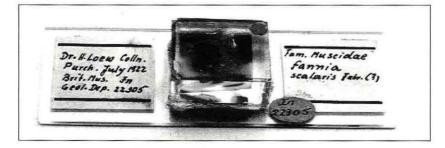


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# A REVIEW OF TECHNIQUES USED IN THE PREPARATION, CURATION AND CONSERVATION OF MICROSCOPE SLIDES AT THE NATURAL HISTORY MUSEUM, LONDON

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### Abstract

Permanent microscope slide mounts have been an integral part of the Natural History Museum's (NHM) collections since microscopes were first used in the study of natural history specimens. This paper discusses the methods, materials and mountants used in the preparation of such slides and their storage based on the findings of a museum-wide survey. Over the years, many recipes for slide mounting media have been tried, the mountants differing in their optical properties and their ageing characteristics. Using the collections as a data base of deterioration dating back to the early 19th century, conclusions are drawn as to which are the most suitable mounting media and which are the most suitable conservation techniques and housing methods. Appended are a list of recipes and a full bibliography.

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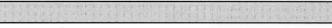
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Curriculum vitae



#### 1. Introduction

Microscopes were rare and little used until the first half of the nineteenth century, when rapid advances in optical research and in manufacturing methods produced cheaper and more reliable instruments. These microscopes became available to the serious biologist and geologist (Allen, 1976: 128) and their availability is reflected in the early 19th century dates for some microscope slides within the NHM's collections. The new microscope slide mounts rapidly developed into collections in the possession of private individuals, universities, hospitals and other institutions. Collections were made of whole organisms as well as sections of minerals, plant stems, worms etc and of microscopic parts of larger organisms such as the cells, pollen grains, spores, hairs, scales, insect genitalia and diseased tissue. Some of this material opened up new avenues for taxonomic research. As their taxonomic value grew, slide preparations increasingly featured in natural history museum collections. In addition to slides made by museum staff, private collections were also transferred to museums for safe keeping, particularly from institutions which no longer had the resources to properly care for such collections.

Deterioration of some mounting media was noted within the NHM aphid (Hemiptera) collection and a programme of survey and rescue of important material was put into operation by the author. As the subject of an MA dissertation, a museum wide study was undertaken to ascertain the condition of other slide collections. Such collections have been thought less prone to deterioration than spirit preserved or dry material and conservation problems relating to them have received little attention. This paper is a revised version of the MA project study.

#### 2. Microscope slide making and curatorial practices.

#### 2.1 Microscope slides.

Initially microscope preparations were made on many different sizes and shapes of glass 'slides'. This can still present a problem in storage geared for the standard and now universally accepted, sized slide which is 75 x 25 x 0.8-1.2 mm. In the examination of most microscope slides it is desirable for transmitted light to pass through the slide, so the slide, mountant (supporting and preserving medium) and coverslip are usually transparent. Coverslips can be square or round, although the latter are preferred as the corners on a square coverslip can catch, causing it to split. Some glass slides can have a shallow cavity ground in the slide to facilitate the mounting of thicker specimens. The higher the magnification used in the examination of the specimen, the thinner should be the coverslip (the thickness varying between 0.085 to 0.35 mm) and the thinner should be the depth of mountant. In addition to glass, slides may be of metal, plastic or wood with a central hole and some dry mounts rely on reflected light so that the slides can be totally opaque. The Cobb slide is an aluminium strip with a hole drilled centrally and is flanged along the long edges. The flange holds card squares with documentation and the specimens are mounted between two coverslips positioned over the central hole (See Fig. 1) (Westheide & Pursche, 1988: 153). The Higgins-Shirayama slide is a further

development made of plastic. Two sheets of plastic, one with a 16 mm wide hole and the other with an 18 mm wide hole, are fused together so that an 18 mm diameter coverslip can be fitted and stuck to the 2 mm wide shelf (Westheide & Purschke, 1988: 153). Similar slides made of perspex are found in the Rothschild insect collection (Fig. 2). The specimen is then arranged in the mountant and another smaller coverslip placed on top so that high magnification viewing is possible from both sides of the slide, unlike a glass slide mount where the thickness of glass on the reverse side hampers or prevents close inspection. High magnification of thick mounts is limited as it is not possible for the microscope objective to be placed close enough to the subject.

