content of the area surrounding the container of alcohol. The unstable estimation can be overcome by placing suitable dehydrating agents in the alcohol container. Dehydrated copper sulphate placed in a container of high grade alcohol will maintain the grade, provided that the open air is not admitted to the container too often. Any increase of hydration will be shown by the return of the blue colour to the white dehydrated copper sulphate being used as a desiccant.

When supplied at a strength of 74° over proof the reagent is often called absolute alcohol and it has the following qualities:-

a) equivalent to 99 percent by volume and as free from water as is absolute ethyl alcohol

b) miscible with xylene in all proportions

c) contains not more than a trace of acetone

d) remains clear in all dilutions

e) completely volatile

f) suitable for most laboratory purposes, including the preparation of elementary organic chemicals

g) satisfactory for most staining purposes and for general microscopic work and for the preservation of organic specimens

Formaldehyde

Pure formaldehyde is an irritating pungent gas readily liquefying at low temperatures. The liquid and the gas readily polymerise into the insoluble form of poly-oxymethylene, which has little scientific application. Formaldehyde gas, however, is soluble in water to form solutions that are relatively stable. Formaldehyde is also used as the solid hydrated polymer paraformaldehyde. The greatest bulk of formaldehyde is supplied as a solution in water, containing 37% by weight of formaldehyde with a small amount of methanol. The solution is often referred to as formalin, 40% formalin, or more correctly as 40% formaldehyde. It is always used diluted as a fixative and then added to other reagents to form preservative media.

Determination of strengths of formaldehyde solutions.

The basis of this method is the quantitative liberation of sodium hydroxide when formaldehyde reacts with sodium sulphite and water. The change in reaction may be followed by using thymol-phthalein as an indicator and the amount of sodium hydroxide liberated is estimated by titration. The amount of formaldehyde present in the fluid may be calculated as follows:-

1 Place 50 ml of a 30% aqueous solution of sodium sulphite in a flask.

2 Add 2 drops of thymol-phthalein indicator solution.

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

4 Add normal sulphuric acid drop by drop until the blue colour just disappears.

5 Weigh out 3 gm of the formaldehyde containing sample. Add this to the sodium sulphite solution, which will turn blue.

6 Shake the solution and titrate with normal sulphuric acid until the blue colour just disappears. (NB: Hydrochloric acid should not be used as the mixture of hydrochloric acid and formaldehyde produces a gas bis-chloromethyl ether, a proven potent carcinogen.)

7 The percentage of formaldehyde may be calculated from the following equation:-

$$\% \text{ formaldehyde} = \frac{\text{Acid titre x normality of acid x 3.003}}{\text{Weight of sample}}$$

1ml normal acid is equivalent to 0.03003gms of formaldehyde therefore to convert to percentage formaldehyde multiply by 100. Thus 0.03003 times 100 is 3.003. In actual practice the titre is the percentage formaldehyde present in the sample.

Formaldehyde Releasers

There are a number of these reagents available and the most commonly used is Dowicil 100. It is a white to cream coloured powder with a high solubility and an aromatic smell. Its full chemical name is 3.5.7.triazo-1-azoniaadamantane chloride. The mechanism of the action of Dowicil is the controlled breakdown of the molecule, the solution liberating formaldehyde in the presence of protein. Solutions should be made up and used within two weeks. A 10% aqueous solution is satisfactory for most cases, although a solution of up to 30% in seawater is used for many invertebrates at the Indian Ocean Biological Centre, Cochin. For whole vertebrates a solution of up to 20% has been used by Australian and Swiss workers quite successfully.

Post fixation preservatives

These reagents have been developed from germicide and fungicide chemicals used in the pharmaceutical industry. They have the property of maintaining tissue in an aqueous solution after it has been well fixed by a standard formula. Although not preservatives in any sense they will retain enzymic activity in the tissues for several days when suitable material is available.

Phenoxetol - A clear viscous fluid which only dissolves in water with difficulty at about 2%.

