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Fluid preservation

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Introduction

Both animal and plant specimens contain tissue elements vital to science and must be carefully preserved to give necessary information for future studies. Correct fluid preservation of these elements is therefore essential. For a fuller understanding of fluid preservation biomechanics refer to Stoddart (1989). Soft-bodied invertebrates especially require fluid preservation to support their body tissues and to prevent the shrinkage and distortion that would occur if they were dried, even by controlled methods. Although some workers prefer to preserve specimens by the alternative method of freeze-drying, there are still many biological tissues that can only be effectively preserved by immersion in suitable fluids.

Preservation (see Figure 5.1)

'Spirit' has probably the longest history of any non-compounded preserving fluid since it acts as both a primary fixative and preservative. 'Spirits of wine' is recorded to have been used by Boyle in London (1662, abridged in 1666) who noted '... that there generally be mixed with the spirit of wine a little spirit of sal ammoniac'. From his writings (1627–1691) there are also indications that concentrated brine and other mild preservatives were in general use prior to 1662 (Reid, 1994). Reid presents a thorough account of the term 'spirit' which was formerly used for chemically diverse fluids such as hydrochloric acid (spirit of salt) and ammonia solution (spirit of sal ammoniac). The first 'spirit' widely known in Europe came from fermented grapes and, under the Arabic name alcohol, it was sold as a medicine in Spain and Italy. Reid (1994) considers its usage now as a general, if archaic, pharmaceutical term for all inflammable liquors obtained by distillation. Swammerdam in Leiden (1637–1680) kept insects in spirits of wine or brandy and Vincent (1719) illustrates holdings of mixed fluid collections in his *Elenchus Tabularum* (see Fig. ii in the Introduction). Reid (1994) also mentions that a core collection of the British Museum made by William Courtine (*c.* 1685) contained a large variety of plants and animals in brandy. Rack, rum and brandy were recommended as preservatives on voyages of discovery (Petiver, *c.* 1700) (see frontispiece).

To understand the full relevance of spirit as a fixative and preservative, a brief history about the use of formaldehyde should be outlined since it largely replaced spirit in the role of fixative for about 100 years. In 1859 the Russian chemist A.M. Butlerov discovered formaldehyde although he failed to identify it. Von Hoffman produced it by the oxidation of methanol in 1867 and this became the standard method for the production of formaldehyde during the 1880s. Blum (1893) discovered its antiseptic property and through investigation of its effect on tissues found that it was a better primary fixative than spirit in relation to cell contents (Edwards and Edwards, 1959). Formaldehyde has since been

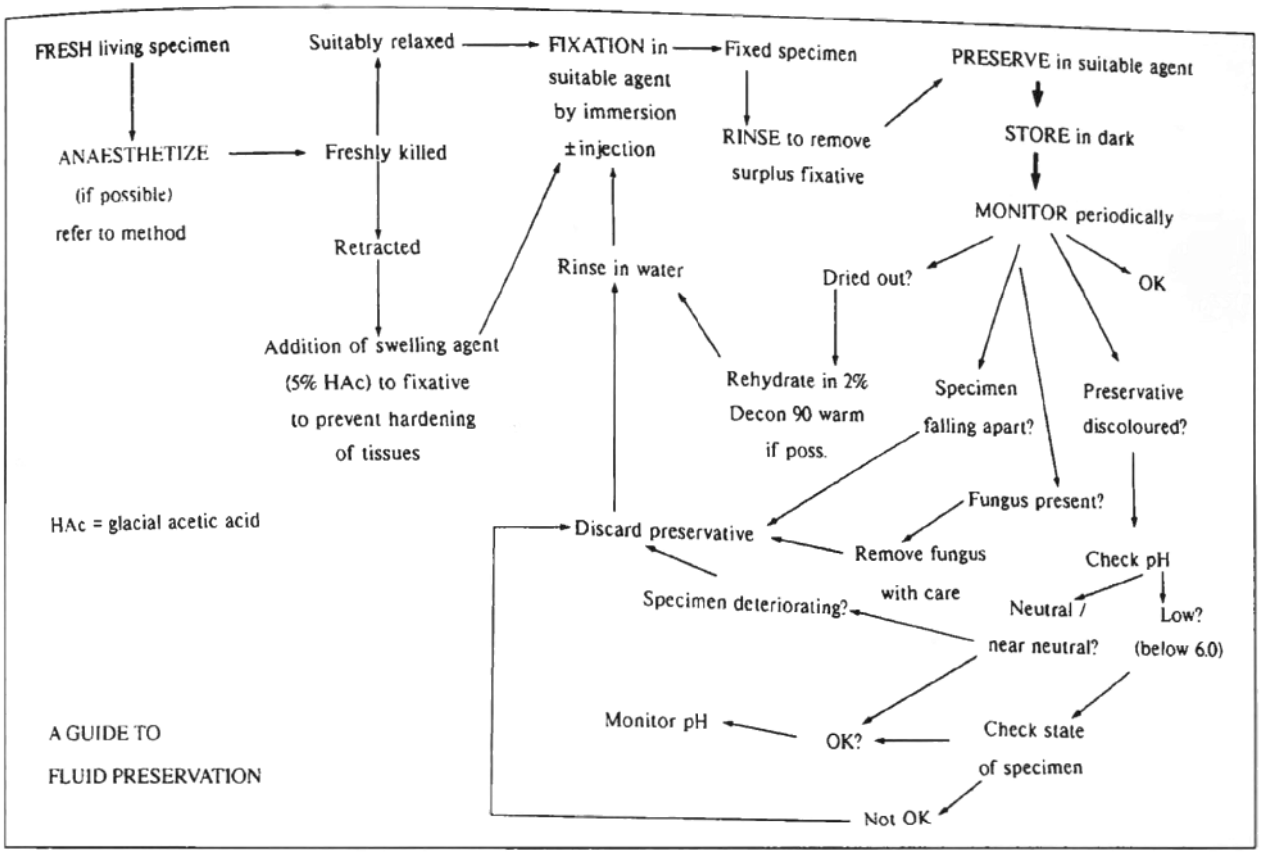

Figure 5.1 Flow chart guide to fluid preservation.

found, however, to be unsuitable for preserving DNA and, ironically, the best-preserved DNA has been found in 100–year-old specimens kept in spirits of wine or other alcoholic fixatives and preservatives (Criscuolo, 1994). For further information about this subject refer to Chapter 6 on genetic material. A 37% aqueous solution of formaldehyde is commonly referred to as formalin (see Formulae at end of chapter).

Despite this and many further discoveries in the use of formaldehyde, few other advances have since been made into the science of gross tissue fixation and preservation. Kaiserling (1922) formulated a compound fixative, colour enhancer and preserving agent (Plate 11) (see Formulae at the end of the chapter, p. 129). More recently, Owen and Steedman (1956) suggested the use of propylene phenoxetol as a preserving agent. Steedman (1976) has since advanced this work and produced a compound fixative and preservative using formaldehyde, with propylene glycol and propylene phenoxetol as humectants – these prevent total desiccation of tissues. Despite these more recent advances, many museum curators still prefer to put everything into alcohol since it seems a safe refuge against using the wrong preservative and causing specimen dissociation; however, the use of alcohol alone can still cause problems, many of which are irreversible (see IMS as a primary fixative, pp. 96). Unbuffered 4% formaldehyde is also useful as a short-term histological fixative. Aqueous-based fixatives are more osmotically compatible with cellular contents, reducing the possibility of cell membrane distortion due to sudden changes in osmotic pressure.

Many older collections have been broken up, neglected, abandoned or lost due to lack of funding for maintenance. However, some collections of medical specimens, carefully prepared using the techniques of Ruysch (1710) and his followers, have been preserved and maintained using some of the old techniques. One of the most famous is the Hunterian Collection at the Royal College of Surgeons, London, which dates from the 1780s (Plate 12).

Figure 5.2 Gastropods and a sabellid worm in Steedman's preservative having been narcotised using menthol.

Despite our progress from using spirits of wine or ship rum to preserve biological specimens, many curators and conservators still disagree as to what grade of alcohol should be used as a fixative or fluid preservative. The use of isopropanol, absolute ethanol or 74 Over Proof (74 OP) industrial methylated spirit (IMS) has been found to dehydrate specimens too severely resulting in embrittlement and, in some cases, gradual erosion of some carbohydrate elements. Between 70 and 90% strength IMS is currently used in museum collections and, provided these parameters are observed, biological collections should show no signs of deterioration.

Narcotization

Many specimens contract violently on disturbance, particularly by the sudden shock of introducing a fixing agent. To produce specimens that are fully extended, displaying their structural features to the best advantage, it is recommended to narcotize them prior to fixation (Fig. 5.2) (see also Smaldon and Lee, 1979). The living specimen is introduced to the narcotizing agent (Tables 5.1 and 5.2) which may either be in the form of a fluid or, in the case of air-breathing animals, a gas or vapour and is allowed to become completely relaxed and immobilized before immersion in a suitable fixative.

Fixation

Fixation is the initial stage of preservation where freshly dead or narcotized material is stabilized by protein coagulation or by the fixative chemically combining with it: this prevents cellular lysis. In order to produce the best results, the fixative should penetrate the tissue rapidly but without distorting it.

The narcotized or dead specimen should be immersed in a suitable fixative. For densely muscled animals or those with a protective skin, injection is advised to increase the rate of fixation both inside and outside the body cavity; this perfusion technique will ensure more thorough and even fixation. The fixative's rate of penetration, by simple immersion, is likely to be too slow to prevent autolysis and some internal putrefaction.

Dead or narcotized specimens should be thoroughly immersed in suitable fixative (in twice the volume of the specimen) (see Table 5.3) for 24 hours to one month. The time taken for fixation depends upon the factors outlined below.

- Size and density of specimen(s) – large and muscled specimens take longer.
- Type of fixative used – formaldehyde-based fixatives are slower to penetrate than alcohol-based fixatives.

Table 5.1 Narcotization of invertebrates

Taxon	*Mg++*	*Menthol*	*Formol drip*	*Other*
Protozoa	✓[1]	✓[1]	O	1–3% chlorbutol[1,6]
Cnidaria:				
Hydrozoa	✓[1]	✓	✓	
Anthozoa	✓[1]	✓	X	†, freezing
Scyphozoa	✓[1]	✓	✓	
'Other jellies'	✓[1]	✓	O	
Ctenophora	✓[1]	✓	X	
Platyhelminthes	✓	✓[1]	X	Freezing
Nemathelminthes	✓	✓[4]	O	
Rotifera	X	O	O	#, ‡, 0.1% chloretone[1]
Annelida:				
Oligochaeta	✓	O	O	20% ethanol in water
Polychaeta	✓	✓	O	MS-222, freezing – *Arenicola*
Bryozoa	✓	✓	O	1% stovaine[2] or 1% β-eucaine[2]
Mollusca	✓	✓	✓	0.5% phenoxetol (marine forms)
Arthropoda:				
Insecta				A
Crustacea	✓	✓	✓	
Arachnida				A
Echinodermata	✓	✓	✓	
Chordata	✓	✓	✓	

Formol drip, 0.5–1% formalin using a burette drip.
Mg++, magnesium salt, usually a saturated solution of magnesium chloride in distilled or sea water.
✓, Found, by author, to give good results.
X, Found, by author, to give poor or inconsistent results.
O, Untested.
†, Refer to Moore (1989b).
‡, Refer to Smaldon and Lee (1979).
A, Air-breathing animals can be anaesthetized using ethyl acetate (good for invertebrates) (Walker and Crosby, 1988).
#, Add to every ml of culture five drops of: 2% benzamidine hydrochloride (three parts), distilled water (six parts) and pure cellosolve (ethylene glycol, mono-ethyl ether–one part). Alternatively, add to culture solution a mixture of eucaine hydrochloride[5] (1 g), 90% alcohol (10 ml) and distilled water (10 ml).
[1]Also known as chloretone and 1,1,1-trichloro-2-methyl propan-2-ol.
[2]If these narcotics are unavailable, a 0.001% solution of MS-222 Sandoz in distilled water may be used.
[4]If unavailable, 0.1 g of MS-222 Sandoz may be substituted.
[6]Narrow parameter between narcotization and autolysis: transfer to freezer if narcotization requires process to be continued overnight, or continue process at < 10°C.

Table 5.2 Narcotization of vertebrates

Taxon	*Mg++*	*Menthol*	*Formol drip*	*Other*
Pisces	X	X	X	Up to 0.5% aqueous MS-222 Sandoz
Amphibia	A	A	X	A
Reptilia	A	A	X	A
Aves	A	A	X	A
Mammalia	A	A	X	A

Formol drip, 0.5–1% formalin using a burette drip.
Mg++, magnesium salt, usually a saturated solution of magnesium chloride in distilled or sea water.
X, Found, by author, to give poor or inconsistent results.
A, Air-breathing animals can be anaesthetized using di-ethyl ether, chloroform or ethyl acetate.

- Fixation takes longer at lower room temperatures (less than 10°C).
- Fixative must be renewed if it becomes contaminated with excess lipid.
- Ensure that fixative is stirred approximately half-way through fixation for water-bodied animals such as jellyfish; this will prevent stratification (diluted layers of fixative from body fluids).

Table 5.3 Fixation and preservation of invertebrates

Taxon	*Fixative*	*Preservative*	*Comments*
Protozoa	Sch	AA	
Cnidaria:			
'Non-jellies'	F, FSal	S	
Hydrozoa	FSal, St	S	1, 2
Anthozoa	F, FSal, St	SPFP, Phx	1
Scyphozoa	B, FSal, O, St	SPFP, S	1
'Other jellies'	FSub, O	S	1
Ctenophora	B, FAA	S	1
Platyhelminthes	F, FSal, FSub	S	
Nemathelminthes			
Annelida:			
Oligochaeta	B, F, FAA, FSal	FAA, S	1, 2
Polychaeta	B, F, FAA, FSal	FAA, S, Per,	1, 2
Bryozoa	B, F, FSal	S	1
Mollusca	B, F, FSal, St	Phx, S, SPFP	1, 2, 5
Arthropoda:			
Insecta	C, FAA, S	S, Pam,	1
Crustacea	C, DB, F, FAA, S	S (+G, 95:5)	1, 5
Arachnida	C, FAA, S	FAA, S	1
Echinodermata	B, F, FAA, FSal	S	1, 2, 5
Chordata	B, BSal, FSal, Inj	S, Lip	1, 2, 5

AA, Absolute alcohol, pure ethanol. [industrial methylated spirit (74 over proof) and isopropanol (propan-2-ol) make cheaper substitutes.]
B, Bouin's fluid.
BSal, Saline Bouin.
C, Carnoy's fluid
DB, Dubosq–Brazil's fixative.
F, 10% formalin (= 4% aqueous formaldehyde) buffered using either a 1% mix of 6.5:3.5 w/v di-sodium hydrogen orthophosphate (Na_2HPO_4) and sodium di-hydrogen orthophosphate (NaH_2PO_4) or (better) 0.05 M sodium β-glycerophosphate ($Na_2C_3H_7(OH)_2PO_4$) until desired pH is reached.
FAA, Formol-acetic-alcohol.
FSal, Formol saline.
FSub, Formol sublimate.
G, Glycerol.
Inj, Must be injected.
Lip, Storage in spirit or formalin must be monitored due to fat leaching out.
O, Osmic acid (1–2% solution).
Pam, May be fixed in Pampl's fluid, not longer than one week.
Per, May be fixed in Perenyi's fluid for three to four minutes (up to one hour for larger specimens).
Phx, Propylene phenoxetol (1% aqueous solution – must be made up in hot water).
S, Museum spirit (80% methylated ethanol).
Sch, Schaudinn's fixative.
St, Steedman's fixative.
SPFP, Steedman's post-fixation preservative.
1, Many colours not easily preserved: store specimen in dark.
2, Kaiserling technique found to be successful for colour preservation if specimen stored in dark.
3, Refer to Crimmen (1989).
4, Refer to Fry (1985).
5, Refer to Stephenson and Riley (1995).
Refer to formulae at end of chapter for details of the above compounds.

IMS (70–90%) as a primary fixative

Although IMS is used as a solvent for compounding other fixatives, it can cause problems if used on its own. Due to its rapid penetrating property and low specific gravity it often causes shrinkage of cellular contents (syneresis) resulting in distortion (Fig. 5.3). Histologists will also be aware of its solvent property in terms of streaming glycogen in liver cells (Fig. 5.4), loss of enzymes and solution of lipid from lipocytes (Fig. 5.5). The latter property can be useful when dissolving unwanted lipid from a specimen but the preservative will need several changes and require regular monitoring (see Lipid leaching, pp. 108). To obviate distortion, specimens should be fixed in a formalin-based fixative and then transferred to a suitable preservative

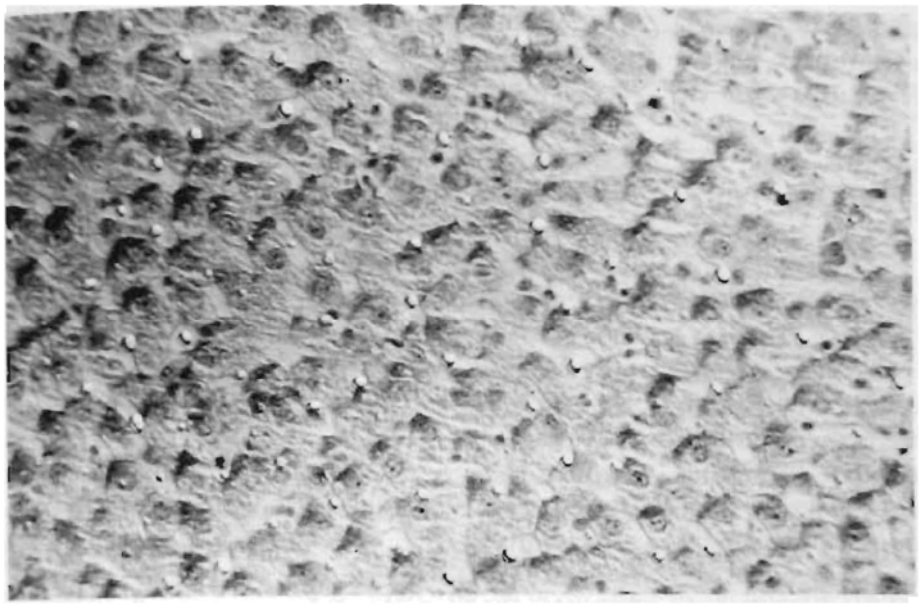

Figure 5.3 Glycogen in liver cells streamed over to one side following alcohol fixation.