#### 2.2 Dry mounts.

Dry mounts are used when a preservative is not required, for example microlepidoptera wings (Fig. 3), and for small opaque specimens such as copepod shells which are viewed using reflected light. These slides need not be transparent so glass can be replaced by stronger opaque materials. Humes & Gooding (1964: 238) discuss the use of wooden slides for copepods. Ostracods are often mounted on black card on wood, cardboard, glass or plastic slides as dry mounts (Fig. 4). The black and white plastic slides figured (Fig. 5) have a clip-on and removable transparent square coverslip to protect the specimens from dust. The dust problem is described by Green for micropalaeontological specimens (1995: 162).

The specimens are attached with spots of adhesive which must not obscure the specimen, and protected in a cavity slide or by solid rings beneath the coverslip. As the coverslip is not supported from below by mountant any pressure exerted from above can cause the glass coverslip to break, exposing the specimen to damage. Such damage can also occur when liquid mounts dry out (due to evaporation through cracked ringing media for example) because the liquid also supports the coverslip. Wooden and card slides with wells cut in them are suitable for housing electron microscopy stubs as found in the Zoology Department of the NHM (Fig. 6).

#### 2.3 The Mounting Medium.

Mounting media may be liquid, gum or resinous, soluble in water, alcohol or other solvents and be sealed from the external atmosphere effect by non-soluble ringing media. The refractive index of the mountant should be chosen to be either similar to, or contrast with, the specimen, depending on the method of viewing of the slide and the nature of the specimen.

#### 2.4 Coverslip supports.

There are a number of methods to support the cover slip when the mount is thick. The support can take the form of a glass or metal ring, cut sections of insect pins, or glass beads or celluloid (Fig. 7) placed around the specimen so as to reduce flattening of the specimen by the coverslip. Cardboard rings (as described by Jobling (1938: 55)) can be cut to the right thickness for the mounted specimens, as used for Euparal mounts of small Hymenoptera, but are not considered suitable by Gray (1954). Problems arise when the

solvent evaporates and the coverslip can be broken as stresses build, so extra mountant should be added at a later date to prevent this. Cavity slides also have this problem with the coverslip distorting in the centre. Celluloid cut into 1 mm. squares is a good support as it 'gives' slightly as the mountant shrinks. Thick square card labels or strips or beads (Fig. 8) can be fixed on each side of the coverslip to act as 'spacers' to protect the mount.

#### 2.5 Liquid mounts.

Liquid mounts require leak proof cells to contain and seal in the liquid. These can be made of card, metal, glass or a suitable water or solvent resistant ringing medium or cement with further layers of soft paraffin wax if required. Such cells can also be used for dry mounts.

#### 2.6 Alternatives to microscope slide mounts.

Most slide mounts are essentially two dimensional with specimens usually flattened dorso-ventrally but not so flat that the difference between the dorsal and ventral surfaces cannot be detected. Some workers dissect specimens and place different parts of the specimen under different small coverslips on the same slide (Fig. 9). As an alternative to slide mounting, associated parts of larger organisms can be placed in vials in liquid preservative to preserve their three dimensional character. The NHM's collection of Butterfly genitalia are placed in vials of glycerine and pinned through the cork on the mounted insect pins. Robinson (1976: 129) considers this technique to be "abhorrent", stressing the likelihood of damage and loss in handling. Vials of liquid preservative may dry out and thus require frequent inspection. For this reason, slide mounts are preferable. Coleoptera genitalia (Cooter, 1991) or Diptera genitalia (Coe, 1966: 19) have been mounted in slide mountant (Euparal and Canada balsam) on small squares of celluloid (cellulose acetate) or card, and also placed on the insect pin (Imms, 1929: 166). Robinson (1976: 129) also condemns this method as "obnoxious" and suggests that genitalia preparations should always be on microscope slides. A further method previously used was to mount the specimen between two coverslips over a hole in card (Walker & Crosby, 1988: 21), sealed with glued paper which can then be pinned or enveloped (Fig. 10). The latter two methods are no longer practised in the NHM Entomology department.