Propylene phenoxetol - Is similar to phenoxetol but has an anaesthetic effect on many invertebrates and is often used for this purpose. Steedman's (1976) suggestion that propylene glycol might be added to these chemicals to effect easier solution in water has proved most successful. The addition of propylene glycol to either phenoxetol or propylene phenoxetol confers the following advantages:-

a) very easy solubility

b) improves clarity by changing the refractive index

c) resistance to complete desiccation

d) improved flexibility of the specimens

e) powerful fungicide action

The addition of phenoxetol to a formaldehyde fixative results in very much quicker penetration of the reagent. The phenoxetol acts as an indifferent salt in the same way that sodium chloride does when used as part of the fixative formol saline.

Keeping solutions

8-hydroxyquinoline sulphate in an aqueous solution is an excellent keeping solution. Useful in field collecting as a dry powder made up in aqueous solutions, it is able to keep plants and animals in a reasonable state for up to one month; after this time proper fixation and preservation should be carried out. Unless specially made it tends to be very acid in reaction, and material should be kept in glass or plastic containers, as most metals are attacked by solutions of the reagent. It is sometimes used as a post fixation preservative in many European museums, where it is known as 'Chinosol'; there is a neutral form available called 'Seraquin'.

Colour preservation

The colours of animals are due to pigments or to the physical structure of the integument. Most pigments are synthesised by the animals, but one important group, the carotenoids, are derived from plant food. The structural colours are caused by Tyndall scattering or by interference with light. Pigments are substances which, due to their chemical nature, absorb light of

certain wavelengths and reflect light of others, and in so doing are coloured. The red robin, yellow canary, green caterpillar and blue lobster are coloured by pigments. There are, however, brilliant colours that are due not to pigments but to the physical properties of the animal's surface, i.e. the skin, hair, scales, feathers etc. These are the structural colours. Tyndall first explained the blue colour of the sky over a century ago. It is blue for the reason that very small particles in the upper air scatter back a higher proportion of the short wave, or blue, light from the sun, than of the longer red/green waves. Feathers are blue for a similar reason, as are blue eyes and the blue faces of certain monkeys. An outer layer of yellow pigment absorbs the blue but allows the green, yellow and red components of white light to enter the tissues. The green light is scattered by minute air spaces in the translucent feather substance to a greater extent than the long wave yellow and red, which are absorbed by a black internal pigment. The green of a tree frog and lizard have a similar origin: living cells near the skin surface contain oily yellow droplets, beneath which are cells containing tiny granules that scatter green light more than yellow or red, which are absorbed by black cells beneath those containing the granules. The Australian Tree Frog *Hyla coerulea* is so called because it is bluish when preserved in alcohol; the yellow pigment is soluble in alcohol, and without it blue as well as green rays of light penetrate the cells containing the granules which scatter the blue light in addition to the green.

Thus the blue colour is a structural colour in feathers, insects and in the eye but it is caused by a carotenoid pigment in the lobster and by bile pigment in some birds eggs. Green is structural in beetles and in certain moths and partly so in feathers and in some frogs; it is a carotenoid pigment in crabs and has some connection with bile pigments with certain insects. Yellow is due to carotenoid pigment in feathers, a melanin in hair, a pterin (first found in butterfly wings and seems to be allied to uric acid) in the salamander and in certain butterflies. Red is structural in beetles, carotinoid pigment in sea anemones, crabs, certain fish (trout) and flamingoes, a pterin in butterflies and in the common frog, and is caused by echinochrome in sea urchins, to haemoglobin in humans, certain worms and molluscs and to a copper porphyrin in turaco feathers. Black is caused by melanin in skin, hair and feathers and by tanned proteins in beetles. There remain many animal colours whose chemical nature and possible physiological origin are still quite unknown.