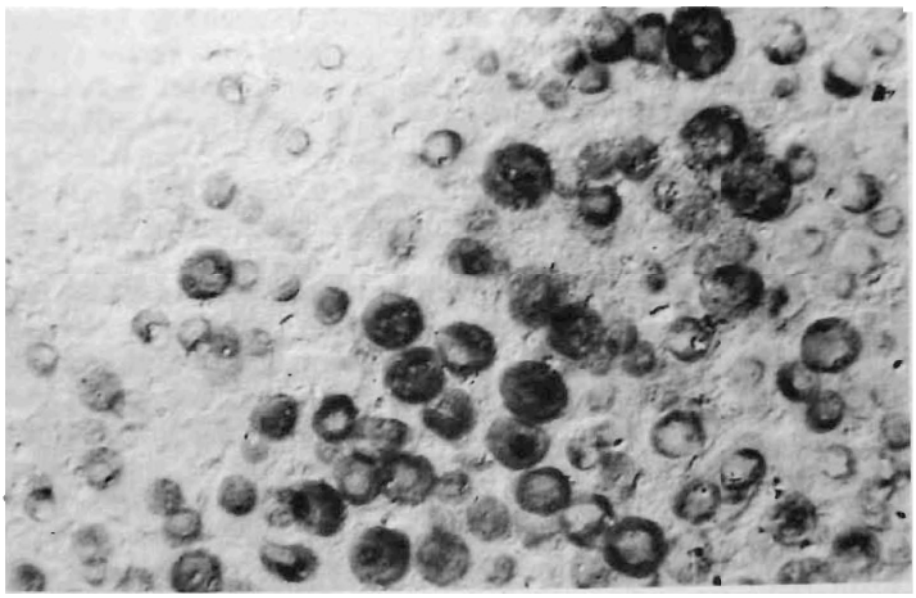

Figure 5.4 Lipid-filled lipocytes in liver stained with oil red O in tri-ethyl phosphate; from a frozen section.

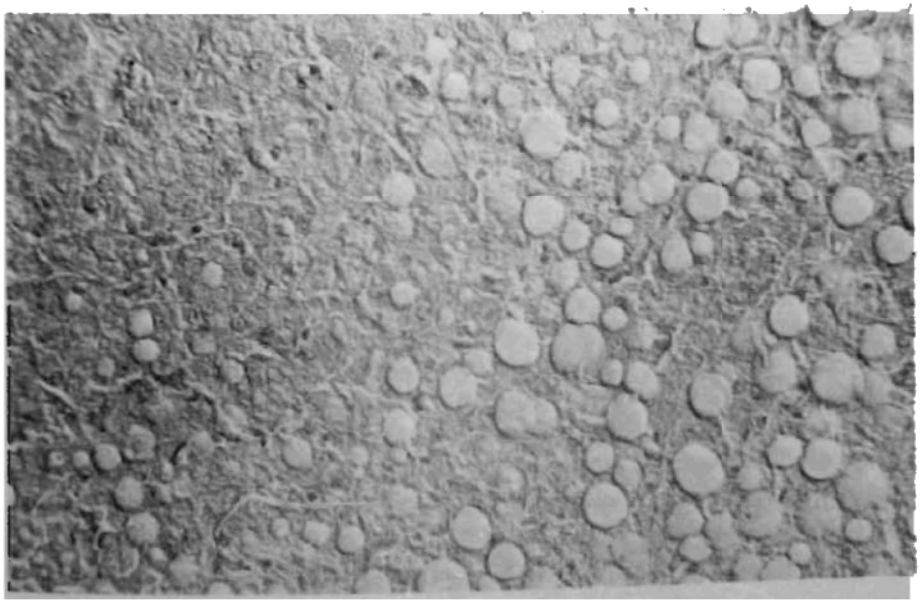

Figure 5.5 Lipocytes in liver, showing cells empty of lipid content – dissolved by alcohol fixation.

such as alcohol or a phenoxetol-based preservative. Even insects or other animals with a reduced tolerance of low pH fixatives can be safely fixed in unbuffered formalin for 24 hours, then transferred to alcohol. Note that buffering formaldehyde reduces its fixative effect.

Further research into the benefits of using alcohol as a primary fixative and long-term preservative have been carried out by Criscuolo (1994) and many other researchers in the USA and Canada. Hawks (pers. commun.) comments that there is considerable anecdotal evidence suggesting that formaldehyde fixation may even be less desirable than alcohol for some groups of animals, especially invertebrates which can show gross tissue deterioration if they are not transferred to a suitable preservative following formaldehyde fixation (Moore, 1990a). However, even slightly prolonged immersion in formaldehyde has been found to cause masking of DNA content. For this reason alcohols are preferred. DNA is well-preserved in dry and alcohol-preserved specimens, whilst formaldehyde causes a cross-bonding reaction in proteins, forming a cage around the DNA and rendering it less easy to extract. Hydroxyapatite has been found to bind DNA thus facilitating extraction from fossil and sub-fossil bones (F. Bonhomme, Montpellier University – pers. commun.).

Formaldehyde as a fixative

Buffering formaldehyde against pH level drop weakens its fixation effect on biological material by reducing formation of the carbonium ion. The carbonium ion is capable of electrophilic attack on protein molecules by reacting with many of the functional groups causing crosslinking of the protein chains (Stoddart, 1989). This leads to the formation of insoluble macromolecular complexes that prevent subsequent protein loss from the tissues. Formaldehyde also reacts with amines in the tissues' amino acids. This in turn produces fatty acids, which is why biological specimens have the effect of lowering the pH of formaldehyde-based fixatives.

To achieve maximum fixation with the best level of preservation, it is advisable to fix in unbuffered formaldehyde for a short time and then 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 and reducing the number of fixative-active carbonium ions and by maintaining a near to neutral pH. Low pH gives rise to protein embrittlement and dissociation and even to decalcification of bone, leading to vertebrate specimens becoming undesirably and unnaturally flexible (Plate 16). High pH leads to the possible gelatinizing of proteins.

Buffers

Buffered formaldehyde is normally used as a preservative since its fixation effect is reduced by buffers. For those who persist in buffering formaldehyde at the fixation stage the choice of buffer is important to maintain biological specimens at the required pH. Carter (1997) investigated the most suitable buffer for formaldehyde and its effect upon the ionic fixation-inducing part of the formaldehyde molecule. His findings, based on a specific type of specimen, are paraphrased here with permission.

Recent conservation work on a fish parasitology collection (at the National Museum of Wales) comprising intestinal tracts, largely from elasmobranchs, has shown that they have remained in their original 4% formaldehyde fixative since they were collected in 1990/1. Checks on pH have revealed levels at 4.0 or slightly below, combined with substantial yellowing of the formaldehyde. This indicates the development of protein dissociation problems due to lipid leaching. Since, for the purposes of light and transmission electron microscopy, the parasites were best preserved in formaldehyde, it was renewed but with an appropriate buffer. Sodium acetate combined with sea water formaldehyde was found to allow the pH levels to rise to neutrality within a few months.

Four suitable buffering agents were tested in both de-ionized and artificial sea water: 3.5:6.5 mix of sodium dihydrogen phosphate and disodium hydrogen phosphate; 4% w/v sodium acetate; 2.5% w/v sodium -β-glycerophosphate (0.01 M) and 15% w/v sodium β-glycerophosphate (0.05 M). The pH of each was measured before and after immersing a fish intestine. Readings were

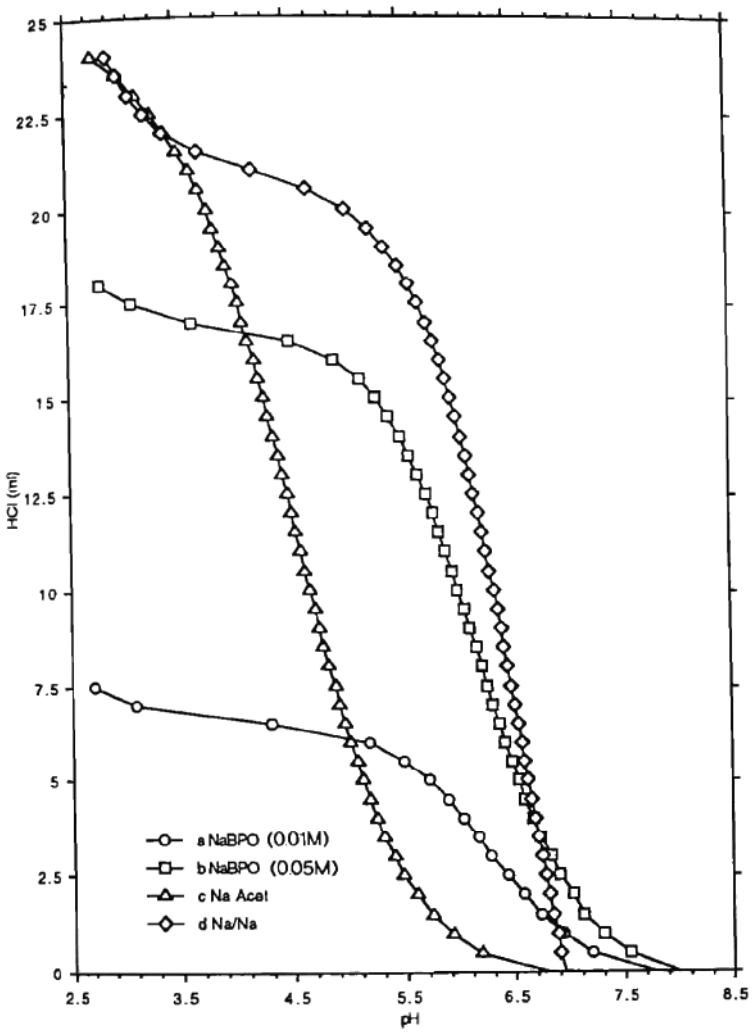

Figure 5.6 Resulting pH curves for buffered formaldehyde solutions when titrated against 0.1 M hydrochloric acid.
NaBPO = sodium β-glycerophosphate
NaAcet = 4% sodium acetate
Na/Na = mix of Na_2HPO_4 and NaH_2PO_4

checked daily for the first week and thereafter weekly. After these studies the buffer range of the used de-ionized water preservatives was measured by titration (Fig. 5.6). The pH of some fresh preservative solutions was compared both when buffered and unbuffered. The pH readings of the buffered solutions was found to be more stable; these were remeasured after three months of preservation time had elapsed. The most effective buffer was found to be the 0.05 M sodium β-glycerophosphate whose pH range was between 5.5 and 6.5 for both de-ionized and saline preservative solutions. The sodium hydrogen phosphate mix was also found to be effective but only in de-ionized water, since it was precipitated in saline.

Long-term preservation

Many fixatives are unsuitable for long-term preservation due to their low pH or oxidizing properties. Tissues are required to be maintained in the fixed state and this is the role of the preservative. If successful preservation is required for the long-term, the tissue must be properly fixed (i.e. all cellular proteins precipitated or combined so that tissue cannot be broken down by bacteria or other denaturing agents) before transferring it to a preservative.

Older preserved specimens

Some fluid-preserved specimens have been preserved in unidentified fluids. Specimens

taken during sea voyages during the eighteenth and nineteenth centuries have occasionally been preserved in ship's rum. The strength of alcohol should be ascertained, using an alcoholometer or a DMA 35 digital density meter, before transferring such a specimen to an appropriate grade of alcohol. Care should also be taken when unsealing jars containing such specimens since many obsolete fixatives contained toxic compounds such as mercury or arsenic salts that can give rise to evolution of arsine gas inside the jar: always use a fume cupboard (refer to Chapter 9 on policies and procedures and see Health and safety, p. 108). Ascertaining what fluid has been used as a preservative can sometimes be hazardous (Moore, 1986, 1990b). If specimens appear to be well preserved, it is better to leave them in the same fluid, as they have already withstood the test of time. However, if the appearance and preservation of the specimen is unsatisfactory, some safe analysis of the preservative is necessary.

Canadian and American museums largely use 70% ethanol as a preservative, following fixation in 10% buffered 4% formaldehyde for one to two weeks; for some years this technique seems to have had the desired effect with no deterioration or traces of tissue in the bottoms of storage jars. Some amino acid leaching has been detected, however, and analysis and prevention methods are expected soon. Some museums prefer to use between 45 and 60% isopropanol as a preservative, since it is slower to evaporate and is not subject to customs regulations. However, isopropanol has been found to embrittle certain specimens and it is definitely not recommended for sponges since it causes gradual dissociation of the spicular skeleton.

Analysis of preserving fluids

Curators are often faced with daunting rows of jars containing (normally) colourless fluids and often resort to analysing the contents by sniffing them in contravention to health and safety considerations. Three analytical methods are suggested:

1. Use of leuco-fuchsin impregnated indicator papers – but bear in mind that they have a limited shelf-life and must be stored in the dark (Waller and McAllister, 1986).
2. Use of a DMA 35 digital density meter which is expensive but precise. Carter (1994) gives examples of fixative and preservative solution specific gravities with all readings taken at 20°C:

Alcohol solution (80% IMS)	0.859 g/ml
Formaldehyde solutions	
(4% aqueous)	1.009 g/ml
(10% aqueous)	1.014 g/ml
(40% saturated)	1.079 g/ml
Formol-saline	
(4% formaldehyde)	1.030 g/ml

3. The following gravimetric method devised by the author. This method does not distinguish between low-grade alcohols (of which there should be none in properly maintained collections), formalin and unconventional preservatives but has the advantages of being faster, cheaper (home-made), much safer and reasonably accurate. A dropping bottle with reservoir,

Figure 5.7 Dropper bottle with pipette reservoir containing colour-coded mapping pin heads of differing weights. The distribution of floating sunk pin heads helps to determine the nature of preservative solutions.

pliers and mapping pins of assorted colours with heads small enough to fit in the pipette reservoir (Fig. 5.7) are required.

(i) Make up a range of preservative solutions for testing.
(ii) Remove pins from heads using pliers.
(iii) Test flotation of pin heads in solutions and replace pins (point first) into pin heads.
(iv) Trim off pins to various lengths so that some will float and some will sink in the various solutions.
(v) When each pin has been trimmed to the correct weight, push the remainder of the pin into the head.
(vi) Put weighted pin heads into pipette reservoir.
(vii) Test – suck up fluid into reservoir, shake pipette to detach adherent air bubbles and note the distribution of floating and sunk pin heads.
(viii) A simple method of distinguishing between 70% alcohol and formalin will only require one ball – it floats in formalin, sinks in alcohol (above 55%).

Recommended technique for preservation

1. Wash fixed specimen in water (use distilled water for small or delicate specimens) and check, by careful examination, that the specimen is completely fixed. NB This stage is often ignored, which can lead to eventual putrefaction in the preservative.
2. Drain off excess water and immerse specimen in a suitable preservative (see Table 5.2).
3. After one week check that no deterioration has occurred (colouring of preservative, tissue deposits on bottom of jar, lipid globules floating on or in preservative).
4. If deterioration is noticed, return the specimen directly to the fixative but take into account the possible osmotic differential between the fixative, rinse bath and preservative.
5. Once deterioration ceases to occur, the specimen may be stored, although it is advisable to check it every three to six months.

Note: Storage in fixative is often unadvisable due to the toxic (hazardous) nature of the fixative and the fact that some fixatives can

Table 5.4 Fixation and preservation of vertebrates

Taxon	*Fixative*	*Preservative*	*Comments*
Pisces	F, FSal, St	Phx', S, SPFP	Inj[1,2,5]
Amphibia	F, FSal, St	Phx, S(60%), SPFP	Inj[1,2,5]
Reptilia	F', FSal, St	Phx, S, SPFP	Inj[1,2,6]
Aves	F, FSal, St	Phx, S, SPFP	Inj, Lip[1,3]
Mammalia	F, FSal, St	Phx, S, SPFP	Inj, Lip[1,2]
DNA	70% ethanol, dried specimens		

F, 10% formalin (= 4% aqueous formaldehyde) buffered using either a 1% mix of 6.5:3.5 w/v di-sodium hydrogen orthophosphate (Na_2HPO_4) and sodium di-hydrogen orthophosphate (NaH_2PO_4) or (better) 0.05 M sodium β-glycerophosphate ($Na_2C_3H_5(OH)_2PO_4$) until desired pH is reached.
FSal, Formol saline.
Inj, Must be injected.
Lip, Storage in spirit or formalin may leach out fat; monitor carefully.
Phx, Propylene phenoxetol (1% aqueous solution – in hot water).
S, 'Museum spirit' (80% methylated ethanol).
St, Steedman's fixative.
SPFP, Steedman's post-fixation preservative.
1, Many colours not easily preserved; store specimen in dark.
2, Kaiserling technique found to be successful for colour preservation if specimen stored in dark.
3, Refer to Crimmen (1989).
4, Refer to Fry (1985).
5, Refer to Stephenson and Riley (1995).
6, Refer to Stuart (1995).
Refer to formulae at end of chapter for details of the above compounds.

ultimately cause tissue breakdown. For details of longer-term storage effects of fixatives and preservatives, see below. See also Table 5.4 giving details of the fixation and preservation of vertebrates.

Propylene phenoxetol as a long-term preservative

During the 1960's, propylene phenoxetol, an embalming fluid, was found to be effective for preserving zoological tissues. It should be made up as a 1% solution in hot water as it will not dissolve in cold. For ease of use as a preservative, Steedman (1976) compounded it with propylene glycol (PG). When used correctly, following thorough fixation, it has found to be an effective preservative for many groups of animals, although it must be remembered that Steedman's solutions were originally devised for preserving marine zooplankton. It swells tissue slightly and thus neutralizes the slight shrinkage occasionally experienced during fixation. Although it appears to preserve tissues in the longer term, it has been observed that some tissues have undergone a gradual deterioration over thirty years. Whether this was due to slightly inadequate fixation is doubtful since tissue breakdown would have been considerably more rapid. Crimmen (1989) first observed this breakdown in phenoxetol-preserved fish specimens after only a few years, although this would have been effected by lipid leaching from fish livers combined with a gradual inability of the preservative to maintain its stabilizing effect on densely muscled areas of tissue. Moore (1997) discusses the pro's and con's of using phenoxetol-based preservatives and warns of the pitfall of its use with tissues that are either too dense to be maintained by phenoxetol or those with dubious fixation histories. Conservators and curators should regularly check long-term phenoxetol-preserved specimens and should transfer those showing signs of deterioration back to a suitable fixative solution. Once refixed the specimens can be returned to the preservative.

Propylene glycol (1,2-propanediol) also acts as a mould inhibitor and humectant for biological tissues; it prevents total desiccation in the case of accidental drying out, and makes the task of rehydration easier and more successful. It makes a useful (5%) additive to alcohol and formalin-based preservatives and additionally has been found both to slow down evaporation and reduce specimen embrittlement and rigidity (Boase and Waller, 1994). Glycerol has also been successfully used but is not a mould inhibitor and is less easy to remove should the need arise.