#### 2.7 Chromosome preparation slides

Chromosome preparation slides can be made from and associated with specimens before preservation of the latter in the main collection. The NHM Aphidoidea chromosome collection numbers some 5,000 slide mounts in DePeX (which are unfortunately deteriorating). A similar collection of simuliid fly chromosome slides mounted in Euparal are also deteriorating but at a slower rate. This deterioration is probably due to the ephemeral nature of chromosomes rather than that of the mountants. Both these collections are considered to be secondary in importance to the collection of photographs of the chromosome preparations made when fresh. The NHM's 10,000 plant chromosome slides are also mounted in Euparal. 2.8 Electron microscopy

Electron microscopy (EM) is increasingly being used in taxonomy, demanding many new preparation techniques and mountants. However, because such preparations are not normally in the form of permanent glass microscope slides, they are not covered in this paper. Some embedding media used in electron microscopy such as polymer resins, as discussed by Smith & Tyler (1984: 260) are suitable for conventional light microscopy. Few new mounting media are now being developed for conventional microscopy as most development is now within the electron microscopy field. The curation of scanning electron microscope stubs is discussed by Julia Golden (1989: 17-26). Stephen Russell (1989) documents a technique for reconditioning SEM treated diatoms for conventional slide mounting. The copepod collection houses SEM stubs in card well slides (Fig 6).

## 2.9 Methods for specimen preparation prior to slide mounting.

#### 2.9.1 Maceration.

Before specimens of insects and some other groups can be made into permanent slide mounts, they must be prepared or 'macerated' using the appropriate method for the chosen mountant. Body tissues, fat, secretions and wax often need to be cleared or denatured, making the organism translucent to facilitate the examination of the surface or exoskeletal structures of the organism. The body contents must not be fixed with formaldehyde which preserves the body tissues so that they then cannot be cleared. Sodium hydroxide or potassium hydroxide are commonly used chemical macerating agents, at varying strengths, for varying periods, and at different temperatures depending on the size and fragility of the specimens. When specimens preserved in aqueous solutions are destined for resinous mounts, they require dehydration as many of the natural and synthetic resin mountants do not mix with water. Dehydration is achieved by placing the specimens progressively through a series of aqueous ethyl alcohol solutions, 50%, 80%, 95% until 100% ethanol is reached. Alternatively, two rinses in glacial acetic acid may suffice (suitable for aphids but not Thysanoptera). Preparation must be done in such a way that the fluid in the specimen is compatible with the mountant thus avoiding opaqueness or osmotic collapse and distortion, which renders the specimen useless for taxonomic study.

#### 2.9.2 Tissue embedding and sectioning.

Thin tissue sections are cut after the tissue is embedded in a medium which holds the tissue together. To make a permanent slide preparation of the section it has to be glued to the slide with a suitable adhesive which should have a similar refractive index to the glass of the slide (RI = 1.51), to the embedding medium and to the mounting medium. Cellulose nitrate has a refractive index of 1.49-1.51 and polyisobutylene adhesive has a refractive index of 1.50-1.51 (Fink, 1987: 97) With possibly three different media involved in the preparation of a plant section one must make sure that they are compatible, both chemically and optically, as the boundary between the media could reduce the visibility of the section. Rawlins (1992: 53) discusses the use

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of embedding media and glass microtome or vibratome cutting tools.

#### 2.9.3 Stains and bleaching agents (see also Appendix 3).

For very translucent specimens, a stain may be required to improve the visibility of the specimen before it is placed into the mountant. There are many stains available which stain different constituent chemical parts of the organism. One must choose the appropriate stain for both the organism and the slide mountant. New (1974: 23) suggests that some stains deteriorate in certain mountants. He suggests that Acid Fuchsin, Fast green and Lignin pink can be fixed in Euparal and Canada balsam, but that Chlorasol Black E is better with polyvinyl lactophenol or methyl cellulose/Carbowax media. This is not the experience of the Microlepidoptera section at the NHM which regularly uses Chlorasol Black E with Euparal and has witnessed little or no deterioration over thirty years. In the NHM Coccoidea collection Acid Fuchsin is used with Canada balsam. Some fading of this stain does occur and remnants of clove oil, the final clearing fluid may be involved as Acid Fuchsin stained specimens stored in clove oil fade within a few days (J. Martin pers. comm.). When using phase contrast, the stain is considered as useful only to locate the organism on the expanse of the slide. It is widely accepted that acid stains will fade in neutral or alkaline mountants but that neutral mountants are better for the survival of more delicate specimens. Rawlins (1992: 56) suggests that glycerol fades many stains. Ramanna (1973: 103) found that Euparal was suitable for preserving fluorescence of Aniline Blue in plant material such as pollen tubes. Partial bleaching with ammonia and hydrogen peroxide may be required if the specimens are intransigently opaque due to dark sclerotisation as in some Aleyrodidae homopteran bugs.