Numerous attempts have been made over the centuries to perfect a method or technique for the preservation of natural colour in animals. For any form of colour preservation it is essential to begin with the living organism, as on death many colours are simply lost. Even those colours that are retained are affected by daylight and the bleaching effects of ultra violet light. Many of the structural colours in animals are transient in life and disappear or assume different phases on the death of the specimen. The only real progress in establishing a reasonable form of colour preservation has

been with the respiratory pigments. Methods of colour preservation have differed widely; for example the common lobster has a specific blue colour in life easily lost in drying or fluid preservation. However, it is possible to inject the animal with a solution of formaldehyde-acetic acid and water and then allow the specimen to dry slowly in the dark. The blue colour will be retained for long periods, even years, although why this happens is not clear. The most successful methods so far employed have been by using alcohols that do not leach out colour substances, the careful use of anti-oxidants, and the preservation of the respiratory pigments in a permanent state.

Tertiary butyl alcohol does not leach out colours in animals and, although there may be colour changes due to chemical interference, preservation will be reasonable in most cases.

Anti-oxidants

The prevention of oxidation and subsequent colour loss has been successful in many cases of colour preservation. Crystalline butylated hydroxytoluene has been used as an emulsion in alcohol and formaldehyde solutions to prevent colour loss in many invertebrate groups and with fish. Vitamin C (ascorbic acid) and pyridine have also been used in preservative solutions with success for fish and amphibia.

Respiratory pigment preservation

From the beginning of the 20th century and with the introduction of the new fluid preservative formaldehyde, research has been carried out in the preservation of the pigment haemoglobin and to a lesser extent the plant pigment chlorophyll. Formaldehyde in the pure state was, and still is, an excellent colour preservative. The problem is that slow oxidation will cause a bleaching effect, hence the use of anti-oxidants. The actual reaction that occurs during the pigment preservation is obscure but what happens seems to be as follows. First, the pigment is converted from haemoglobin to temporary acid haematein, using a solution of sodium salts with formaldehyde. After treatment with alcohol the specimen is transferred into a sodium salt-glycerin-water solution and the temporary acid haematein is converted finally into the permanent alkaline haematein in which state it is morphologically indistinguishable from the fresh, untreated tissue. The conversion change in the pigment can easily be followed by the use of a spectroscope, the absorption bands varying as the conversion progresses. This has proved to be the most efficient and lasting technique for fluid colour preservation.

Metallic salts

Many metallic salts are used in fluid preservation, including mercuric chloride, potassium dichromate, osmium tetroxide etc. They are almost all excellent fixatives but have no value as long term preservatives and they have

many disadvantages, for example mercuric salts are poisonous, corrosive and give rise to precipitates within the tissues. Dichromate and alum initiate oxidising and acidic reactions, and osmium has a decolourising effect. These substances are best restricted to fixative solutions and for use in histological reagents.

Neutralising agents

Sodium acetate - Frequently used to keep formaldehyde solutions at a pH of 6.9 to 7.3. Without specimens immersed in it will keep a solution of formaldehyde at pH 7 for months; usually used as a 5% solution.

Pyridine	40% Formaldehyde	25ml
	Distilled water	75ml
	Pyridine	5ml pH approx 7.8

For marine plankton 10 parts to 90 parts fixing fluid will maintain a pH of 7 to 7.6. Disadvantage: poisonous pungent vapour unsuitable for open dish or close work. Confine to fume chamber. Very good for nerve examination and general neurological investigations.

Sodium hexametaphosphate - This is the basic ingredient of many water softeners, and its solutions usually have a pH of 8 to 8.6; they may also have a slightly solvent action on calcium carbonate.

Hexamine - This reagent is produced as a reaction between ammonia and formaldehyde. Smith in 1944 suggested using a 20% solution with 40% formaldehyde, which maintains a pH of approximately 8.4 and has a softening effect on some tissues. Not widely used in general practice these days.

Sodium glycerophosphate - Used as a buffer for formaldehyde solutions. Has certain advantages; maintains a pH of 7 with a 2% solution in 40% formaldehyde. Usually precipitation follows the addition of phosphates to water (especially sea water), but this is not the case with sodium glycerophosphate. Tissues showed no change, and the solution remains clear after a year in the buffered fixative.