Effects of preservatives on mammalian tissue

While it is advisable to test both the long-term and short-term effects of preservatives on tissues, it is difficult to achieve the former without the test of time. A long-term and short-term experiment using rat and gerbil organs has established a useful technique for assessing the success of different preservation regimes. The following notes and Tables 5.5 and 5.6 present the results of experiments carried out by the author.

Long-term experiment

The problem of testing preservatives for long-term effect is obviously the time needed to elapse before such an investigation can be satisfactorily completed. However, several well-known preservatives were tested, using various fixatives, on rat organs fixed on 16th September 1957, transferred to preservative ten days later and sectioned and stained twenty years later. The detailed results are shown in Table 5.5, but can be summarized as shown below:

- **10% formalin to 75% ethylene glycol**. Considering the period of time in preservative the tissues showed a good result for ethylene glycol as a preservative for formalin-fixed material. There was a slight fall-off except for cell contents (poor), and gross appearance was fair.
- **10% formalin to 80% ethanol**. For the tissues that were stainable, the results were good. Cell contents were poorly preserved although gross appearance was fair to good.

Short-term experiment

A more thorough but shorter-term experiment, using gerbil organs, was performed with fixation lasting only 20 hours. Preservation was monitored by sectioning and staining samples

Table 5.5 Results after a twenty year experiment testing fixatives to preservatives using rat organs: ratings from 1 (worst) to 5 (best); tested on liver/kidney/ileum

10% formalin to 75% ethylene glycol:						
A	B	C	D	E	F	G
3/2/3	3/3/3	4/4/3	4/4/4	3/2/3	2/2/4	3/2/3
10% formalin to 80% ethanol:						
A	B	C	D	E	F	G
≠/2/2	≠/3/3	≠/4/4	≠/4/4	≠/2/3	≠/4/4	≠/3/4

≠, Liver infected with fungus – tissue would not stain.
A, Colour: colour of organ after preservation.
B, Texture: organ soft (normal) to hardened.
C, Nucleus: good to poor staining reaction.
D, Cytoplasm: good to poor staining reaction.
E, Masson trichrome: good balance of the three stains to total inbalance (all stained green).
F, Histochemical: normal to faint reaction.
G, Evenness: preservation even to patchy.

Table 5.6 Results of a two year experiment fixatives to preservatives using gerbil organs: ratings from 1 (worst) to 5 (best): tested on liver/kidney/ileum.

10% formalin to 75% ethylene glycol:							
Year	A	B	C	D	E	F	G
1st	1/1/1	4/4/4	4/4/4	3/5/5	2/2/2	2/3/3	3/3/3
2nd	1/1/1	4/4/4	3/3/3	2/3/3	1/2/1	1/2/1	3/3/3
10% formalin to 80% ethanol:							
	A	B	C	D	E	F	G
1st	1/1/1	2/2/2	3/3/3	3/3/2	2/3/2	2/3/2	2/2/2
2nd	1/1/1	2/2/2	2/2/2	2/2/2	2/2/1	1/1/1	2/2/1
Steedman's fixative to Steedman's preservative:							
	A	B	C	D	E	F	G
1st	3/3/3	5/5/5	5/4/4	5/5/5	2/2/3	2/2/3	3/3/1
2nd	3/3/3	5/5/5	3/4/4	4/5/5	2/1/2	2/2/3	3/3/3
80% ethanol to Steedman's preservative:							
	A	B	C	D	E	F	G
1st	2/2/2	4/4/4	4/4/4	3/3/3	2/3/3	3/2/4	3/3/3
2nd	2/2/2	4/4/4	3/3/3	3/3/3	2/2/2	1/2/2	3/3/3
Carnoys fixative to Steedman's preservative:							
	A	B	C	D	E	F	G
1st	1/1/1	4/4/4	3/4/4	4/4/4	2/2/2	2/3/4	4/4/4
2nd	3/3/3	5/5/5	3/3/3	1/1/1	2/1/1	2/2/3	2/3/2
Dubosq–Brazil's fixative to Steedman's preservative:							
	A	B	C	D	E	F	G
1st	1/1/1*	4/4/4	4/4/4	4/4/4	2/2/3	2/3/3	3/3/3
2nd	1/1/1*	5/5/5	3/4/4	4/4/4	2/2/2	1/2/2	2/2/2

*, Stained yellow by picric acid.
A, Colour: colour of organ after preservation.
B, Texture: organ soft (normal) to hardened.
C, Nucleus: good to poor staining reaction.
D, Cytoplasm: good to poor staining reaction.
E, Masson trichrome: good balance of the three stains to total inbalance (all stained green).
F, Histochemical: normal to faint reaction.
G, Evenness: preservation even to patchy.

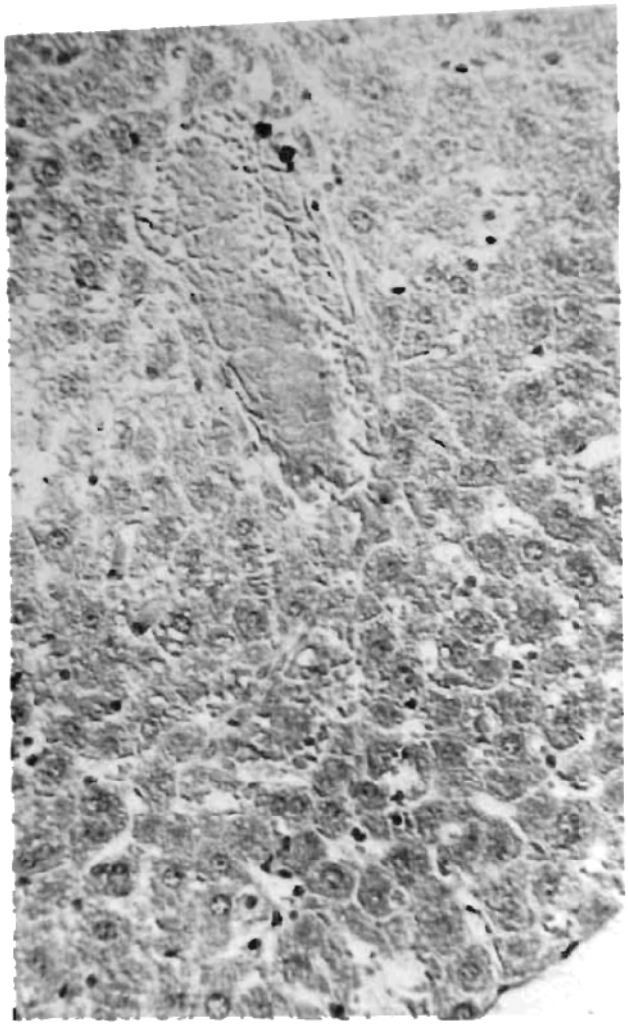

Figure 5.8 Rat liver sections (results of preservation experiment.). Alcohol fixation showing slight shrinkage between cells.

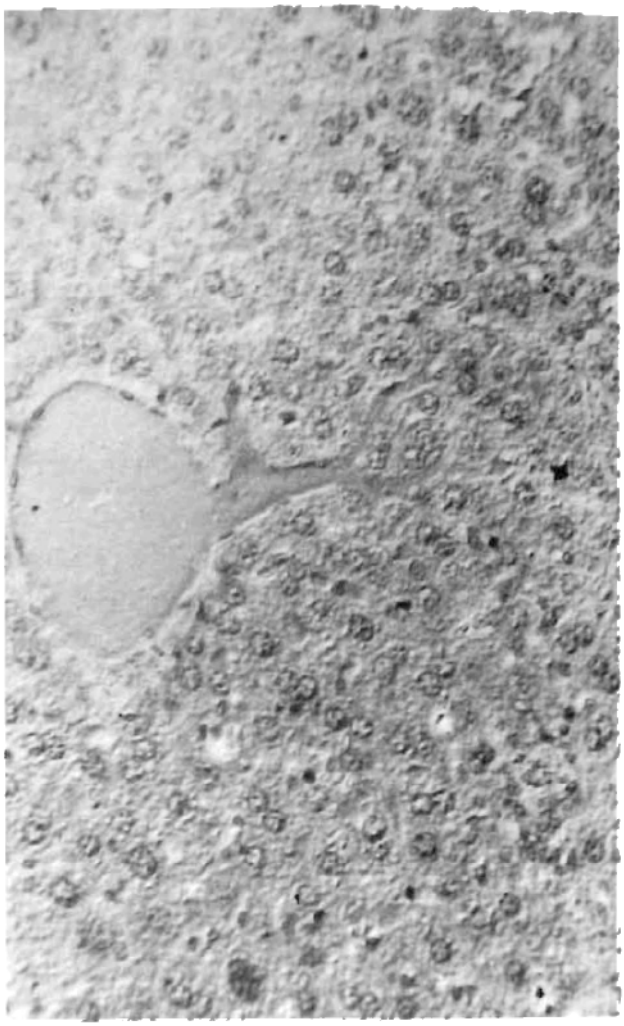

Figure 5.9 Formalin fixation showing no intercellular shrinkage: nuclei well defined.

after periods of one and two years (Figs 5.8–5.11). The detailed results are shown in Table 5.6 and are summarized as shown below:

- **10% formalin to 5% formalin**. Preservation poor and some fall-off in preservation over one year.
- **10% formalin to 80% ethanol**. Poor preservation and some fall-off over one year. Result inconsistent with 20 year experiment.
- **Steedman's fixative to Steedman's preservative**. Poor penetration of ileum sample, but good gross preservation. Less good for cell contents with slight fall-off during test period. Especially good for gross preservation.
- **80% ethanol to Steedman's preservative**. Poor cytoplasm result for year 1 due to slight shrinkage during fixation. Gross preservation good.
- **Carnoy's fixative to Steedman's preservative.** Better and more even fixation took place at 4°C. Preservative enhanced gross colour and texture of organs but cytology results fell off during second year.
- **Dubosq–Brazil's fixative to Steedman's preservative.** Improvement of gross texture of tissues, some cytological fall-off.

From the above experiment Steedman's preservative was concluded to be good for maintaining the gross appearance of tissues but fair to poor for maintaining good cytological staining reaction unless tissue was fixed in Steedman's fixative. Tissues were softened by the preservative but not to the point of disso-

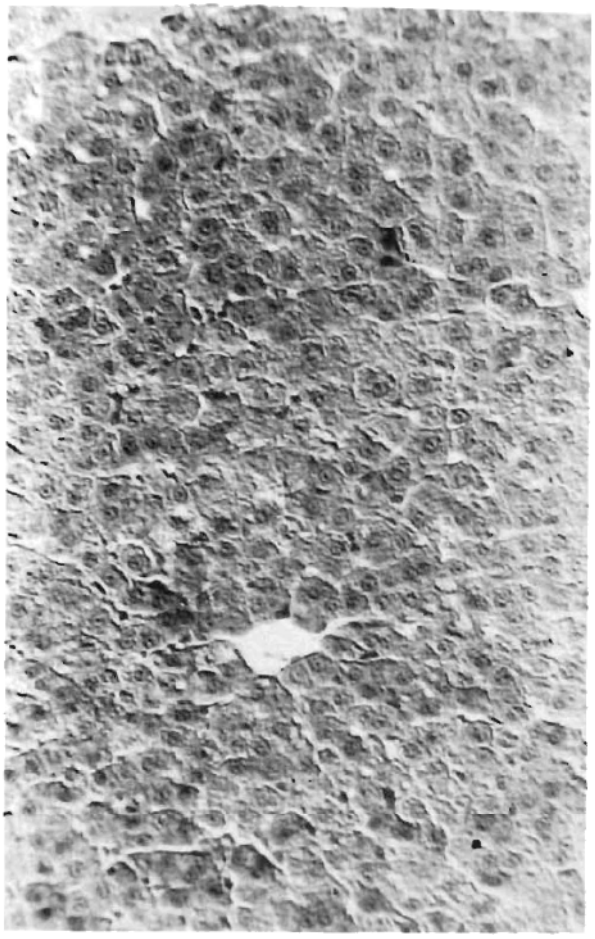

Figure 5.10 Alcohol fixation followed by alcohol preservation after one year: nuclei less well defined.

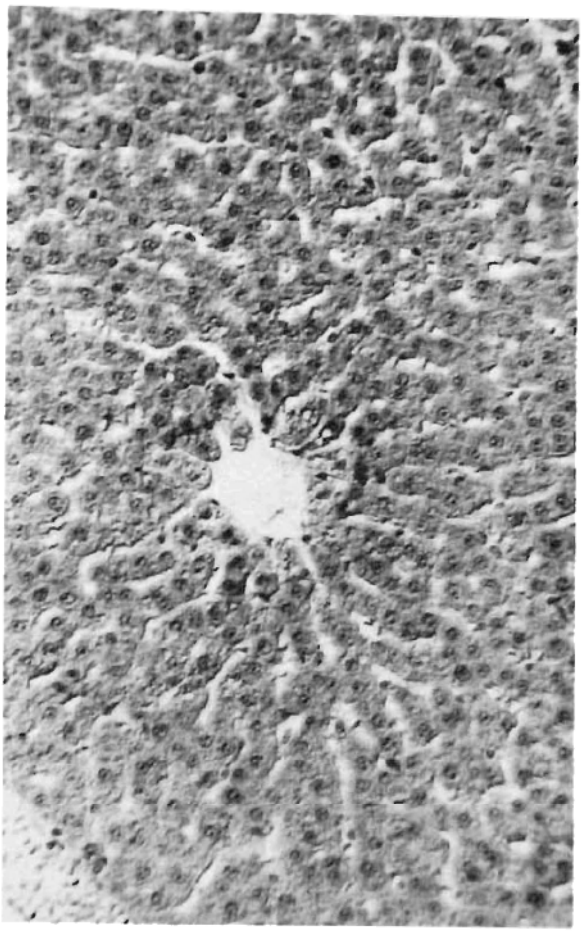

Figure 5.11 Alcohol fixation followed by two years in Steedman's preservative: intercellular shrinkage still present although nuclei are better defined – the preservative has maintained this sample perfectly since fixation.

ciation. Ethanol was found to be fair to good for gross preservation, depending on fixation, but poor for cytology.

Preserving colour

In 1922 Kaiserling published a revised technique for preserving colour in biological specimens that he initially developed in 1896. Judah (1922) also published a revision of the technique in English. This technique has been successfully used on sea anemones (Plate 11), particularly the cloak anemone, *Adamsia carciniopados*, whose magenta spots fade in most fixatives and preservatives in a few days. The spots have now been preserved for fifteen years by the Kaiserling technique and are still just visible suggesting that, although finite, the technique preserves some fugitive colours considerably better than standard preservatives.

The technique uses three fluids: Kaiserling I, II and III (see Formulae at the end of the chapter, p. 130).

- Kaiserling I (fixative) – due to its slow penetration, injection is advised as well as immersion.
- Kaiserling II (enhancer) – brings back the colour faded by the fixative within half to one and a half hours.
- Kaiserling III (preservative)

The author has found that tissues begin to shrivel due to the osmotic pressure difference between I and II and some colours are leached by the alcohol within ten to twenty

minutes! This stage must be monitored and it has to be judged when is the best time to transfer to the preservative III which must have already been made up. Bear in mind the osmotic pressure differential of II and III. A brief rinse in distilled water between these stages will help to equilibrate the osmotic pressure and will reduce the osmotic shock to the tissue or specimen. The specimen stays in the preservative, preferably stored in the dark or the colours will be faded by UV.

There have been many modifications to this technique since Kaiserling first published it in 1896 and these can be traced in Harris (1984). More recently Lee (1989) has experimented with both this and the more recent Wentworth (1938, 1957) technique which utilizes additional sodium phosphate buffers and eliminates the need for glycerol.

Botanical specimens (see Table 5.7)

Most gross preservation techniques applied to botanical specimens involve controlled drying. Apart from a few groups, fluid fixation and preservation is normally relevant as a preparation for cytological techniques and use of the transmission electron microscope. Some fixation and preservation methods are listed for preservation of cytological elements (indicated by appropriate symbol). Fluid preserving techniques for zoological specimens can also, by and large, be applied to botanical material: the same problems can

Table 5.7 Modifications and specialized techniques for certain groups of plants (fungi have been included here for convenience)

Taxon	*Fixative*	*Preservative*	*Comments*
Slime moulds	FAA	S	
Freshwater macroalgae	CAA, F	C, S, Kew	=
Desmids	[F] +HAc	S	Few drops of HAc
Charophytes	CuAc, F	C, S, Kew	=
Euglenophytes	Sch.†*	S	Fix at 60°C for 5–10 minutes
Diatoms	Sch, †*	S	
Marine algae	CAA, FSal	C, S, Kew	Store in dark
Phytoplankton	F, FI	S	May be stored in 30% PG
Fungi	F, FSub	C, S, Kew	=
Lichens	FAA	C, S, Kew	
Bryophytes	FAA	FAA, C, S, Kew	
Pteridophytes	FAA	C, S, Kew	
Flowering plants and gymnosperms	F, FAA	FAA, C, S, Kew	=
Fossil seeds	FAA	Alc, G	Remove salts in distilled water

For plant chromosomes in pollen mother cells and fern spore mother cells: fix three parts absolute ethanol to one part glacial acetic acid and store in freezer at –10°C or, for faster penetration, fix in six parts absolute ethanol, three parts chloroform and one part glacial acetic acid and store material in 70% ethanol at 4°C after 24 hours of fixation. Pollen grains and spores can be fixed in the above mixture of FAA and then stored in glutaraldehyde; if required for transmission electron microscopy the fixatives below should be used.

For transmission electron microscopy the following fixatives and buffers have been effective on bryophytes and diatoms and will probably work for other plant groups: †*, 2–3% glutaraldehyde in 0.05–2.0 M sodium cacodylate for two to four hours at 20°C; †*, glutaraldehyde and osmic acid in 0.05–0.1 M 2% each of Sodium orthophosphate mix – 6.5 di-sodium hydrogen orthophosphate (Na_2HPO_4): 3.5 sodium di-hydrogen orthophosphate (NaH_2PO_4) for one hour at 4°C in the dark.

Alc, 70–80% industrial methylated spirit (IMS 'museum spirit').
C, Copenhagen mixture.
CAA, Chromo-acetic-alcohol.
CuAc, Acetic cupric acetate.
F, 10% formalin (4% formaldehyde).
[F], 2–3% formalin.
FAA, Formol-acetic-alcohol.
FSal, Formol saline.
FSub, Formol sublimate.
G, Glycerol (mixed 50:50 with Alc).
HAc, Glacial acetic acid.
Kew, Kew mixture.
PG, Propylene glycol.
S, 'Museum spirit' (80% methylated ethanol).
Sch, Schaudinn's fixative.
†, Suitable as a cytological fixative/preservative.
*, Special fluids can be compounded for colour preservation (see preservatives of plant colour, p. 131).