#### 2.10 Microscope slide mounting methods.

To select the most suitable slide mountant one must refer to literature covering the study of the organisms concerned (as listed in the bibliography) Great care is needed in selecting methods to balance the requirements for visibility with the longevity of the mount and the specimen within the mount. For example, doubts about the permanence of gum chloral mounts have now been widely published (Noyes, 1989, Upton, 1993 and see discussion below).

The mountant used in a slide preparation should always be noted on the slide label. Names used should also indicate a specific recipe if possible, and the name of the preparator and the date. Gum chloral mounts have indiscriminately been called Berlese, Hoyer's or Faure's even when the recipe is quite different from the original in the concentration of constituent parts. A deteriorating slide without exact details of the mountant can prove difficult to repair, and may involve trial and error soakings in different solvents which may damage the specimens and waste much time. Often slides were made as temporary mounts and were not meant to last, but a change in status of the specimens, for example to 'type' series, dictates the use of a more permanent mount so as to preserve the type for future taxonomic study.

Many slide mountants require a period in a curing oven at 30-40 degrees centigrade to harden the mount by evaporation

of the solvent especially if the slides are to be stored vertically as a soft mountant will creep under the influence of gravity. If the mountant is soft, later handling can also move the coverslip possibly rolling and ruining the specimens. The use of wire springs to hold the coverslip, as described by Wagstaffe & Fidler (1970: 197), is employed by some workers to compress the specimens while the mountant hardens.

#### 2.11 Ringing the coverslip.

Liquid, glycerine and gum chloral mounts need to be ringed round the edge of the cover slip to seal the mountant and prevent its escape, loss through evaporation, or oxidation through contact with air. Excess mountant from round the edge of the coverslip should be removed by scalpel before ringing. Ringing of round mounts is easily accomplished using a mounting turntable (Fig.11). The slide is clipped onto the revolving stage and a small amount of ringing medium such as Euparal, Glyceel, Glyptal (Travis, 1968) or Murrayite is applied with a fine brush as the slide revolves. Wu (1986) describes his method of applying Glyptal to nematode 'Hoyer's' mounts using a plastic bottle. The many sealants used are listed in Appendix 1. Cutex and other brands of nail varnish have been used for both ringing and as a mountant. Wells (1978) describes nail varnish used with butyl acetate-acetone to mount mollusc radulae and pollen grains. The use of more than one coverslip on a slide (Figs. 8 & 9) can make ringing difficult and tedious.

If the mountant is prone to shrinkage, ringing may not stop the ingress of air as any stress applied to the ring or the coverslip may cause them to fail. A further application of the mountant at a later date may be necessary to replace the shrinkage loss, before a ring is applied. Resin mounts do not normally need ringing as the solvent in the resin needs to evaporate in order for the mountant to harden.

#### 2.12 Labels.

Bridson & Forman (1992) state that gummed labels for microscope slides should be of "archival" quality, be they foil backed, gummed self adhesive or manually glued. Non-archival gummed and self-adhesive labels have often been used in the past and have sometimes become detached or become transparent after a few years. Where slides are stored vertically in contact with each other, the most suitable labels consist of thick card squares glued to the glass with a polyvinyl acetate glue. These have the advantage that they also act as 'spacers' between the slides to protect the coverslip and the slides can be stacked for drying without fear of them sticking together and becoming useless. A diamond-tipped engraving scribe can be used to write the locality details and accession number directly on the glass slide beneath the labels. This provides the means of maintaining the identity of the slide when labels become accidentally detached or the writing on labels fades. The Microlepidoptera genitalia slides, plant chromosomes and pollen slides and Protozoa slides (in the NHM collections) are marked in this way.