Borax (Sodium tetraborate) - Soluble to about 6% in water, it is a good neutralising agent. Used in excess in the fixative, it will maintain a pH of 8 to 8.4. Some bleaching occurs, however, and it is not suitable for colour preservation formulae.

Fixative solutions

It is essential for research to ensure that adequate fixation precedes any preservation technique. Often the two operations are combined but occasionally a fixative must be completely removed before preservation can commence. Formaldehyde, alcohols of various kinds and metallic salt additives have already been mentioned. Here is a list of common reagents normally used in fixation.

14   *R. H. Harris*

Formaldehyde

Mercuric chloride

Picric acid

Potassium dichromate

Chromic acid

Osmium tetroxide

(NB: This reagent has a very harmful vapour, it can fix the mucous membranes of eye and nose, and must only be used in an appropriate fume cupboard.)

Industrial methylated spirit

Acetic acid

Formaldehyde formulae:

Formol saline

40% Formaldehyde	10ml
Distilled water	90ml
Sodium chloride	0.9gm

As alternative to the sodium chloride the solution may be buffered or sea water added in place of the distilled water for the treatment of marine invertebrates.

Bouin's Fluid

Picric acid (saturated aqueous solution)	75ml
40% Formaldehyde	25ml
Glacial acetic acid	5ml

This solution keeps indefinitely. It is sometimes diluted for delicate tissues (embryos etc.) with distilled water. The yellow stain of picric acid can be removed by immersion in a solution of saturated lithium carbonate in 70% alcohol.

Mercuric chloride formulae:

Heidenhain's 'Susa'

Mercuric chloride	45gm
Distilled water	800ml
Sodium chloride	5gm
Trichloroacetic acid	20gm
Glacial acetic acid	5ml
40% Formaldehyde	200ml

Heidenhain's 'Susa' is one of the best fixatives available. Tissues should be transferred to alcohol after fixation; usually not more than 24 hours for large specimens and down to a few hours for small pieces of tissue. It is necessary to remove mercuric salts which form a precipitate in the tissues when immersed in this fixative. The precipitate is removed with alcoholic iodine. The nature of the precipitate is unknown.

Zenker's Fluid

Mercuric chloride	5gm
Potassium dichromate	2.5gm
Sodium sulphate	1gm
Glacial acetic acid	5ml
Distilled water	100ml

This solution does not keep, so it must be made up fresh just before use. However, one can make up a stock solution which keeps well by leaving out the acetic acid, which is added just before use.

Zenker-formol: (Helly's Fluid)

This is a variation of the Zenker formulation in which the acetic acid is omitted and 5ml of 40% Formaldehyde is substituted just before use.

Chromic acid formulae:
Champy's Fluid

3% Potassium dichromate	7ml
1% Chromic acid	7ml
2% Osmium tetroxide	4ml

Flemming's Fluid

1% Chromic acid	15ml
2% Osmium tetroxide	4ml
Glacial acetic acid	1ml

This solution is sometimes used minus the acetic acid.

Alcohol and acetic acid formulae:
Carnoy's Fluid

Absolute alcohol (74OP)	60ml
Chloroform	30ml
Glacial acetic acid	10ml

Rapid penetration, fix for 10 minutes to 1 hour.

Schaudinn's Fluid

Saturated aqueous mercuric chloride   2 parts
Absolute alcohol (74OP)   1 part
Glacial acetic acid to 5% added just before use.

Mainly used for smear fixation for single cells, protozoa etc.

Colour preservation fixatives:

Kaiserling's Fixative (1903)

Potassium acetate	85gm
Potassium nitrate	45gm
Formalin	800ml
Distilled water	4000ml

Jore's Fixative

Sodium chloride	1 part
Magnesium sulphate	2 parts
Sodium sulphate	2 parts
Formalin	5 - 10 parts
Distilled water	100 parts

Jore also used a formula in which artificial Carlsbad salts were used:

Formaldehyde	5 parts
Saturated solution of chloral hydrate	5 parts
Artificial Carlsbad salts	5 parts
Distilled water	1000 parts

Pick's Fixative

Formaldehyde 5% aqueous	100 parts
Artificial Carlsbad salts (by weight)	5 parts

Artificial Carlsbad salts

Sodium sulphate	22gm
Potassium sulphate	1gm
Sodium chloride	9gm
Sodium bicarbonate	18gm

Wentworth's Fixative

Formaldehyde	80 - 100ml
Sodium acetate	40gm
Distilled water	1000ml

Wentworth's fixative makes use of the anti-oxidant sodium hydrosulphite in the final solution which also has marked reducing properties.