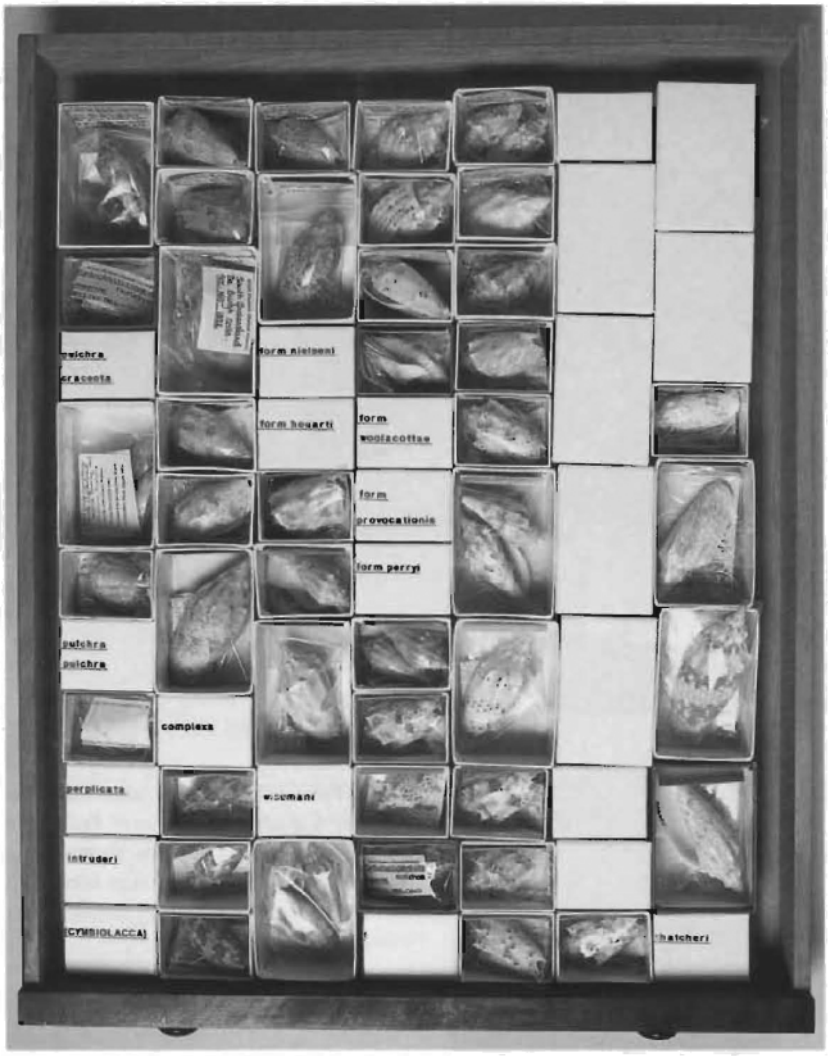

Plate 8 A shell collection with individual specimens enclosed in polythene bags and stored in unit trays (The Natural History Museum).

Plate 10 Cork stoppers immersed in fluid showing the first signs of deterioration. Over time they will either crumble when removed or pop out, releasing specimens into the fluid. The cork will also become increasingly acidic over time and may contaminate the preservative (The Natural History Museum).

Plate 9 Rubber stoppers that have perished with age to the point where it is almost impossible to retrieve specimens within the vial without damaging them (The Natural History Museum).

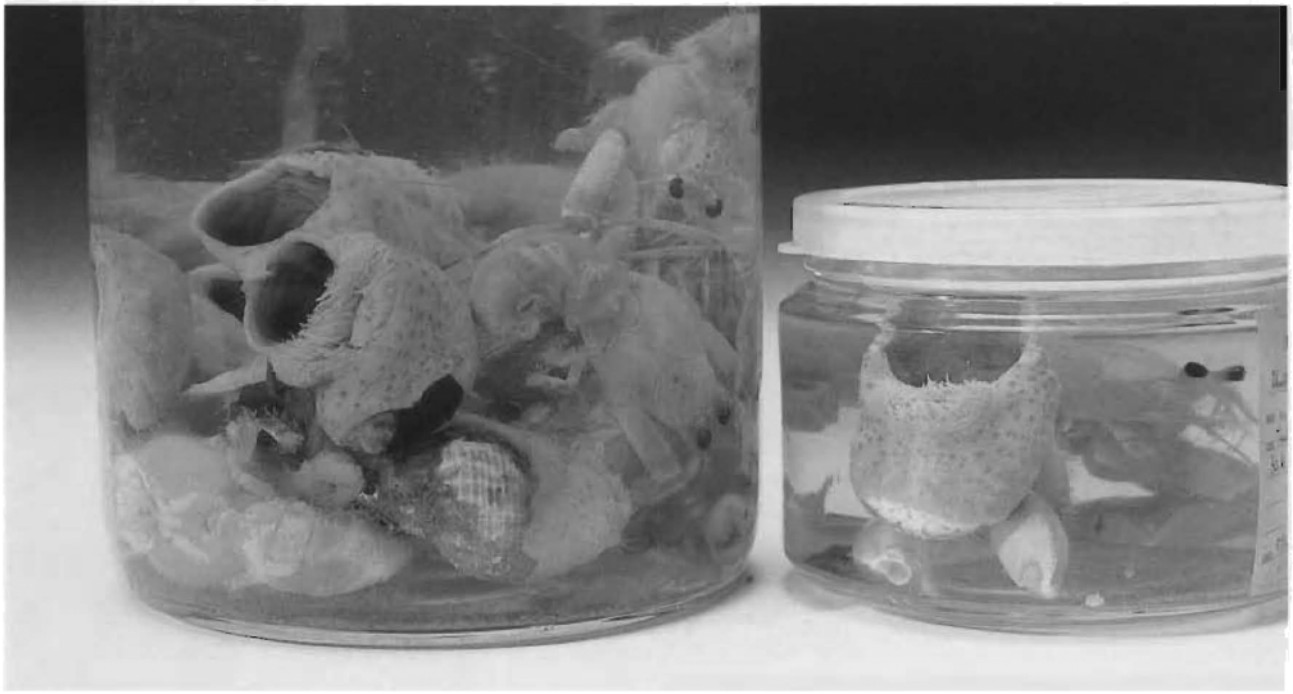

Plate 11 Cloak anemones (*Adamsia*) preserved by the Kaiserling colour-preserving technique and still showing their fugitive magenta spots seven years after fixation (Simon Moore).

Plate 12 A selection of John Hunter specimens on display at the Hunterian Museum. In the background pathological specimens are displayed. (Hunterian Museum, Royal College of Surgeons, London).

Plate 13 *Left:* Cubomedusan jellyfish mounted with an acetate disc inside the bell and attached to the roof of the jar using a wooden bar.
Right: two lion's mane jellyfish, one collapsed at the bottom of the jar, the other attached with an acetate disc under the bell. An acetate disk is also shown outside the jar (Simon Moore).

Plate 14 Spirit collection jars stored in a metal sliding-drawer system (The Natural History Museum).

Plate 15 The LeParfait jar is the preferred choice of jar in some museums. The jar is available in a number of sizes and there is a good seal by way of a rubber gasket. The glass is sturdy, the lid cannot be separated from the jar and the sides are straight which allows for easy storage and retrieval of small glass vials (The Natural History Museum).

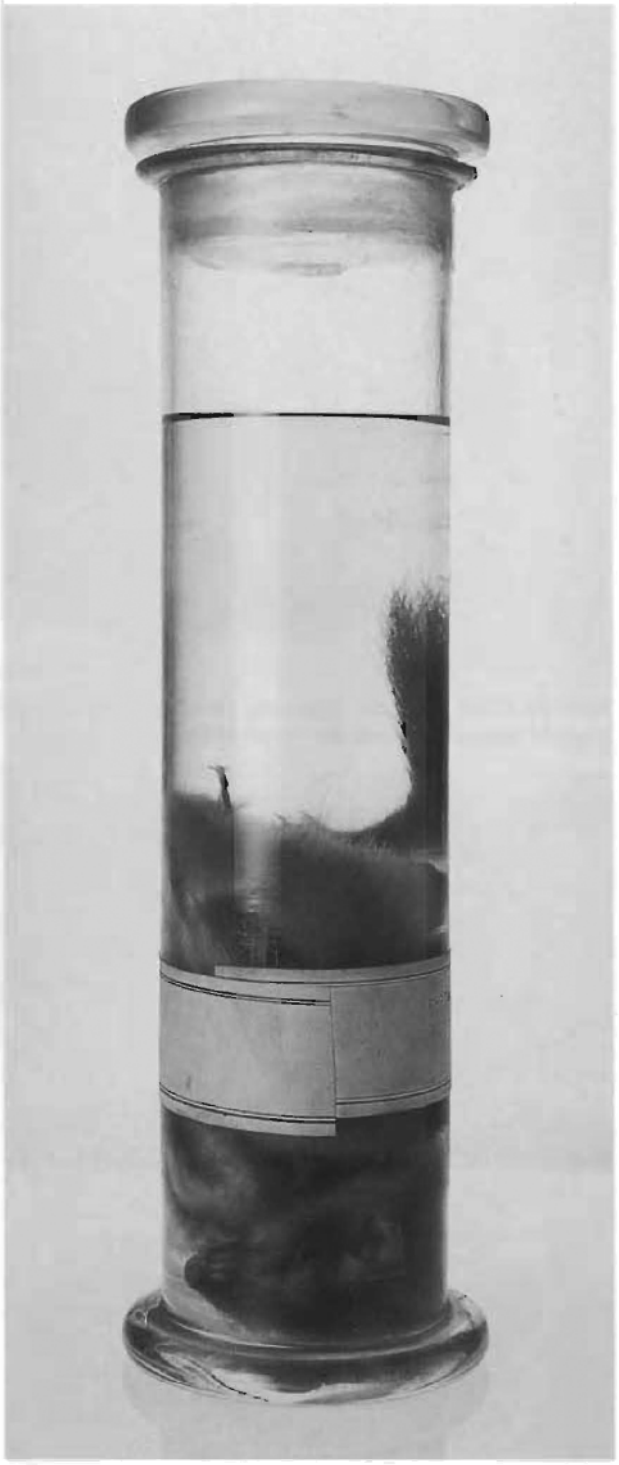

Plate 16 Preserved stoat that has collapsed into the lower part of the jar as a result of the acidity of the preservative brought about by oxidation of the lipids, which has decalcified the skeleton. The jar has been refilled with alcohol to replace lipid-contaminated preservative but some discolouration is still noticeable (The Natural History Museum).

Plate 17 The glass jars stacked on this old museum trolley are more likely to be knocked over and broken (Annette Walker).

Plate 18 An old trolley which has been modified to transport tall glass jars safely. The specimens are cephalopods from the *Discovery* expedition (The Natural History Museum).

Plate 19 Large glass jars have to be stored with care. The jars illustrated are stored in an isolated room where they are less likely to be damaged or broken (The Natural History Museum).

occur (see Maintenance and Remedial conservation *et seq.*, p. 108) and the same remedies can be applied. Formalin is widely used as a gross fixative and preservative for plants although formol-acetic-alcohol gives better general results; Copenhagen and Kew mixtures (see Formulae at the end of the chapter) are even better for non-microscopic plants and fungi. Although recognized as a separate kingdom, fungi have been included here for convenience.

Specimen handling

Most fluid-preserved specimens are fragile and must be handled with extreme care. The containers themselves must be handled with caution and where they must be transported any distance, specially designed trolleys with pneumatic tyres should be used. Old glass jars can be extremely fragile and should be handled with safety gauntlets in case of sudden breakage (Clark *et al.*, 1994) (see also Fig. 5.26).

Moving fluid-preserved specimens is often straightforward but moving one metre high or taller jars and other bulky specimens requires specialized equipment that has to comply with health and safety regulations. Clark *et al.* (1994) have designed a trolley that not only protects the jars from bumps and uneven floors and prevents jostling between the jars but also protects the curator by minimizing the possibility of breakage and accidental spillage. The trolley itself was made of oak so that it would not absorb any spilled fluids, caulked with Sirosil-25 and fitted with a double tubular rubber sealing strip at the loading end. It was fitted with six solid tyre wheels, the corner wheels being on casters. A wooded lattice was built over the trolley and attached with wing-nuts for easy removal if required (Plate 18). The wooded horizontals were fitted with elasticated straps to prevent taller jars from toppling.

Smaller but tall, unstable jars can be transported in bottle crates on standard trolleys. For museums with many tall or broad jars, or large collections, the additional luxury of pneumatic tyres may seem expensive but it is recommended to ensure a smooth and safe journey.

Fluid-mounted (non-alcohol) specimens that are to be used for teaching and demonstration purposes should be securely mounted in perspex containers (often called 'visijars'). Embedding delicate specimens in clear resin is useful for teaching material, provided that many specimens are available since there is a slight failure rate despite advances in the technique and the formulation of the resin itself (Voss, *c.* 1970). Some resin blocks also deteriorate after about twenty years (Plate 22), others chip or crack if dropped and all can become scratched through frequent handling or misted if stored near solvents. Scratches and surface misting can be removed by grinding on wet and dry grit paper (grade 800) and then polishing with a high speed mop.

Specimen labelling and documentation

Labels inside jars

Labels inside jars must be made from paper that will withstand long-term immersion in fluid preservative without softening or discolouring (e.g. Resistall, Goatskin Parchment or other material such as Tyvek). Traditionally, information is written in black 'Indian ink' although alcohol-proof inks have recently been developed for use with computer printers. Full details of paper and inks can be found in Appendix II on papers, ink and label conservation.

Ordinary writing paper and pencil can be used as a temporary substitute in the field but such paper may soon soften and dissociate, particularly in formalin, but also in phenoxetol-based preservatives and in alcohol.

Attachment of display labels to fluid-preserved dissections

Spirit-preserved specimen labels can be attached using the celloidin technique (see pp. 111 and 117). Label pointers (to show specific areas) can be cut from Goatskin Parchment or Resistall paper and attached at either end using the same technique. The gelatin technique can be similarly used for attaching labels and pointers to specimens in formalin or aqueous-based preservatives.

Treatment of old labels

Many older jars have labels glued to the outside. Ultraviolet light gradually discolours them over the years and fades the ink. Such labels are frequently found in a fragmentary condition and can be treated as follows:

1. Before carrying out any treatment record all available data from the label, using an infrared lamp if the ink has faded to illegibility.
2. Remove the specimen and stand the jar (upside down if the label is at the top) in warm water for about five to ten minutes.
3. Remove fragments of label as they become detached and place them (together) between two discs of filter paper with a weight on top.
4. Once the entire label has been removed it should be dried in filter paper with a weight on top to keep it flat. Allow it to air-dry for several days.
5. The old label can be removed to an archival file where it should be mounted on acid-free board and a new label placed on the jar with a cross-reference to the old label in the file.
6. Repairs to old labels can be made using Japanese Kozo tissue.

Kishinami (1989) has developed an archival technique for storing old labels from fluid-preserved specimens and which is now standard practice at the Bernice P. Bishop Museum. This involves using Mylar negative storage sleeves in acid-free folders.

Maintenance and remedial conservation

There are many problems in maintaining a fluid-preserved specimen in good order: lipid leaching leading to alteration of pH and bacterial decay, fungal attack leading to decay and/or collapse of specimen, drying out and bleaching due to ultraviolet light. However there is usually something that can be done to restore a deteriorated specimen.

Lipid leaching

Vertebrate specimens, especially cetaceans, contain large areas of adipose tissue. Whereas formalin preserves lipid, it does not fix it, which leads to lipid leaching from specimens and contaminating the preserving fluid with glycerides and non-linked fatty acids. If neglected, the glycerides in the contaminated preservative oxidize and release further fatty acids causing a lowering of pH which in turn leads to gradual decay and dissociation of the preserved specimen. Similar problems can occur to specimens preserved in alcohol, causing it to become dirty brown in colour (see also Dingerkus, 1982). Specimens known to contain adipose tissue should be checked at least twice a year, especially if freshly preserved. Contaminated preservative can easily be renewed and fresh alcohol will solvent-clean specimens clogged with fat globules (Plate 16). Specimens that have begun to dissociate will need further treatment (see pp. 111 *et seq*).

If slight contamination has occurred, the pH of the fluid must be checked with a pH meter or with a strip of pH indicator paper (pH should not fall below 5.0). This check should also be carried out if the preservative has been renewed. Alcohol and phenoxetol-based preservatives should be checked regularly for lipid content, indicated by discolouring or clouding of the liquid or, in high concentrations, the appoearance of visible fat globules.

Fungal attack

Specimen jars containing either formalin or alcohol-preserved specimens are sometimes found to be contaminated with a suspension of growing hyphae of fungus (*Penicillium* spp.). This fungus has been found to grow in alcohol, even formalin, especially if specimen jars have become at all contaminated or diluted by lazy curators topping them up with water! If the specimen is unaffected it should be removed and the jar cleaned with 5% potassium permanganate or copper sulphate solution. A small amount of phenol, menthol or thymol added to a specimen jar acts as a useful fungus preventor (about 0.01 g per litre) but a well-sealed jar is the most effective method of preventing fungal growth. If the specimen is enmeshed in fungal hyphae it should be dissected out. Organic fungicides such as thymol and menthol have been found to be slow in counteracting fungal infection

and, as mentioned above, are best used as preventatives. A stronger fungicide such as *ortho*-phenol, is advised but caution is required when handling, as such compounds may blister skin. If the specimen is decaying due to fungal contamination it must be re-fixed.

Glycerine-preserved specimens, such as alizarin transparencies, are particularly prone to fungal attack and it is vital to ensure that jars are well sealed to prevent ingress of fungal spores. While a few crystals of menthol or thymol will provide additional protection, phenol is not advisable since it gradually removes the alizarin dye.

Rehydration (Figs. 5.12–5.14)

Careful consideration should be given as to whether dried-out specimens are worth rehydration. In some cases they may be equally well kept as dry specimens. Rehydration is brought about by gradual re-expansion of cytoplasm and cell membranes with a water-based soapy fluid. Solutions of middling to fairly alkaline pH are ideal and their soapy property slowly permeates and rehydrates membranes and cell contents in the manner of fat liquors (Remsynol, Eskatan FSN)

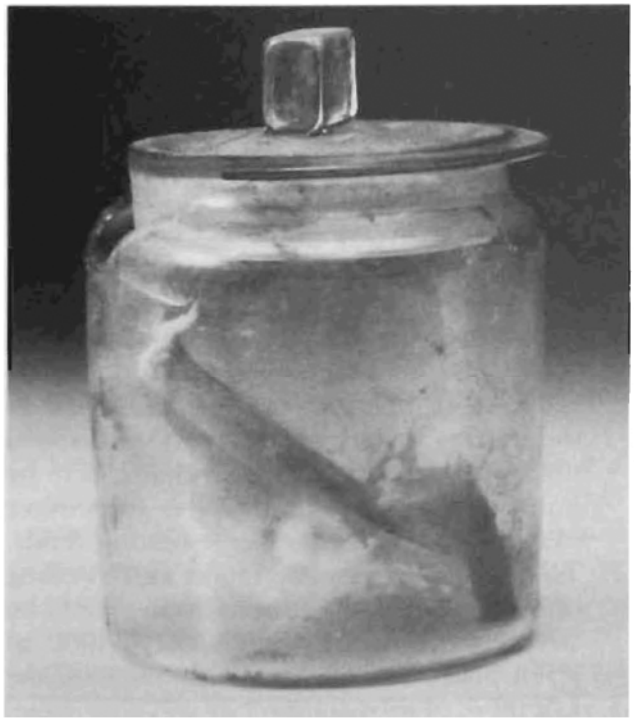

Figure 5.12 Dried-out noctule bat. Note cracked lid.