#### 2.13 Immersion oils.

For viewing microscope slides at high magnification, an immersion medium is required between the microscope's high power objective lens and the coverslip. Such immersion oils are available from a number of microscope manufacturers and usually have a refractive index close to glass at about 1.5. Immersion oil should be cleaned off the slide after examination as it forms an unsightly smeer on the glass and might cause deterioration of the mountant or the sealing ring (M.G.C., 1992: 52).

#### 2.14 Storage.

Collection storage has often been dictated by that already in use for major donated collections, so that, for example slide storage at the NHM is not standardised. Storage of slides can be horizontal (slides laid flat) on shallow slats and trays in boxes (Fig. 12) or cabinets; or vertical within slotted boxes (Fig. 13) or drawers (Fig. 14) which are a little deeper than the width of the slides. At the NHM slides are stored in a variety of wooden and metal cabinets, either vertically orientated in drawers (about 800,000 slides, mostly in Entomology Department) or horizontally in wooden (Fig. 15) and metal cabinets (Fig. 16) and in boxes with slats (about 1,100,000 slides).

When considering the particular needs of a large microscope slide collection one can quote Mound (1992: 10) who describes the 400,000 NHM Homoptera and Thysanoptera slides combined, as a solid block of glass of 12 cubic metres. This weighs about 8 metric tonnes and exerts a load of 0.3 tonnes/square metre or about 4 kiloneutons/square metre. Such weight must be carefully considered when floor loadings are concerned! The regulation minimum floor loading is 5 kiloneutons but the NHM Entomology block floor has been recorded as capable of carrying 17 kiloneutons (Colin Farmiloe, pers. comm.).

One must decide on the right type of cabinet in which to store slides. In the NHM, generally the thinner the slide mounts, the more likely they will be stored vertically so that the slide collection can become a self indexing system. The thicker the mounts, the more likely they will "creep" under the influence of gravity when stored vertically, so such slides are better stored horizontally. Liquid mounts are also stored horizontally as the seal is more prone to damage by jostling with other slides and the specimens will sink to the lower edge and be damaged against each other or against the edge of the mount. However, slides stored horizontally take up more space.

In the NHM, standard wooden cabinets (so called Hill units) with horizontal slide drawers hold 5250 slides (Fig. 15) and those with vertical slide drawers can hold 10,000 slides when full (Fig. 14). Before storing slides vertically they must be baked hard in an oven at 30-40 degrees centigrade to avoid the mountant "creeping". The vertically stored Homoptera slides (Entomology Dept.) are carded (the thickness of the card label on each side of the mount gives some protection) and enveloped to give extra protection from dust and physical damage (Fig. 18). Previously, manilla envelopes specially manufactured were used to house slides individually (Fig. 18), though the time spent duplicating information was considered excessive, but is still used for type and other important specimens especially when that material is loaned, so that the information remains within the collection. In the past, plastic sleeves have been manufactured from unplasticised polyvinyl chloride which is not of archival quality and which are turning yellow and becoming brittle. New plastic sleeves are being made of archival quality polyester (by Preservation Equipment Ltd.) Such vertically stored collections can be arranged taxonomically with closely related families, genera and species together (and with associated indices), or they can be arranged alphabetically (by family, genus and species in unrelated order), and because they are thin and card-like, they can be their own index obviating the need for a separate card index (Fig. 16). With vertical slide storage it is possible to incorporate short bottles of specimens in spirit, pinned dry specimens in unit trays and dried host plant samples in the same drawer, as in the NHM Diptera collection (Fig. 19). Unit trays holding about 25 slides each are used in some vertically stored collections to facilitate easy removal of slides (Fig. 16). At the Royal Botanic Gardens at Kew, plant anatomy slides are housed in Gallenkamp metal clips (4 to a clip) which fit vertically in standard card file drawers, the slides standing upright on end.