Fixation: Preliminary treatment of specimens before preservation.

It is essential to treat all specimens as something rare and valuable, since in many cases subsequent microscopic examination of an entire animal has

often revealed new and important information of research value. The care taken of any specimen at the earliest stage is of the utmost importance as specimens damaged at this time are often beyond any further conservation or repair. Fresh specimens should never be placed in formol-saline unless circumstances require the sample to be kept for some time before treatment. Saline acts as an indifferent salt and the specimen could easily become over-fixed and subsequently distorted and damaged. Organs should be suspended in the fixative and not allowed to lie on flat surfaces or they will adopt the flat appearance on fixation and many organs have been spoiled in this way. Gauze etc. should not be left on a specimen or it might leave an impression when fixation is completed.

All whole vertebrate animals of any size should be injected intra-pleurally and intra-peritoneally and an opening made in the skull to allow the fixative to reach the brain. Better still, the animal should be injected through the blood vascular system thus ensuring complete fixation of all tissues and organs. Again the injection technique may be adequate but it is necessary sometimes to open the pleural and peritoneal cavities and move the organs manually to allow the fixative to penetrate and bathe the organs and tissues thoroughly. Entire animal specimens that have been fluid preserved are often spoiled by insufficient fixation of the abdominal organs especially the liver and parts of the alimentary tract.

Preservation: Treatment of specimens after fixation.

Assuming that the specimen has been adequately fixed it is now ready for general preservation. Care must be taken in the selection of suitable containers. Glass jars with clip on lids similar to those used for bottling fruit are probably the most efficient for small and medium sized animal material. For larger and bulkier specimens tanks of some kind are required; ones with tightly fitting lids should be selected. Large plastic tanks may appear ideal but their efficiency has been called into question over lengthy periods of time. There is some evidence that they tend to develop splits and other surface degeneration after some years especially when formaldehyde containing solutions are employed. This may be due to the original polymerisation during tank production proving unstable in the long term. Certainly most plastic tanks will not stand prolonged contact with solutions containing alcohol or glycerine. There are many containers which are said to resist volatile chemicals, petrol and other solvents. This seems evident providing the vessel is kept constantly filled. It is when the container is partially filled that the deterioration seems to take place.

It now seems certain that the use of glass containers for fluid preserved biological material will never be entirely superseded until the resistant properties of plastics have been thoroughly investigated. Some form of lining might well be the answer. Paraffin wax has been used on many occasions experimentally to prevent the etching of glass when using hydrofluoric acid

and it seems reasonable to suggest that a similar form of lining of a plastic tank might improve its resistance to solvent and other deterioration effects.

Mounting fluid preserved specimens for museum and study purposes.

Historical:

The earliest containers for fluid preserved specimens were made of glass. These were hand blown into moulds to give a slightly oval shape with a lip on the top to secure some form of seal. In most cases this was quite complicated. A pig or sheep bladder was wetted and laid over the surface of the filled jar and this was covered with a layer of lead, followed by another layer of bladder. When quite dry the bladder-lead sequence gave a fairly tight and secure seal provided the jar was not moved about too much. This was one of the reasons why the earlier fluid preserved collections were kept under lock and key in glass fronted cupboards.

Later on the jars became more sophisticated and were made into rectangular or square shapes with one or more faces polished to give a clearer view of the specimen. Actual optical cells were made for specimens but these were always very expensive and very few were used in museum collections.