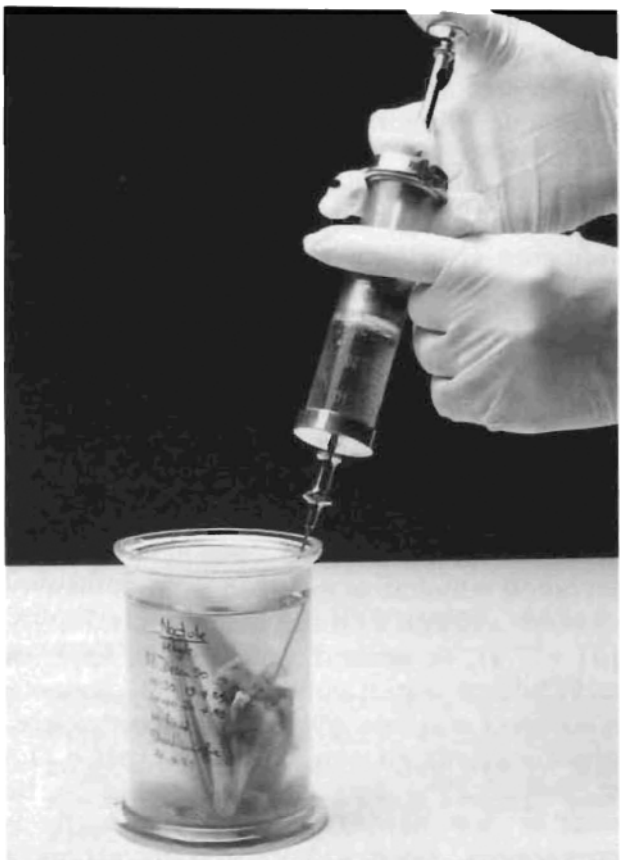

Figure 5.13 Restored specimen following rehydration in 5% Decon-90 injected with Steedman's fixative.

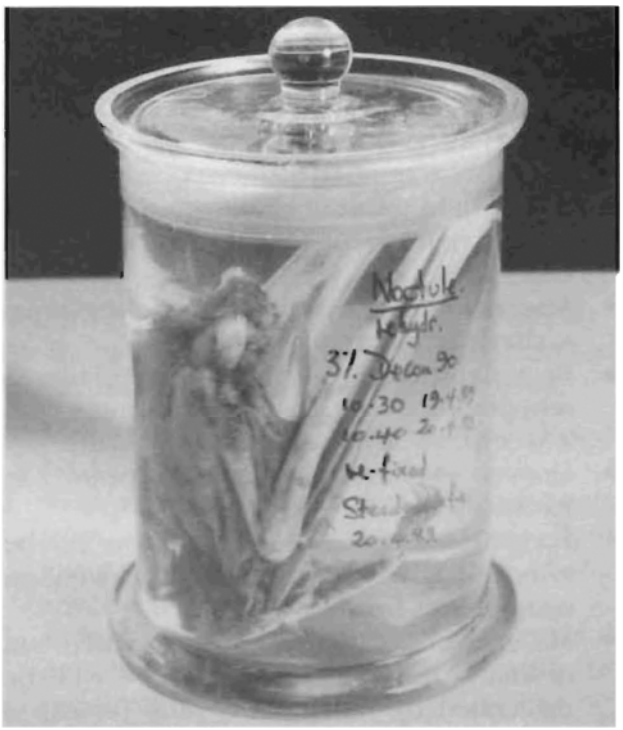

Figure 5.14 Fully reconstituted noctule bat in Steedman's fixative.

which are used to revive desiccating skins. Since van Cleave and Ross (1947) published the trisodium orthophosphate rehydration technique, which has subsequently been a standby for all curators of fluid-preserved specimens, even better results have been obtained using other rehydrating fluids. Jeppesen (1988) reviewed the types of fluid used in rehydrating dried-out invertebrates. For crustaceans and nereid polychaetes he particularly favoured the use of a 3% solution in de-ionized water of the surfactant Decon 90 (pH = 7.5–8.0, pH of concentrate = 12–13). This has been tried by the author on a wide range of animal specimens and has been found to give even better results than the sodium orthophosphate technique. Other favoured rehydrators included a 2% solution of di-octyl sodium sulfosuccinate ($C_{20}H_{37}NaO\text{-}S$, pH = 7.4), de-ionized water on its own, or artificial sea water alone, and a 5% aqueous solution of ethylene glycol. Sodium orthophosphate is highly alkaline in solution (pH = 11.4 at only 0.25%) and was not found to be suitable for hard-bodied animals such as crustaceans, since it appeared to act as a chelating agent, softening the exoskeleton.

Having decided which rehydrator to use, the following points should be considered, since this process can give rise to problems.

- Loss of colour – the result of exposure to air, and further colour loss can occur during the process.
- Air bubbles trapped inside specimen – treat with a mild vacuum or diffuse the trapped air by briefly immersing specimen in 100% IMS.
- Maceration/dissociation of fragile specimen – check regularly during process.
- Bacterial attack – possible if specimen is rehydrating over a long period (longer than a week), so it must be checked daily.
- Uneven swelling of tissues (see point 2 in list below).
- Formation of insoluble salts – these can be removed in a water-bath after rehydration using a soft brush.
- Maceration of labels – if written on ordinary non-rag paper, labels will be macerated by the process (any deposited salts should be brushed away using a soft brush with de-ionized water).

Rehydrating solutions should be used at different concentrations depending on the density of the tissue. The following recommendations apply to most rehydrating solutions.

- Membranous tissues: 0.5–1% solution.
- Dense muscle: Up to 5% solution.
- Combination of muscle and membrane (e.g. bat): – 1% solution, will require warming.
- Size/weight of specimen: Experience required; do not exceed 5% strength of solution and monitor frequently. Do not leave fragile specimens rehydrating overnight, instead leave in fixative; rehydration can be continued next day.
- Presence of outer skin: 2% solution – injection may be necessary after rehydration of skin. Wash specimen thoroughly afterwards to remove rehydrating fluid from body cavity.
- Exoskeleton: Better to leave dry? If the exoskeleton is fragile it would be better to rehydrate it since surrounding fluid will give it both internal and external support.
- Small, fragile or invertebrate specimens will not require warming.

If specimens are worth saving and rehydrating, the following process is recommended:

1. Place specimen in a container capable of being warmed to 40°C without loss of fluid vapour and cover completely with rehydrating fluid. If the specimen floats and needs weighing down, use lead-shot in sealed polythene bags. The concentration of rehydrating fluids should be gauged with respect to the nature of the specimen (see above). If the specimen has adhered to the side of its jar it will have to be rehydrated *in situ*. Labels should not be rehydrated, although if they are stuck to the inside of the jar they can be processed until they detach. They should then be immersed in de-ionized water to remove salts absorbed from the rehydrating fluid.
2. To avoid uneven penetration and swelling of tissue the rehydrating solution should be covered and warmed to about 30–40°C on a hot-plate. This will not only increase the evenness of rehydration but will also speed up the process. It is unwise to leave a

rehydrating specimen on a hot-plate overnight as the specimen may have started to macerate by the next morning. Alternatively, the specimen may be put under mild vacuum at this stage which will also help to remove trapped air bubbles.

3. Agitate the specimen from time to time to release gas bubbles. If the specimen shows signs of falling apart before rehydration has been completed then the specimen must be refixed before rehydration is continued.
4. Once the specimen appears to have been rehydrated, remove it from the fluid and immerse it in de-ionized water. Check that all external features are as pliable as they should have been before the specimen dried out and that the overall appearance of the specimen is satisfactory. If the rehydration process does not appear to be progressing (i.e. the specimen is not fully rehydrated) the rehydrating solution can be increased by up to 50% of its original concentration, but not above 5%.
5. If the specimen has been too long in the rehydrating fluid, carefully pour or siphon away the fluid and replace it with a formalin-based fixative. Do not use alcohol above 20% strength as the sudden dehydration may distort the specimen.
6. Bathe specimen in de-ionized water to remove surplus rehydrating fluid. The presence of residual dehydrating fluid is indicated by a clouding of the water. Also check pH against a control of de-ionized water (the latter is sometimes slightly acid).
7. Deposition of crystals on the specimen may occur if using a salt rehydrator such as sodium orthophosphate. These crystals may be removed by soft brushing in a bath of de-ionized water. Be careful not to brush too hard as the specimen will be fragile at this stage. Delicate specimens should be fixed before removal of any salt deposit.
8. The specimen can now be refixed using a suitable gross anatomical fixative. This stage is only necessary if the rehydrated specimen is unusually fragile or to be stored in an aqueous-based preservative such as phenoxetol. For alcohol-preserved material it can be transferred, following washing in de-ionized water, directly to a graded alcohol series starting at 10%.
9. The rehydration process should be recorded on a separate label inside the jar and should include such details as: accession number or scientific name of specimen; date when found dry; rehydrating fluid used; date of processing; fixative used; preservative used (if relevant); name of curator/conservator.

Repairing damaged fluid-preserved specimens, including dissections

Frequent handling of specimens increases the risk of damage and this becomes noticeable as labels and fragments of the specimens gather on the bottom of the container.

Celloidin

Celloidin is effective for repairing alcohol-preserved specimens.

1. Remove specimen and detached pieces from container and drain excess alcohol away, but do not allow any part of the specimen to dry out – keep a pipette and some alcohol nearby.
2. Position detached fragments or labels as required and apply ether-alcohol mixture to each in turn.
3. Carefully apply some 1% celloidin to the damaged areas, enough to effect the repair. Allow to gel but not dry out and be careful not to breathe on this area or it will form a white insoluble colloid.
4. When the celloidin has gelled, pipette some alcohol over it and check that the bond is firm.
5. Return specimen to jar.

Gelatin

For formalin or other aqueous-based preserved specimens hydrated gelatin must be used.

1. Melt some gelatin in a hot water-jacketed beaker (60–70°C).
2. Remove specimen and detached fragments from container and drain excess fluid away, but do not allow any part of the specimen to dry out.
3. Rinse the specimen in water for several minutes to remove surface formalin or preservative.
4. Reposition loose fragments or labels and, using a camel-hair brush, apply molten

gelatin to affected areas. Leave to gel but not to dry out.
5. Check that the bond is firm and return specimen to jar.

Fragile specimens

The celloidin technique for repairing and attaching damaged alcohol-preserved specimens to glass (refer to end of chapter for details of reagents) can be used for fragile specimens.

1. Arrange specimen on a glass plate and drain off excess alcohol.
2. Moisten damaged area (or edge of specimen) with ether-alcohol mixture.
3. Drip 1% celloidin onto damaged area and leave to gel. Do not allow to dry out – moisten with ether-alcohol if necessary. Avoid breathing on celloidin as condensation will react with it forming an opaque colloidal film.
4. When celloidin surface has gelled (after about one to three minutes it takes on a crinkled appearance), slowly immerse plate and specimen into alcohol. Leave for ten minutes for celloidin to harden. The celloidin should be completely transparent; if not, redissolve with solvent and allow to regel.
5. Attach any parts of specimen trailing in fluid.
6. Leave specimen in alcohol overnight (at least) to check that the bond is firm before sealing jar.

Super glues

These glues have been used with great success for joining tissues in surgical operations and can equally well be used for repairing specimens. However, this process is irreversible and therefore unacceptable from a conservation point of view, particularly since alternative techniques are available.

Dense tissue specimens

Glass-rod needles are used and their preparation should be carried out on a large ceramic tile or ceramic surface.

1. Heat the middle of a 3–5 mm diameter glass rod in a bunsen flame; when it starts to melt draw it out until it is about 0.5–1 mm in diameter. Allow it to cool on a ceramic surface and then cut into desired lengths ('needles') using a glass-saw, or nip off with pliers.
2. Insert glass needles through specimen where it needs support and carefully nip off any protruding ends with pliers.
3. Dendritic specimens such as hydroid colonies or soft corals can also be repaired by this method, or alternatively can be bound with non-ferrous wire (Fig. 5.15).

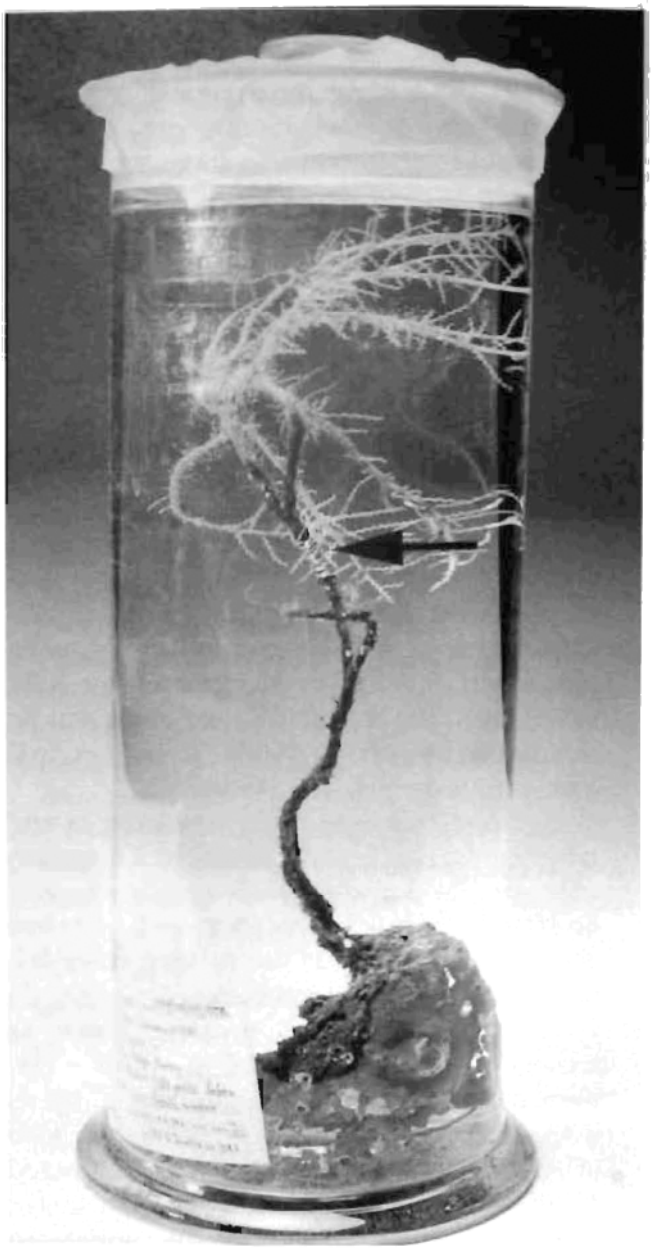

Figure 5.15 Large hydroid colony repaired using silver-plated wire.

Copper or ferrous-based (even stainless steel) metallic pins are to be avoided as they

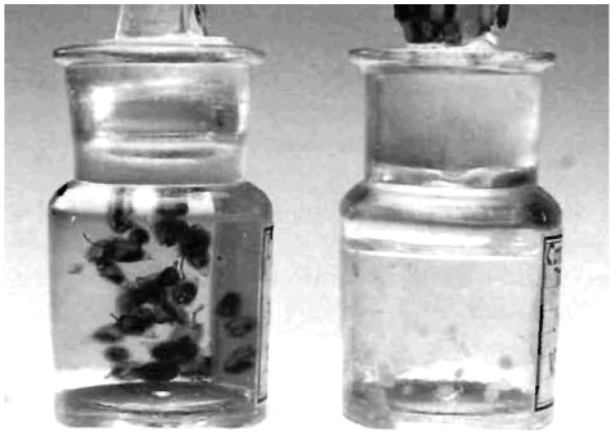

Figure 5.16 Hundred year old, fragile specimens of hydromedusae successfully transferred from osmic acid fixative to alcohol preservative (left jar) as opposed to formalin fixation followed by transference to alcohol (right jar).

will corrode and produce corrosion salts which can colour the preservative.

Transferring specimens from one preservative to another

Preservatives can sometimes be unsuitable for certain types of zoological specimens and must be tested by monitoring the specimen at regular intervals to see if any deterioration takes place. If necessary, specimens can generally be transferred to a more suitable preservative without any problem provided that adequate care is taken (Fig. 5.16).

Transferring formalin-fixed specimens to 1% phenoxetol is straightforward and only requires the specimens to be immersed in running water until no formalin can be detected (Waller and McAllister, 1986). Transferring a specimen from a phenoxetol-containing preservative to alcohol may require some refixation if the specimen is at all frail.

Aqueous-preserved specimen to alcohol

1. Immerse specimen in de-ionized water for about thirty minutes.
2. Transfer to 20% alcohol for at least one hour. For more densely muscled or dense tissue specimens (vertebrates), three hours is an average time for the alcohol to penetrate although this can be assisted by subcutaneous injection.
3. Continue the dehydration by steps of 10% strength of alcohol for frail specimens and by 20–25% for more robust specimens until the required grade is reached. When the alcohol concentration has been changed, check that no distortion, wrinkling of surface membranes (due to syneresis), or bending of the specimen has occurred, in which case some rehydration will be required, but only if simple down-grading of the alcohol is insufficient to restore the specimen to its normal state. Progress of equilibration can be monitored by using an alcoholometer or DMA-35 meter until consistent readings are obtained.
4. Once the specimen has been successfully transferred to 80% strength, it should be checked over a 24-hour period before storing.

NB If dehydration stages are short-cut, a small amount of heat is generated by the interaction of the changed fluids which releases air dissolved in the fluids as bubbles; these form in the tissues and can only be effectively removed by vacuum treatment.

Alcohol specimen to an aqueous preservative

- Hydration is carried out by taking frail specimens down by steps of 10% strength and more robust specimens by 20–25% steps.
- Curators that have taken specimens down the alcohol ladder too rapidly have reported some swelling of tissues in robust specimens. Fragile specimens can fall apart!
- The specimen will float and gradually sink down to the bottom of the container as the tissues become accommodated to the denser fluid.
- Leave the specimen for at least thirty minutes after it has sunk and then transfer it to the next grade.

Slide mounts

Older microscope slide collections often contain fluid mounts – mainly pieces of internal organs or small whole animals and plants usually preserved in saline inside a glass micro-case or ring mount. Moore (1979) demonstrated how damaged and dried-out

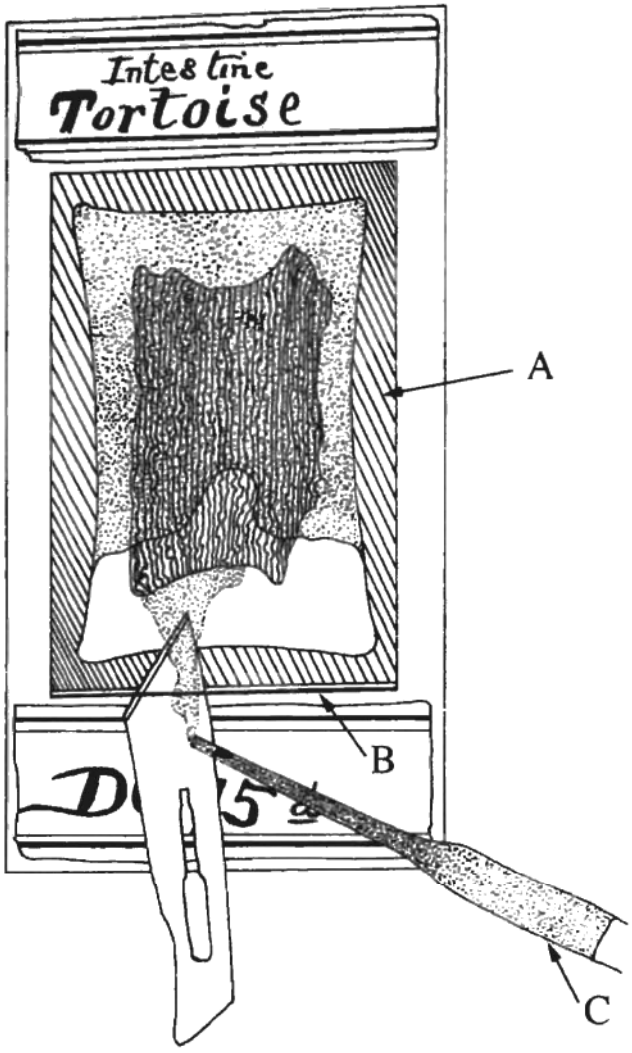

Figure 5.17 Filling a fluid mount using a no. 25 Swann–Morton scalpel blade. A; Ground glass cell surface coated with gold size; B, edge of coverslip; C, Steedman's preservative in pipette.

fluid mount specimens from the J. Quekett collection at the Royal College of Surgeons, London, were conserved. Damaged mounts were repaired with epoxy adhesive and then, using a scalpel blade and pipette, Steedman's preservative was flooded into the mount to refill it before resealing the mount with gold size. The edges were later coated with gold paint (Fig. 5.17).

Recording treatment (see also Chapter 9 on policies and procedures)

Any treatment of a specimen involving specific techniques from rehydration to topping up a jar should be recorded. A long label glued to the back of the jar can be marked with appropriate symbols as to the type of treatment, the date and the curator's initials (see below). Such activities should also be recorded in a ledger together with photographs of the treatment if required.

Storage jars must be topped up with the correct fluid. Details of preservatives should be recorded on the specimen label but with older collections such details have often been omitted. Having established the type of preserving fluid and refilled the jar, a spirit-pen mark can be recorded on a label glued to the back of the jar: A for alcohol (IMS), F for formalin (4% aqueous formaldehyde), FS for formol-saline (for marine collections), SF for Steedmans fixative, SP for Steedman's preservative, and so on. Likewise for mixtures: F + A for formalin and alcohol or F→A for formalin being superseded by alcohol. This method has the advantage of being non-permanent, if desired, and prevents the jars from becoming cluttered with too many internal (treatment) labels. This also gives a record of which jars have been topped up. Care should be taken not to splash alcohol on to the marks recorded on the outside of the jars!

Topping up

On the second occasion of topping up, previously treated jars should have their gaskets or lids (for plastic-lidded jars) renewed. Ground-glass jars should be smeared with Paraffin Soft White (BDH) around the lid and the lid rotated when put back into position to ensure an even and thorough seal. Be sure to check the strength of the preservative as evaporation of alcohol can cause its dilution (Carter, 1995).

Specimens in glass display jars

Both anatomical and natural history specimens have been mounted in fluid preservative and sealed in glass jars since the mid–seventeenth century. Illustrations in Levin Vincent's *Elenchus Tabularum* of 1719 show a range of natural history specimens in cylindrical jars sealed with pigs' bladders (Fig. 5.18). Ruysch's *Thesaurus Animalium* of 1710 also shows that sealed jars could be decorated with dry speci-

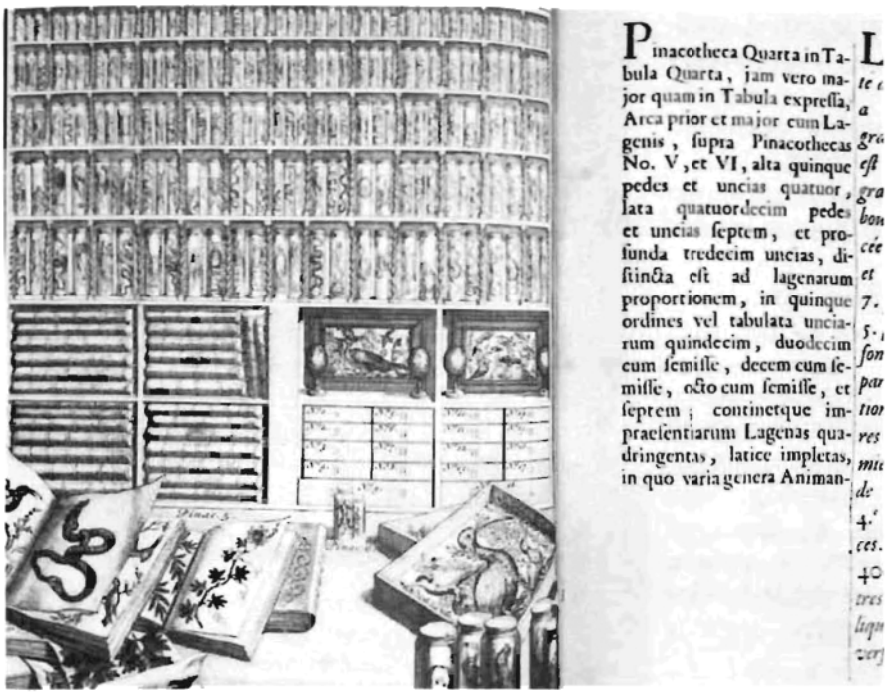

Figure 5.18 Early eighteenth century natural history study cabinet illustrating birds and reptiles in fluid preservative, the jars sealed with pigs' bladders. From Levin Vincent's *Elenchus Tabularum*, 1719.

mens mounted on the lid (Fig. 5.19). By the nineteenth century museum jars were being sealed with bituminous compounds such as bitumen or Stockholm tar and lead sesquioxide (see pp. 117 and 120), and in the early twentieth century the gutta percha technique was being used. Another sealant found to be successful in the nineteenth century was gelatin and, with some modification, this is still effective. Many museums still have specimens in jars sealed by all of these methods. These techniques relate to alcohol-preserved specimens in glass jars. Formalin-preserved material is referred to below.

For the successful mounting of biological specimens in glass display (battery) jars the following items will be required:

- Celloidin pyroxylin or gelatin sealant (see Formulae at the end of the chapter and the section below).
- Glass drill bits, which can either be purchased (diamond dust burrs are ideal) or home-made from an old triangular cross-section file (3–5 mm sides) cut up into two inch lengths and the ends ground to tetrahedral points on a grinding wheel. These are tempered in a flame until glowing dark red (correct temperature for tempering) and then quenched by plunging into cold water. When blunt the bits

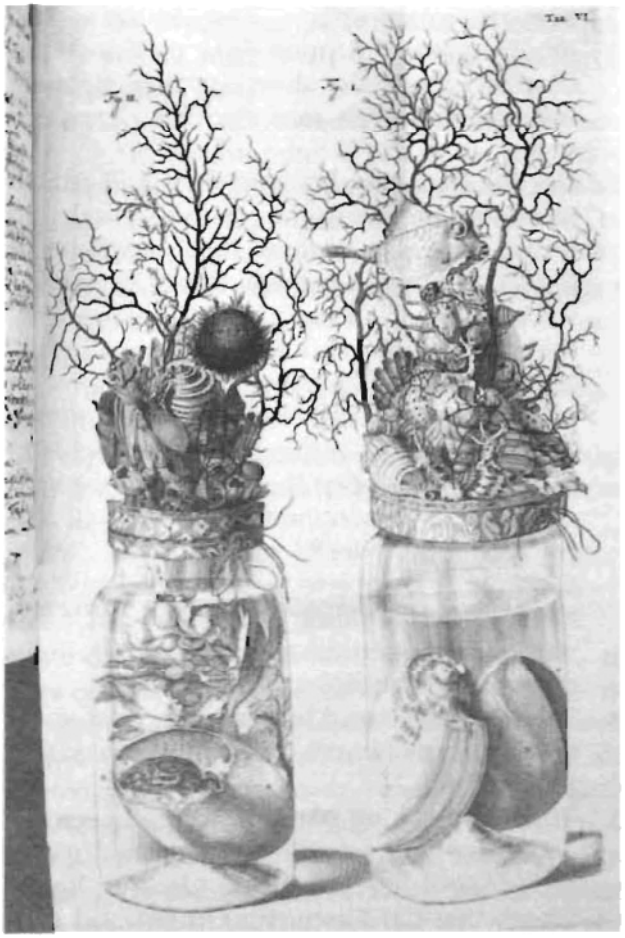

Figure 5.19 Marine specimens in fluid preservative with the jar lids decorated with Florentine paper and a montage of dried specimens (Ruysch, 1710).

sealant – see p. 18) is used for mounting small specimens instead of celloidin and is gelled by contact with formalin.

Soft-bodied, pelagic specimens, such as jellyfish

These can be supported by a small, centrally pierced circle of clear acetate sheet placed centrally under the bell and inside the stomach. A piece of monofilament knotted at the lower end is then passed upwards through the medusa and acetate disc using a needle (Plate 13 and Fig. 5.21). The specimen is then carefully transferred to its preservative and inverted to remove any air pockets. Air trapped inside the canal system can be removed by carefully pricking the affected areas with a needle. Vacuum treatment can exacerbate the problem.

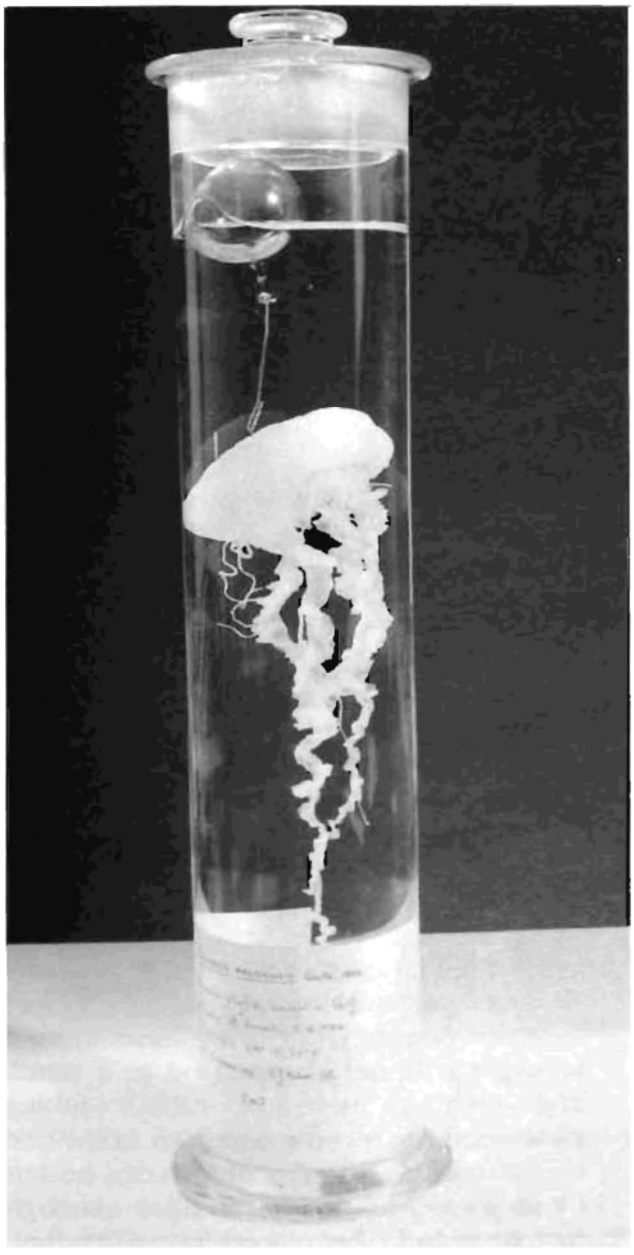

Figure 5.21 Jellyfish similarly mounted to the specimens in Plate 13 and suspended by a glass float.

Celloidin technique (refer to Formulae at the end of the chapter for details of reagents)

The following method is recommended for attaching alcohol-preserved specimens to glass:

1. Arrange specimen on a glass plate and drain off excess alcohol.
2. Moisten attachment points (or edges of specimen) with ether-alcohol mixture.
3. Drip 1% celloidin onto attachment points and leave to gel. Do not allow to dry out – moisten with ether-alcohol if necessary; avoid breathing on celloidin as condensation will react with it forming an opaque colloidal film.
4. When celloidin surface has gelled (after about one minute it takes on a crinkled appearance), slowly immerse the plate and specimen into alcohol. Leave for ten minutes for the celloidin to harden. The celloidin should be completely transparent; if not, redissolve with solvent and allow to regel.
5. Attach any parts of the specimen trailing in fluid.
6. Leave the specimen in alcohol overnight (at least) to check the bond is firm before sealing the jar.

Sealing glass display jars

The following processes should lead to the successful sealing of glass lids to display jars (battery jars): the gelatin method has been tried and tested and a well-sealed jar can remain sealed for over twenty years. Other sealants such as bitumen and Stockholm tar with lead sesquioxide are both hazardous and messy; paraffin wax is ideal for short-term sealing and the lids are easier to remove than those sealed with gelatin. Alternatively Dow Corning silicones (available from Merck) provide a good seal for jars although these

products have only been tested for up to fifteen years and not over the longer term.

Gelatin sealing technique

This is a tried and tested method of unknown origin but the recipe for the sealant was discovered in an index museum day-book dating from the mid–nineteenth century at The Natural History Museum, London. The technique for its use has since been improved by Moore (1980).

Preparation of sealant

The sealant is made up from gelatin coignets – wafer-thin sheets that are used in preparing food. Commercial powdered and fibrous gelatin, although cheaper, are unsuitable as they do not melt below 100°C following hydration.

1. Weigh out 24 g of gelatin sheet and leave to hydrate in tap water. The coignets should be adequately hydrated in twenty minutes; thicker sheet gelatin will take longer (overnight).
2. Melt hydrated gelatin in a water-bath (gelatin will char if brought into direct contact with heat). If the molten gelatin is as mobile as water, it will have absorbed too much water during hydration – leave it for a further twenty to forty minutes (preferably using a magnetic stirrer) to evaporate the surplus water.
3. Using fume removal facilities, take the gelatin from the heat and stir in 6 ml of glycerol and 3 ml of glacial acetic acid.
4. Pour the mixture into a metal tray and leave to set (several hours).
5. Cut it into squares and store in an air-tight jar with a crystal of menthol as a mould inhibitor.

Sealing technique

1. With the specimen in the jar, fill the jar about three-quarters full with alcohol preservative.
2. Ensure that the lid has a filler hole drilled in one corner not too near the edge, and that areas in contact with sealant have been ground to give some key – the top edge of the jar and lower edge of the lid. These should be clean, grease-free and dry. Dried gelatin can be scraped off with a scalpel or rehydrated for easier removal.

2a. Lid grinding is essential, otherwise the sealant has no key for attachment. Pour about 5 ml of water and glycerine on to a sheet of scrap plate-glass and sprinkle on some medium grit carborundum powder. Grind away any sharp edges to the lid and then press it on to the grit and glycerine mixture and move the lid around on this mixture in a circular motion until the glass surface has been sufficiently ground. Wash and dry the lid.

3. Melt some gelatin sealant in a hot water-jacketed beaker and heat the lid in a tray of hot water to about 80°C.
4. Brush sealant fairly generously on to the outer edges of the jar. Press down the pre-heated lid (drained) on to the applied gelatin seal with the filler hole at the front. Heat from the lid should remelt the gelatin if it has cooled and gelled; brush in further gelatin if any gaps appear.
5. Apply weights to the lid and leave overnight.
6. Inspect for white streamers of excess gelatin which may have run down into the alcohol. If present, the jar will have to be cleaned and then resealed using less sealant.

6a. To removed, invert the jar into a shallow, hot water-bath and leave until the sealant has hydrated and swelled. Take care that the lid does not fall off!

7. If appearance is satisfactory, leave for a further 24 hours until sealant takes on a frosted appearance. If the sealant is opaque white, then some alcohol has affected it (the jar may be too full) and the jar should be resealed.
8. If the jar seal looks good, top up the level of alcohol in the jar to 3–5 mm below the lid, using a syringe, through the filling hole. If the jar is filled to the brim, the seal will be put under pressure in warmer weather. (Larger jars can be filled using a separating funnel clamped on to a stand, and with a piece of rubber tubing connected to a needle wired on to the end of the tubing. This apparatus can be left unattended while the jar is filled although the jar should be placed in an alcohol-proof dish in case of accidental overflow – see Fig. 5.22.)