In the late 1940's plastics were employed for the production of museum jars and perspex sheet was made available for workers to make their own containers. The preparation of a perspex museum jar is and was a difficult process to do well, and there were unforseen problems. The sealing of a plastic container left much to be desired. Welding was eventually used, and Tompsett (1970) working at the Royal College of Surgeons described a useful technique which could be repeated quite successfully. Then there is the problem of suitable preserving fluids for use with plastic containers. Alcohol and glycerine cannot be used except on a very temporary basis because the plastic sheet deteriorates when in contact with these reagents. Any preservative used in plastic jars must be based on a formaldehyde formulation. However, where specimens are used for teaching and consequently are handled and moved a great deal, perspex museum jars are not recommended as they are prone to leak. Bostic glue applied to the surfaces (which must be dry) where the leak occurs can effect a useful temporary repair (R.Down, *in litt*).

There have been attempts to place fluid preserved material into plastic blocks for teaching and museum purposes. This has had a limited success as the eventual breakdown of many of the resins used has been a barrier to the use of valuable or irreplaceable material. It seems that for the foreseeable future glass jars will be the best means of keeping fluid preserved material, with the less efficient but more easily obtainable plastic containers playing an increasingly important role in this field.

Procedure for mounting a fluid preserved specimen in a glass jar.

Selection of a suitable container: Most of the early jars fitted the specimen quite tightly and the samples were suspended with fine silk from two points on the lid which were pressed down by the sealing leaving a suspended specimen in the preserving fluid. With the introduction of the rectangular and square glass jars internal slips were cut to fit inside the jars so that the specimens could be sewn on. This involved the drilling of the glass slip. Although there are tungsten carbide bits which cut through glass quite easily, the earliest and in some ways the most efficient method of drilling holes in glass is to use bits broken from a small triangular file the ends of which have been ground to a pyramidal point, then heated in a bunsen flame to cherry red and plunged into water. Using a flux made from camphor and turpentine the drill will easily go through glass of medium thickness (up to 22 oz). Drill through until a slight click is heard. This indicates that the bit has just penetrated to the other side of the slip. Turn the glass slip over and finish off the hole by countersinking; this also leaves a depression in the glass. When the specimen is attached by fine silk to the glass slip the knot is tied over a small glass bead which fits snugly into the countersunk hole. Most specimens can easily be sewn through but on occasions a tissue or specimen might be too friable to be held in the normal way. Take for example, a brain, either entire or in sections. It would be impossible to sew through such a tissue and keep it firmly on a glass slip. The procedure in these cases is to insert a very fine glass rod along the length of the brain or section so that a thread can be passed over the rod and drawn back into the tissue leaving no trace, but firmly attached to the glass slip.

Alternatively a specimen may be attached to a slip by sewing through the specimen and running the silk round the slip. First make sure the edges of the glass slip have been rubbed smooth with a fine grade carborundum (emery paper will do for this). This is the general procedure for a specimen mounted in either an alcohol or formaldehyde formulation. Sometimes it is necessary to use other fluids. For cleared specimens, transparencies and calcium and cartilage specific stained samples, the preserving fluid may be a solvent or clearing agent. The same procedure is adopted for the mounting, but the sealing agent for the lid will have to be resistant to the action of the preserving reagent.

Cements for glass museum jars

Hot cements

1. *Asphalt and guttapercha.* - The guttapercha is obtained from the outer covering of pre-plastic electric wiring. It is made into a mixture by heating on a sand bath. Use a spatula or a table knife broken off at right angles to form a straight surface. Heat the lid of the jar and continue the seal

by heating a piece of glass and using this to press the lid into position until a seal has been obtained.

2. **Bitumen.** - Melted in an old kidney dish or other enamelled container. Keep melted by placing on the bypass of a bunsen burner flame or spirit burner. Apply with a knife as for 1. Seals may be made using an old electric iron which gives continuous heat and is very efficient.

3. **Rubber and wax.** - Scrap rubber 2 parts, paraffin wax (high melting point) 1 part. Melt together on a sand bath. Used for sealing specimens with an excess of fat.