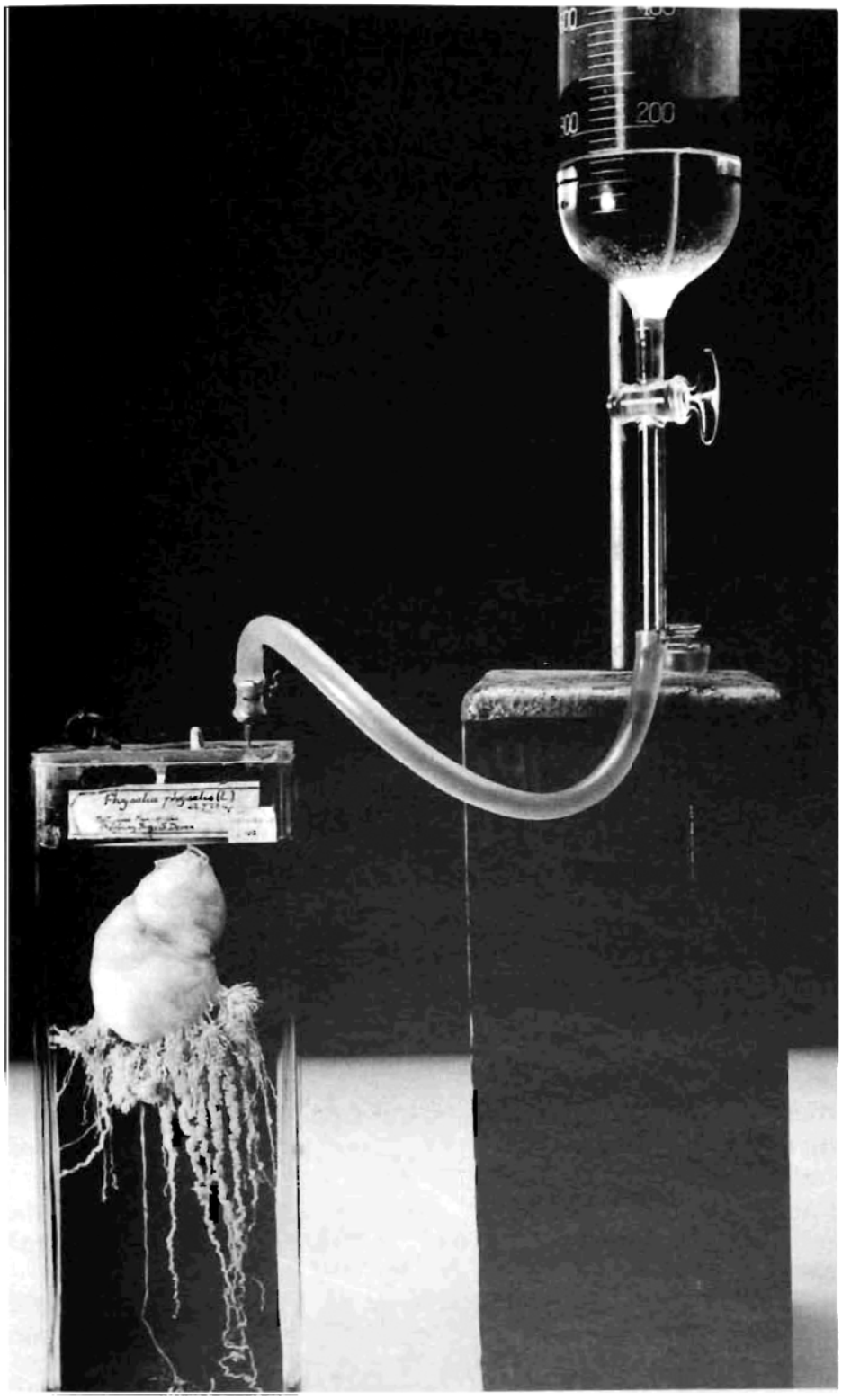

Figure 5.22 Separating funnel adapted for battery jar filling.

8a. If the jar becomes overfilled, remove surplus alcohol using a syringe.
9. Insert a piece of polypropylene rod into the filler hole and trim it flush with a sharp blade. Polypropylene rod. Its diameter can be reduced in diameter by rotating and stretching it over a hot flame. The rod itself will burn gently (blow it out) if overheated. The rod can be stretched when sufficiently hot – it becomes transparent and then opaque as it cools.
10. Seal the hole with a blob of molten gelatin.

Other sealants (for Formulae see the end of the chapter)

Gutta percha

This technique was first devised by Schorr in 1907 and has been improved over the years. Although it is considered to be old-fashioned, some curators still use it by tradition in preference to the even older gelatin technique.

1. Apply cement evenly to the edge of the jar using a spatula.
2. Warm the lid in hot water, dry it and apply it to the cemented edge of the jar.
3. Apply weights and leave for three days to a week for cement to harden.
4. Trim off excess cement using a pre-warmed spatula.

Stockholm tar and red lead

This technique can be used in conjunction with a pig's bladder. The sealant has a life of five to ten years, becoming increasingly brittle.

1. Pour Stockholm tar into a shallow dish and mix in lead sesquioxide (this is a health hazard and usage should be checked under local health and safety regulations) until the mixture forms a sticky paste, firm enough not to flow.
2. Apply cement evenly to the edge of the jar using a spatula.
3. Warm the lid in hot water, dry it and apply it to the cemented edge of the jar.
4. Apply weights and leave for three days to a week for cement to harden.
5. Trim off excess cement using a pre-warmed spatula.

Pig's bladder technique

The pig's bladder technique considerably extends the life of a jar seal and gives the jar a better finish. It is possibly the oldest and most traditional way of sealing a museum jar (Figs 5.18 and 5.23) but usually requires some experience before a successful result is achieved. Pigs' bladders can be obtained from slaughterhouses.

1. Cut the pig's bladder open and pin it out in a dissecting tray; immerse it with formalin-based fixative. Cover the container and place it into a fume cupboard.

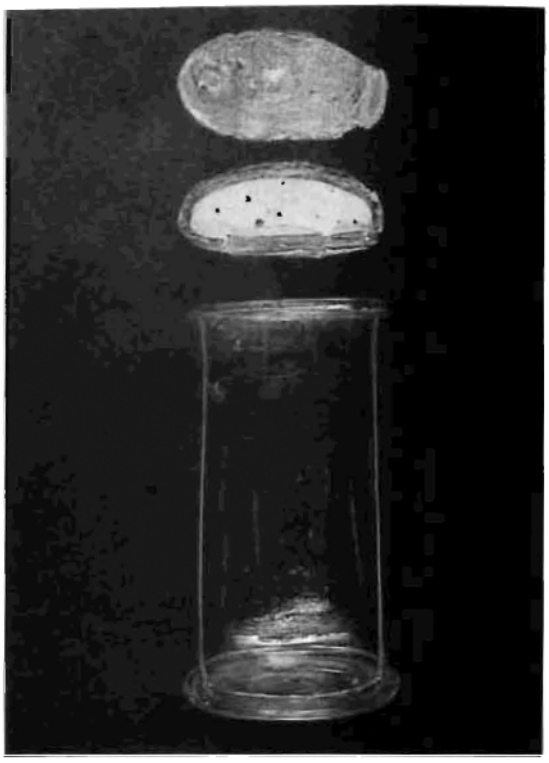

Figure 5.23 An early glass museum jar with the lid dismantled to show the pig's bladder seal and a lead foil luting cap. Note the holes in the bladder and lead, which have been bored as entry points for 'topping up'.

2. After fixation unpin and remove the bladder and wash it in running water until formalin is undetectable (Waller and McAllister, 1986).
3. Stretch the bladder over the top of the jar and attach the corners of the bladder to the side of the jar using adhesive tape. Experienced curators fold the corners in just below the jar rim to give a thicker bed for ligaturing.
4. Bind the bladder to the jar just below the rim using several turns of fine twine and tie it off.
5. Apply some varnish or button polish to the twine to prevent it from working loose. Allow the bladder to dry.

5a. If the resulting seal fails then remove the bladder and rehydrate it using Decon-90 or 2% sodium orthophosphate; go back to stage 1.

6. When completely dried, remove the tape and trim corners off the bladder just below the twine.
7. The bladder can be painted black using bitumen dissolved (20%) in toluene

(carried out in a fume cupboard), if desired. If it shows signs of becoming brittle some lanolin cream, gently applied, can slightly extend the life of the bladder and improve its appearance.

Silicone sealing

Dow Corning silicone is suitable for glass jars containing formalin, alcohol, phenoxetol-based preservatives and glycerol-acetate mixtures, although Horie (pers. commun.) states that formalin will eventually break down a silicone seal.

1. Check that areas to be sealed are free from grease, dirt and old sealant and that the jar is clean.
2. Areas to be sealed are primed with coupling agent (catalyst) and the jar is half-filled with preservative.
3. When the coupling agent has dried, apply a thin bead of sealant, about 2–4 mm, around the rim and press the lid into place. Leave to cure for 24 hours.
4. The jar can be filled using a syringe or filler to within 3–5 mm of the lid and the hole is sealed with polypropylene rod and a blob of gelatin.
5. Silicone-sealed jars can be reopened with a scalpel and resealed simply by applying a further layer of sealant.

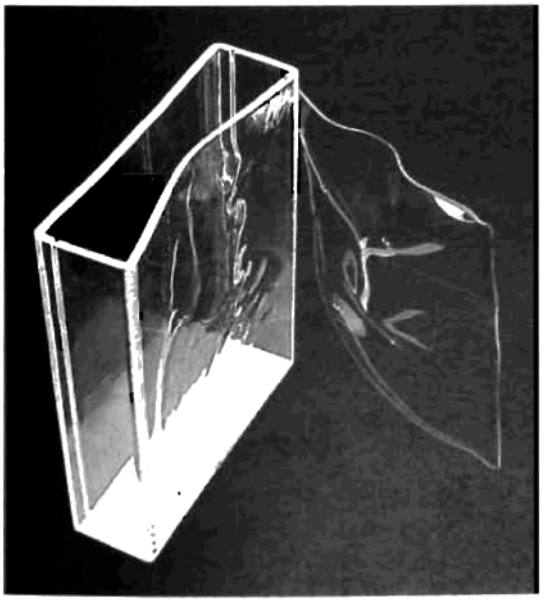

Figure 5.24 A perspex jar which has softened and distorted due to the mild solvent action of an alcohol preservative.

Formalin-preserved material

Material in formalin or some other aqueous-based preservative is best displayed in acrylic sheet perspex 'visijars'. These are easily made (Mahoney, 1973) or can be purchased through a supplier (Holloway Plastics) who also market a useful kit for enabling such jars to be topped up; this comprises a drill, tap and wrench, nylon screws and O–ring seals. Plastic jars should never contain spirit since alcohol preservatives have a mild solvent action on the perspex and within 24 hours can soften and distort a jar (Fig. 5.24).

Acrylic jar sealing

Acrylic jars must be sealed using either Tensol cement (see notes below) or a perspex cement. Perspex cement is a fast-setting glue made by dissolving perspex in a 50:50 mixture of chloroform (trichloroethylene is a slightly safer alternative) and glacial acetic acid; its mobility should be fairly fluid. Due to the toxic and unpleasant nature of the solvent, a metal-surfaced fume bench is advised. Some conservators prefer to use Tensol cement since it is slower to set and allows more error-adjustment time. Tensol cement is available from ICI in two forms: Reactive (more difficult to use but makes a proper perspex bond) and Solvent (makes a less effective seal but is easier to use). For the inexperienced a trial run, using scrap perspex, is essential! The following method requires the use of large clamps or heavy weights:

1. Check that the lid fits exactly on the jar and lies flat.
2. Drill a hole in a front corner of the lid, not too near the edge. If the jar has been freshly made do not proceed beyond this stage for three days – this will help to prevent concave bowing of the jar sides (see notes below).
3. Fill the jar and insert the specimen. Leave for several hours, then brush away any air bubbles that have formed.
4. Brush some perspex solvent (50:50 w/v mixture of glacial acetic acid and either chloroform or trichloroethylene) on to the surfaces to be sealed and then apply some glue to the edges of both the jar and lid. NB. Great care must be taken not to let glue or solvent run down the sides of the jar – the solvent will mark the jar after three seconds!

5. Apply the lid at once and press it down on to the glued surface. Since the glue is fast setting, speed is important; if the glue starts to cure before you are ready, keep it moist with solvent applied with a brush.
6. Apply weights or clamps as desired. NB Clamps should only be used in conjunction with rigid blocks of wood or the clamps may mark the perspex.
7. Leave overnight and, if satisfactory 24 hours later, top up and seal with polypropylene rod and a blob of perspex cement, or a nylon screw if using a commercial sealing kit (see Sealing technique above).

Preservative can still evaporate from a museum jar through the seal, particularly from perspex jars. Most sealed-in specimens will create a slight vacuum in the jar. A hiss is often heard when removing a filler plug from a jar. Although glass jars are able to withstand this vacuum, plastic jars will bow inwards – they have been known to implode in extreme conditions. The vacuum must be released, in this case, by drilling through the lid or plastic plug. Bari Logan (pers. commun.) suggests that lacquering the seal with pitch will prevent this evaporation and vacuum build-up. He also states that collections housed in perspex jars need regular maintenance. Although more expensive, 5 mm perspex is much less prone to bowing and is more robust for handling by students. The vacuum must still be periodically released, ideally every six months if kept in warm storage/display conditions, and the jar replugged. Van Dam (1997) discusses the possible physical interactions between preservatives and museum jars and their gas permeability, and how this leads to the accumulation of negative and positive pressures that cause bowing or cracking of containers.

Sealing screw-top and ground glass-lidded jars

Steigerwald and Laframboise (1994) have developed a tape for effectively halting evaporation from jars of alcohol-preserved material. The tape, made of polypropylene acrylic (PPA), is easy to use and is the most effective sealing tape to halt or at least slow down evaporation. It is manufactured by 3M (no. 5086 sealing tape), conforming to the specifications of Steigerwald and Laframboise. It contains an alcohol-resistant acrylic adhesive and comes in a one-inch-wide roll, 0.07 mm thick and 2.74 m per roll.

Storage
(see also Chapter 9 on policies and procedures and Chapter 7 on the collection environment)

An ideal storage area for biological fluid-preserved collections should be dark, well ventilated, uncarpeted in case of spills, and with an adequate drainage area to cope with accidents. Formalin and alcohol-containing jars should be colour-coded either with coloured circles or (better) with painted circles to quickly distinguish alcohol from formalin etc. and stored either in open top, easy-to-handle polypropylene boxes or in a subdivided, metal sliding drawer system (Plate 14). If specimen containers are stored on shelves, there should be a raised lip at the front to prevent jars from slipping off.

Store-rooms housing specimens preserved in fluids that are fire hazards must be appropriately equipped. Fire regulations, correct extinguishers (i.e. CO_2 and foam) and hazard notices, quick exits to avoid asphyxia and eyewash solutions in an appropriate place are all obligatory in areas of this kind. Larger facilities may have fully automated monitoring and alarm systems for levels of alcohol and other volatiles in the atmosphere. Horie (1994) and the Museums and Galleries Commission (1992) provide guidelines and standards for collections storage.

Containers

There are many types and sizes of museum jar available to curators these days. The problem of choice has been substantially diminished by research into the efficiency of these containers. Clark (1993) particularly and Suzumoto (1992) have carried out tests on jars and gaskets found in the collections of The Natural History Museum, London and the Bernice P. Bishop Museum in Hawaii and whose collections and containers cover a time-span of 150 years (Table 5.8). Plates 15, 34, 36 and Figs 5.25–5.30 illustrate the advantages and disadvantages of various jar designs used in natural history collections.

Table 5.8 Summary of storage jar designs (after Clark, 1992 and Suzumoto, 1992)

Jar	*Formalin*	*Alcohol*	*Expensive?*	*Problems*
Ground glass	✓	✓	✓	2
Kilner	✓	✓	=	5, 7
Pig's bladder-sealed	✓	✓	=	3
Battery	✓	✓	✓	3
Visijars (perspex)	✓	X	X	3, 4
LeParfait gasket	✓	✓	X	5, 8
Bakelite-lidded	✓	✓	X	1, 9
Danish	✓	✓	X	1•
Stoneware	✓	✓	=	2
Palmer	✓	✓	X	4A
Fibreglass bins	✓	✓	✓	5
Polyethylene bins	✓	✓	X	6

=, No longer available as standard.
1, Lids have shelf-life of less than twenty years. •Now improved but tested only ten years so far.
2, Lids sometimes difficult to remove.
3, Seal is broken, requires resealing process.
4, Perspex can craze after twenty years.
4A, Found to split after twenty to thirty years.
5, Gaskets vulcanize and need replacing after five years.
6, Plastic gradually deteriorates in contact with fluid preservatives (five to ten years).
7, Screw rings corrode/deteriorate.
8, Gaskets since improved but untested beyond five years.
9, Now improved, with polypropylene lids (especially used in USA/Canada).

Figure 5.25 Ground glass stoppered jars are used extensively in zoological collections. The jar and stopper are made of tough borosilicate glass and manufactured in a wide range of sizes. The jar is straight-sided, allowing for easy storage and removal of specimens and the evaporation rate of the preservative is low. When 'topping up', ground glass jars should be smeared with Paraffin Soft White (BDH) around the lid and the lid rotated back into position to ensure an even and thorough seal (see text).

Figure 5.26 One of the problems faced with this design is the difficulty of removing the lid if the knob is knocked off. Moreover, with time the jar may be difficult to open safely as dust seems to cement the jar and stopper together. Stoppers cannot be interchanged and the jars are expensive.

Figure 5.27 Danish jars are inexpensive and are available in a variety of sizes.

Figure 5.28 The lids of a Danish jar have a limited shelf-life and the plastic lids become brittle and split after ten to twenty years.

Other points to consider with respect to storage jars are listed below.

- Soda-glass in older jars is fragile and internal stresses may cause larger jars to crack. More expensive borosilicate jars have been found to be much tougher.

Figure 5.29 A 'honey jar' is useful for storing small glass tubes as the sides are straight and the jar is very inexpensive. The metal lid is enamel-coated on the outside and lined inside with cellulose to ensure a good seal and to protect the metal against corrosion. Evaporation is slow.

Figure 5.30 Bakelite-lidded jars with straight sides are also used for the storage of small glass vials. However, the evaporation rate is very fast and the lids have a short shelf-life of under twenty years. The Bakelite becomes brittle and, if the lid is difficult to open, it may crack or split. An improved polypropylene lid is now available to replace bakelite.

- Jars need to have an open and unrestricted neck.
- Ensure that ground glass jars have tight-fitting lids (no leakage) and have a strong enough bar on the lid to remove them safely and easily.
- Jars should have a broad and flat base, especially for jars taller than 500 cm.

Risks to collections

Preventive conservation is vital and the following risks must be eliminated if a fluid-preserved collection of biological specimens is to survive in good condition. Fluid-preserved collections can be adversely affected by the following external agents: light and UV radiation, temperature, relative humidity, pollutants and mechanical stress (Horie, 1994; Rose and de Torres, 1992; and Simmons, 1995). See also Chapter 9 on policies and procedures.

Ultraviolet bleaching

Specimens stored where sunlight or powerful artificial lighting reaches them will ultimately show signs of bleaching or at least a general lightening of colour. Specimens that have suffered from bleaching cannot be effectively restored to their original colour. Prevention is the only alternative and this can be achieved by excluding light in a variety of ways (see Chapter 7 on the collection environment).