4. **Gelatin.** - Use the best sheet gelatin. Soak in water until soft and squeeze out excess water. Melt on a sand bath adding a few drops of glacial acetic acid when melted. Pour into a flat tin and allow to set. Cut into small squares and keep in an airtight jar. Use only enough for the immediate purpose. Will not allow more than three consecutive meltings, after which it will no longer melt effectively as it turns into metagelatin.

These hot cements should only be used for formaldehyde and other aqueous formulations. Alcohol being inflammable would be dangerous except if an iron was used to seal the lids. If circumstances permit gelatin could be used, provided that the lid was warmed with hot water or on a hot plate and then applied to the gelatin smeared surface of the top of the jar. If successful full optical contact is made resulting in a good seal.

Cold Cements.

1. **Lead oxide (red lead or litharge) and Stockholm tar.** - Mix into a stiff paste and smear on the edges of the jar. Allow at least a week for hardening and in some cases (very large containers) several weeks. Formulated for use with large jars and those containers which for various reasons cannot be treated by other means.

2. **Spateholtz cement.**
Gum arabic 50gms
Sugar 50gms
Isinglass................ 2gms
Formaldehyde 30mls

Mix into a paste, apply and leave for at least a week. Used for containers containing various organic solvents such as oil of wintergreen, methyl benzoate, liquid paraffin and benzyl alcohol. This reagent has been used with success for most solvents.

3 **Silicone rubber.** has been used for some time and has proved a very efficient sealant.

Method of attachment of a specimen to an internal glass slip when it is too fragile or soft to be sewn or tied on.

Alcohol preserved specimens - Carefully clean and dry the internal plate and prepare the following two reagents, a solution of equal parts of ether and absolute ethyl alcohol and 'celloidin' (Necoloidine BDH). This is an 8% solution of pyroxylin in ether/absolute alcohol and before use is diluted to 1% with the first solution. Apply the ether alcohol solution to the dry plate in the area of intended attachment. Place some diluted celloidin solution to the area and then gently press the specimen down on the slip. Allow to dry naturally (breath will cause opacity). After 20 seconds wash over the slip with alcohol and the specimen may then be sealed into a jar in the usual way. The alcohol will gel the celloidin into a colourless film and form a firm attachment.

Formaldehyde preserved specimens - The slip is dried as for the previous method. A solution of 3% gelatin is melted ready for use. An area of melted gelatin is placed on the slip and the specimen gently pressed onto it. Formaldehyde preservative is then poured over the attachment and the action of the fluid will convert the gelatin to an irreversible gel holding the specimen firmly in position.

Dealing with fluid preserved specimens of unknown history; repair and conservation.

First check the sealing of the jar, there will often be a straight forward leak from around the lid.

Sealing agents

These may be one of the following:-
Gold size and shellac

Glycerin and zinc oxide
Red lead and Stockholm tar
Isinglass and sugar
Gelatin and agar
Agar

In most of the above cases the lid will have to be broken to remove the specimen, though occasionally a lid may be removed by easing off with a sharp scalpel.
Glycerin and gelatin
Bitumen
Rubber and wax
Bitumen and wax

In all the above cases gentle heat should be used to remove the lid. Take care with alcohol preserved material, immersion in hot water or the application of hot cloths are probably the best methods to adopt.

Supporting slips

These may be:-
>Plaster of Paris
>Natural mica
>Glass
>Celluloid (pre-perspex)
>Perspex (not with alcohol preserved specimens)
>Specimens may have come adrift from supporting slips.
>Their method of attachment may have been:-
>Silk (plain surgical or waxed)
>Silver wire
>Horse hair
>Nylon monofilament (usually in plastic containers)

Specimens are sometimes reconstructed in several places so be prepared to carry out delicate and complicated repair. Probe soft specimens to find supporting rods for re-attachment.Take great care as specimens become progressively fragile in preservative media.

Mounting a fluid preserved specimen in a plastic jar.

The use of plastic museum jars should be restricted to fluid preserved material based on a formaldehyde formulation and not alcohol which will attack most plastics in time. Post fixation reagents are also acceptable for this purpose. One advantage of a plastic jar is that it can be constructed to fit a specimen. In the case of manufactured glass jars a large range of sizes may be needed in order to find a suitable fit.

The construction of plastic jars is well described by Tompsett (1970) and should be followed carefully if such work is contemplated. Jars may be purchased from biological supply houses but tend to be rather expensive. A choice of coloured internal glass slips are also available. Specimens are usually attached to the internal slip and then the jar is sealed and the container filled through a small hole drilled in the lid; this is to avoid 'fluid creep' which is the cause of so much leakage in this type of jar. These filling-holes are covered with coverslips and sealed with any slide mountant. Plastic jars are easily scratched and are also subject to dirt and grease due to the problems of static electricity generation. There are a number of anti-static polishes available for the treatment of this situation. Plastic jars seem to last longer in better condition if they are filled with fluid preservative and used. Jars kept in store tend to show evidence of drying out of the adhesive from the edges of the container and this problem has not yet been satisfactorily dealt with. Construct or purchase a jar and use it as soon as possible.

Tissue reconstitution.

One of the problems with a fluid preserved collection is the possibility of a leak in a container or a breakage, somehow unnoticed, in which the fluid runs away or evaporates leaving the specimen to desiccate and generally

deteriorate. In extreme cases, mould damage and even insect infestation can occur. The main aim in reconstitution is to regain the exact shape and dimensions of the specimen ·before drying out and to try to regain, if at all possible, some resemblance to the cellular integrity of the tissues. Based on archaeological studies of dried and mummified tissues and their possible resuscitation, a number of useful reconstitution formulae have been worked out.

1. Solutions of sodium tetraborate. 2 to 5 % aqueous
2. Solutions of sodium and potassium hydroxide. 1 to 2% aqueous
3. Solutions of sodium orthophosphate. 1 to a maximum of 14% aqueous
4. Solutions of sodium sulphate. 5% aqueous
5. Solutions of sodium acetate. 2 to 10% aqueous
6. Trichloracetic acid. 1 to 3% aqueous
7. Sodium carbonate 2% and formaldehyde 0.5% aqueous
8. Saline. 1 to 2% aqueous
9. Glycerol and 10% acetic acid equal parts
10. 30% alcohol
11. Formaldehyde. 1% aqueous
12. Citric acid 2% and sodium citrate 20% aqueous
13. Clove oil (mainly for naturally desiccated material)
14. Alcohol 90%, 30 volumes; formaldehyde 1%, 50 volumes and sodium carbonate 5% aqueous, 20 volumes
15. Glacial acetic acid. 2% aqueous

The most commonly used reagent in this list is No. 3 sodium orthophosphate (or tri-basic sodium phosphate). It often has remarkable results with dried up tissues. Sometimes a little gentle heat or incubation helps the reconstitution along quite well. The strength of the solution can be varied according to the specimen involved. For very small or delicate specimens a 1 to 5% solution will be suitable. The time taken will vary according to the size and condition of the desiccated sample. Definite progress is usually observed in a few hours. Specimens treated in this way are vulnerable to breakdown in the reconstituting fluid if left for longer than the optimum time. The time in the solution can only be determined by trial and error coupled with careful observation. They must then be washed briefly in several changes of distilled water before returning to the original fixative. They remain in this for the same amount of time as is necessary for the fixation of fresh specimens. They may then, if necessary, be transferred to a preservative (if different from the fixative) or a post fixation preservative.

All solutions containing acetic acid are particularly good for the care and treatment of insect and general arthropod material, whilst the sodium and

potassium solutions are good for most invertebrates. It has been the practice in the past few years to include propylene glycol in preserving fluids as this reagent acts as a humectant and any specimen preserved in solutions containing it will never dry out completely even if all the fluid has evaporated away.

If for any reason a specimen is found to have dried out and it is not possible to treat it for some time, it may be kept in a stable condition and even slightly improved, by placing it in a container over crystals of tri-chlorphenol. Specimens have been made safe for over six months under these conditions, until the appropriate restorative treatment can be given.