Adverse temperature and relative humidity

High temperatures and low humidity will lead to rapid evaporation and drying out of specimens, while other adverse conditions of temperature and humidity may catalyse deteriorative processes such as oxidation of leached lipids. An ideal storage temperature is 10°C but this is only possible where storage is separate from working areas. A more practical aim in most facilities is to keep the temperature below 20°C, keeping particularly sensitive materials at a lower temperature in a refrigerator (or, for some alcohol-preserved material for molecular studies, in a freezer).

Relative humidity should be maintained at 45–55% to prevent deterioration of external labels (and seals of old jars with pig's bladder covers), either due to growth of moulds (> 60%) or embrittlement due to drying out (< 40%). Drier conditions may also accelerate drying out, although this will be considerably slower than for high temperature. For details on environmental monitoring see Chapter 7 on the collection environment.

Pollutants

The most obvious pollutants are dirt and dust, which are mostly evident as layers of grime on jars and labels. Not only is this unsightly, rendering the labels difficult to read, but it also makes handling hazardous. Even where it is not possible to install full air conditioning and filtration, good storage units, regular cleaning and inspection regimes will help to overcome this problem. Less obvious pollutants such as oxides of nitrogen and sulphur will not only affect labels but may also diffuse into jars. Siting of the building is an important factor (see Chapter 7 on the collection environment).

Mechanical damage

Although fluid-preserved specimens are protected to some degree from mechanical damage by the fluid itself, they must still be carefully stored to avoid unnecessary jolting and vibration. Great care must be taken if compactor units are used to ensure that they are smooth-running and protected from impacting with one another by use of pneumatic buffers. Similarly, great care must be taken when moving jars around the store and trolleys should have pneumatic tyres and retaining supports, particularly for large jars (Clark *et al.*, 1994).

Large or exceptionally heavy jars should be stored on pallets and tall jars (over 1 m) should be secured with linen tapes to prevent toppling. In earthquake zones, retaining bars are placed across the open ends of shelving to prevent jars from being shaken off during minor tremors.

Transportation and postage

Sending fluid-preserved specimens by post or by commercial carrier can be a hazardous process and should not be done without adequate justification. Nevertheless, it is often

necessary to send specimens and this must be achieved with the minimum risk. The first essential is to minimize movement and consequent physical damage to the specimen during transit. This can be achieved either by packing around the fluid-containing jar or draining off as much fluid as possible and wrapping or packing the specimen in preservative-soaked material. McCoy (1993) recommends the use of cheesecloth (grade 10) to wrap specimens since it absorbs just the right amount of preservative fluid to keep the specimens moist in transit and does not adhere to protruding claws or rough surfaces.

Specimens must never be allowed to dry out during transit. If specimens are sent in tubes or jars of preservative, the containers should be absolutely full to avoid air bubbles moving around during transit and damag specimens. The containers should be carefully sealed using laboratory Parafilm (obtained from laboratory suppliers) or a sealing tape which will not dissolve if the fluid leaks. Enclose the container in stout polythene bags as an added precaution in case of possible leakage and pack around with several layers of protective packing such as polystyrene chips. If the fluid is drained off, this should be done immediately prior to sending and arrangements should be made for the specimens to be returned to preserving fluid immediately on arrival. Such specimens may be sealed in polythene bags and surrounded by packing material as for material in fluid.

Very delicate specimens can be sent through the post by embedding them in agar (after Harris, 1965) as this process is reversible.

Reversible embedding technique

Small fluid-preserved eggs, cartilaginous skeletons and Victoria blue transparencies and cnidarian medusae are examples of zoological preparations that can be stored in agar. The embedding technique is as follows:

1. Ensure that specimens for embedding have been appropriately and thoroughly fixed.
2. Make up a 4% aqueous solution of agar by steaming or heating in a hot water-jacketed beaker. Leave to cool to about 40°C.
3. Pour a thin layer of cooled agar on to a watch glass or cavity dish and arrange the specimens as desired on the agar surface. Ensure that no moisture is left on the surface of the agar (this may cause separation of the two layers) by wiping the surface with a piece of absorbent paper.
4. Pour on a second layer of agar so that no surface of the specimen is left exposed.
5. Allow the agar to gel completely at 15–20°C, cast the opaque block out of the container and trim the block with a razor-blade ensuring that there is at least 1 mm of agar between the outside edge of the specimen and the exterior of the block.

Specimens for posting can be sent in tubes with a moist cotton material wad to prevent agar from drying out and a crystal of menthol or thymol as a fungus preventer. To reverse the process, the block should be warmed in water until the agar dissolves away.

Irreversible embedding technique

For permanent storage, agar can be rendered insoluble by dehydration, but this is an irreversible process:

1. Transfer the agar block directly to 70% alcohol, in which it can be stored.
2. To clear the block dehydrate it in two changes of isopropanol for one day (minimum) and then transfer it to a 50:50 mixture of isopropanol and benzyl alcohol for a few hours.
3. Transfer the block to pure benzyl alcohol, methyl benzoate or a similar non semi-volatile clearing agent in a sealed container since most clearing agents are carcinogenic.
4. Depending on the density and size of the block it should be cleared in one to six hours.
5. Store the block in the clearing agent.

Higher alcohols that crystallize at room temperature can also be used as a storage medium to send specimens through the post:

1. Specimens should be in alcoholic preservative.
2. Warm some n-dodecanol or tertiary butyl alcohol in a water-bath until melted (about 23°C).
3. Place specimen into fluid in container for posting and allow to cool. This can be

accelerated by refrigeration but care must be taken to avoid freezing as this may cause shrinkage.

4. To release the specimen from the crystalline alcohol, remove the stopper and place the tube into some warm water.
5. Transfer the specimen to preserving spirit.

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Suppliers

Acid-free board, envelopes and boxes

Coutts Ltd, Violet Road, London E3 3QL, UK.

Preservation Equipment Ltd, Church Road, Shelfanger, Diss. Norfolk, IP22 2DG, UK.

Chemicals

MERCK Ltd (BDH), Magna Park, Lutterworth, Leics LE17 4XN, UK.

Aldrich Chemicals Co. Ltd, (formerly Sigma), The Old Brickyard, New Road, Gillingham, Dorset SP8 4JL, UK.

Goatskin Parchment and Resistall (see Appendix 1 on Documentation)

Japanese tissue

Arjo Wiggins Ltd, Gateway House, Basing Vw, PO Box 88, Basingstoke, Hants, UK.

Preservation Equipment Ltd, Church Road, Shelfanger, Diss, Norfolk, IP22 2DG, UK.

Jars

Battery glass jars (available as 'glass tanks'): **Parlane International**, Pagoda Park, West Mead, Swindon, Wiltshire SN5 7TT, UK.

Danish jars: **Kay R. Grathwol**, Glosemosevej 20, DK-2600, Glostrup, Denmark.

Perspex jars (known as 'visijars') and cement, also supplying drilling and sealing kit with threaded plugs: **Holloway Plastics**, Units 5 and 6, Willenhall Industrial Estate, Willenhall Lane Bloxwich, Walsall WS3 2XN, UK.

Paraffin Soft White

MERCK Ltd (BDH), Magna Park, Lutterworth, Leics LE17 4XN, UK.

Polyester resin and accessories

Alec Tiranti Ltd, 70 High Street, Theale, Berks RG7 5AR or Warren Street, London W1, UK.

Propylene phenoxetol

NIPA Laboratories Ltd, Llantwit Fardre, Pontypridd, Mid Glamorgan CF38 2SN, Wales.

Whole biological specimens, fluid-preserved and jar-mounted

John Dunlop, 12 Tideway, Littlehampton, W. Sussex BN17 6QT, UK.

Spirit sealing tape 5086

3M (UK) PLC, PO Box 1, Market Place, Bracknell, Berkshire RG12 1JU, UK and at: Gorseinon Road, Penllegaer, Swansea SA4 1GD, Wales.

Stockholm tar

Scats Ltd, Northgate House, Staple Garden, Winchester, Hants SO23 8ST, UK.

Tensol cement

ICI Petroleum and Plastics Division, PO Box 34, Darwen, Lancs BB3 1QB, UK.

UV fluorescent light filters

The Morden Company, Belt Cottage, Somerley, Ringwood, Hants, UK.

UV protected cases in VA or VE grade acrylic

Quadrant 4, Shakenhurst, Cleobury Mortimer, Worcs. DY14 9AR, UK.

Formulae

All measurements are in ml unless otherwise indicated. Threshold limit values (TLV) are given in square brackets for hazardous fluids and denote the legal/safe limit in milligrams per cubic metre of air. An asterisk (*) denotes those fixing or preserving agents used in the past (see Davie, 1894 and Lee, 1885). These have either gone out of fashion or, for health and safety reasons, are actively discouraged. Note that arsenic salts are listed as Schedule 1 poisons in the UK.

Acetic cupric acetate: Distilled water (200), glacial acetic acid (8) [tlv 10], cupric acetate (1 g).

***Arsenic paste:** Mix 1000 g crystallized arsenic (arsenic trioxide [tlv 0.1]) with 500 g sodium bicarbonate and dissolve them in 3.5 litres water. In a fume cupboard boil the mixture down to 2 litres. For use mix with whiting powder.

***Arsenical soap:** Slice 1000 g white soap (not too hard), melt it in enough water to cover it and simmer until melted. Add 190 g powdered potassium subcarbonate and stir into soap solution. Stir in 1000 mg powdered arsenic. Dissolve 150 g camphor in 250 ml 96% ethanol and add to above mixture. Stir thoroughly and boil down to tarry consistency. Pour into earthenware jar to harden and stir occasionally to prevent arsenic from settling at the bottom. The soap should have a buttery consistency.

Bouin's fluid: Saturated aqueous picric acid (75) – can be made up in 1% saline if

required, 10% formalin (25) [tlv 30], glacial acetic acid (5) [tlv 10].

Buffers for 4% formaldehyde (Carter, 1997; Mahoney, 1973; Steedman, 1976)

Sodium orthophosphate mix: 6.5 g di-sodium hydrogen orthophosphate (Na_2HPO_4) and 3.5 g sodium di-hydrogen orthophosphate (NaH_2PO_4) in 1 litre of 4% formaldehyde.

Sodium β-glycerophosphate (0.05M): 150 g sodium β-glycerophosphate ($Na_2C_3H_5(OH)_2PO_4$) in 1 litre of 4% formaldehyde.

Carnoy's fluid: Absolute alcohol [tlv 1000] or isopropanol (propan-2–ol) (60) [tlv 980], chloroform (30) [tlv 50], glacial acetic acid (10) [tlv 10].

Celloidin: 8% nitrocellulose (pyroxylin) in ether-alcohol (supplied by Merck-BDH under the name Necoloidine), diluted to 1% with the solvent below. (Should be stored in a refrigerator which is, preferably, explosion-proof.) Celloidin solvent mixture of di-ethyl ether [tlv 1200] and isopropanol (50:50 vv). This mixture is highly flammable and should be stored in a refrigerator.

Chromo-acetic acid: Sea water (400), chromic acid (1 g), glacial acetic acid (0.4) [tlv10].

Copenhagen mixture: IMS (76), distilled water (18), glycerol (5).

Dubosq–Brazil's fluid: 80% alcohol (150), 40% formaldehyde (60) [tlv 3], glacial acetic acid (10), picric acid (1g).

Formalin: Saturated solution (37%) of formaldehyde gas in water [tlv 3.0]. Standard fixation strength of formalin is 10% = 4% (3.7%) formaldehyde.

Formol-acetic alcohol (FAA): Isopropanol (90) [tlv 980], 40% formaldehyde (25) [tlv 3], glacial acetic acid (5) [tlv 10].

Formol saline: 10% formalin in 1% saline [tlv 30].

Formol sublimate: Saturated mercuric chloride [tlv 0.05] in 10% formalin [tlv 30].

Gelatin sealant: 112 g of gelatin sheet soaked twenty minutes to overnight in water to hydrate it to a soft, rubbery consistency. In a fume cupboard melt hydrated gelatin using a water bath and when molten add 24 ml glycerol and 12 ml of glacial acetic acid [tlv 10]. Mix together, remove from heat and pour into a metal tray. When set (several hours but this can be reduced by refrigerating), cut into squares and store in an air-tight jar with a crystal of thymol or menthol.

***Gilson's fluid:** 60% alcohol (60), glycerol (30), distilled water (30), acetic acid (15), mercuric chloride (0.15 g).

Gutta percha sealant: Gutta percha (100 g), Stockholm tar or pitch (100 g), paraffin wax (54°C congealing point) (25 g).

Kaiserling I (fixative): 40% formaldehyde (400) [tlv 3], potassium acetate (50 g), potassium nitrate (30 g), distilled water (1000).

Kaiserling II (enhancer): 80–90% ethanol.

Kaiserling III (preservative): Potassium acetate (200 g), glycerol (300), distilled water (900).

Kew mixture: IMS (53), distilled water (37), 40% formaldehyde (5), glycerol (5).

Osmic Acid [tlv 0.002]: 1–2% solution in deionized water, usually buffered with sodium cacodylate (which contains an arsenic salt).

Pampl's fluid: Distilled water (30), 95% alcohol (15), 40% formaldehyde (6) [tlv 3], glacial acetic acid (4) [tlv 10].

Perenyi's fluid: 10% nitric acid (4) [tlv 100], 0.5% aqueous chromium trioxide (3) [tlv 0.05], 80% IMS (3).

Phenoxetol: 1% dissolved in hot water.

***Pictet's fluid:** 5–10% solution of manganous chloride (marine animals); 1–3% manganous chloride (terrestrial animals).

Schaudinn's fixative: Saturated aqueous mercuric chloride (2) [tlv 0.05], isopropanol (propan-2-ol) (1) [tlv 980]. Add glacial acetic acid [tlv 10] to 5% concentration just before use.

Steedman's fixative: 100 ml propylene phenoxetol dissolved in 500 ml propylene glycol (propane-1,2-diol). Add 500 ml formalin (40% formaldehyde [tlv 3]) and dissolve 110 ml of this concentrate in 890 ml of distilled water or saline, pH 6.8–7.0.

Steedman's post-fixation preservative: 50 ml of propylene phenoxetol dissolved in 500 ml of propylene glycol. Dissolve 110 ml of concentrate in distilled or de-ionized water, pH 7.0–7.4.

Stockholm tar jar sealant: Mix a quantity of Stockholm tar with enough lead sesquioxide (poison!) until a fairly stiff paste is obtained.

Preservatives of plant colour (from Wagstaffe and Fidler, 1968 except for 'General fixative and preservative')

Green algae: Dissolve 0.5–1 g of cupric acetate in 2% formaldehyde [tlv 100]. Keep specimens in solution for one day, then store in 10% formalin (4% formaldehyde).

Fungi: (Basidiomycetes) for fresh specimens only. For those with slightly water-soluble colours, use mercuric acetic: Dissolve 1 g of mercuric acetate in 1 litre water and add 5 ml glacial acetic acid [tlv 10]. May be used for storage. For fungi with highly water-soluble colours: Dissolve 1 g mercuric acetate and 10 g lead acetate in 1 litre of 90% alcohol and add 10 ml glacial acetic acid [tlv 10]. May be used for storage.

Chlorophyll

(a) Bubble sulphur dioxide [tlv 5] through 750 ml distilled water until volume has increased to 755 ml. Add 15 g cupric sulphate and dissolve. Seal the specimen, completely immersed in fluid, in a jar for three to seven days, then store in 2–5% formalin [tlv 50]. NB An undesirable blue-green colour may be imparted to some plants.

(b) Dissolve cupric acetate in 50% glacial acetic acid [tlv 10] until saturated. For use dilute this stock solution 1:4 in water. Immerse specimen and leave for three weeks. For faster results the plant may be immersed in the stock solution and heated to 90°C for ten minutes. Wash in tap water, store in 2% formaldehyde [tlv 60].

(c) Dissolve 10 g of cupric chloride and 1.5 g of uranium nitrate [tlv 0.2] in 90 ml of 50% alcohol, add 5 ml 40% formaldehyde [tlv 3], 2.5 ml glycerine and 2.5 ml glacial acetic acid [tlv 10]. Immerse specimens for three to ten days depending on size and store in 4% formaldehyde (10% formalin). Better green colour than (a) above. NB This is a hazardous mixture, gloves will be required and a label indicating the nature of the preservative.

Red apples: Dissolve 50 g zinc chloride in 1 litre of warm water. When cool add 25 ml 40% formaldehyde [tlv 3] and 25 ml glycerine. There may be a precipitate which should be filtered out before using as a preservative.

Yellow apples with red streaks: Mix 5 ml of 40% formaldehyde in 750 ml of colourless paraffin oil (liquid paraffin). Immerse inside sealed container for two weeks, then store in chlorophyll preserving solution (see (c) above).

Red and blue flowers: These can be difficult to preserve but some success has been obtained by using two solutions. Solution (a) is suitable for blue flowers with a high pH value and solution (b) for red flowers with low pH. Intermediates can be treated with a mixture of the two solutions.

Solution (a): Dissolve 1 g of thiourea and 2 g of sodium citrate in 100 g of tertiary butanol [tlv 300]. If the solution solidifies at (cool) room temperature 10 ml of n-butanol [tlv 150] can be added.

Solution (b): Dissolve 1 g of thiourea and 2 g of citric acid in 100 g of tertiary butanol [tlv 300].

General fixative and preservative: This has found to be particularly effective, especially with Orchidaceae and is used at Kew. IMS (53), water (37), 40% formaldehyde (5) [tlv 3], glycerine (5). The glycerine helps to maintain flexibility in specimens.

Glossary

Autolysis – Breakdown of dead tissue caused by its own enzymatic/acidic secretions (self-digestion).

Cheesecloth – a loosely woven cotton material initially used to wrap cheese but now sold in the UK to the building trade.

Dissociation – Breakdown of tissue leading to its dissolution into a mass of disconnected cells.

Fix – To stabilize specimens by changing their chemistry while preserving as far as possible their microscopic structure.

Fixation – Initial stage of preservation involving precipitation or chemical combining with cellular proteins to prevent decay, lysis or osmotic collapse.

Fixative – Chemical compound which initiates fixation.

Humectant – Fluid additive that prevents total desiccation of preserved biological tissue.

IMS – Industrial methylated spirit, 74 OP (over proof) methylated ethanol.

Maceration – Softening of tissue leading to dissocation.

Narcotization – Blocking neural pathways by using a narcotizing agent. This prevents contraction of a specimen upon fixation.

Penetration – Rate at which a fixative can fix tissue.

PG – Propylene glycol.

Preservation – Maintenance of state of fixation.

Preservative – Chemical compound which maintains preserved state.

Rehydration – Gradual fluid replacement in cytoplasm and other cell contents.

Spirit – Generic name for various concentrations of ethanol with or without the addition of methanol; see also IMS.

Syneresis – Shrivelling of cells caused by osmotic pressure differential between two fluids.

TLV – Threshold limit value. See Formulae on page 129.