As with most natural history collections, microscope slides require a controlled environment. There are four lines of defence - the sealant ring, the envelope, the cabinet and the room in which the cabinet is housed. The Museums & Galleries Commission's Standards in the care of Biological Collections (1992: 52) makes little mention of the specific needs of microscope slides, except to list them as being prone to damage from variation in relative humidity. In high relative humidity, fungal attack can occur in dry, liquid and other aqueous mounts and creep and sweating can occur in aqueous mounts. In low relative humidity, discolouration and cracking can occur in these same mountants (see section on Gum chloral mounts below.) Sometimes, slides can be damaged when they are lent to institutions in which the environment is not controlled or when they are subjected to varying conditions in transit. Slides should be stored in total darkness except when being examined as high light level is suspected in the deterioration of gum chloral mounts and in the fading of stains. Routine freezing of incoming parcels to kill insect pests may damage aqueous micro-slide mounts.

#### 3. Which microscope slide mountants should be used ?

The general museum literature gives little in the way of guidance on the merits of different microscope slide mountants, with no mention of drawbacks in Walker and Crosby (1988) or in Stansfield (1992: 443) for example. General works covering the choices in the making of microscope slide mounts for biological specimens include Gray's Microtomist's Formulary and Guide (1954), Wagstaffe & Fidler's Preservation of Natural History Specimens (1970) and Knudsen's Collecting and Preserving plants and animals (1972). Often, to find the latest preferred method, one has to consult the texts for the specific organisms with which one is dealing. Some preparators have no knowledge of why they used their chosen mountant, apart from the fact that they had always used it by tradition, which might indicate that the mountant is suitable but possibly not the best available. Individuals working in relative isolation

may not be aware of better techniques which are worth experimenting with. Recipes and techniques have been published in 'special interest' journals which are not read by other workers studying in different disciplines. Specialists also have a tendancy to develop their own, often complex techniques, with little regard to archival quality, compatibility, standardisation, or ease of future handling, curation or conservation.

Rawlins (1992: 56) suggests that there are two categories of mountants, the permanent and the semi-permanent (which do not set hard). Gutierrez (1985: 352) states that "no mounting media are fully satisfactory" for spider mites. Lillie et al, (1953: 71) came to a similar conclusion after carrying out an exhaustive survey of histology mounting media. Many workers would agree with this statement but most workers who wish their slide mounts to remain "permanent", choose a favourite mountant which both suits their viewing requirements and preserves the specimens for future research. Such workers have frequently made up their own mountants and given them "pet" names, often not indicating the recipe used for a particular slide and sometimes not bothering to publish the recipe. There is much confusion as to the correct recipes for mountants and when they were first used, as discussed by Upton (1993) for gum chloral mounts. Some proprietary brands of mountants are and have been made to secret recipes whose names or recipes have been changed and have been copied by others, which has added to the confusion. Since the last century, collections have grown and have been amalgamated into major museum collections, some of which now require conservation. The conservator may be faced with the problem of not knowing what the deteriorating mountant consists of, even if the name of the mountant is written on the slide, as it may not be the published recipe. With the passage of time, the evidence of degradation of some mountants has slowly and, sometimes too quickly, become apparent, adding another dimension to the discussion about the best refractive indices and the short term effect of the mountant on the objects.

#### 3.1 Refractive indices (see Appendix 2).

"The ideal mounting medium for stained preparations should have the same refractive index as the mounted object." (Pantin: 1964, 21). Conversely, if the specimens are colourless, as are diatoms or mites, then visibility in bright field microscopy is enhanced by a difference in refractive index. A diatom of approximate refractive index of 1.4 when mounted in a mountant of 1.5 will have a visibility proportional to the difference between 1.4 and 1.5 (Fleming, 1943: 34). Knudsen (1966: 500) states that diatoms and mites become 'lost' in Canada balsam because the refractive index is close to that of the mountant. Acarologists such as Norton of Syracuse New York State University, continue to use gum chloral mounts because they offer a "better" contrast with a lower RI. Diatom researchers use mountants with a higher RI of 1.6-1.7 (such as Naphrax) than diatom silica at 1.43, (McLaughlin 1986: 287). The use of stains and of phase contrast has largely overcome this problem. Walker & Crosby (1988: 22) mention that Canada balsam is "particularly recommended as a mountant because its refractive index (1.53) is very close to that of glass". They

do not refer to the problem of reduced visibility of small and transparent organisms in bright field. It is important to remember that refractive index changes with temperature of the mountant and with the percentage loss of solvent from it. Canada balsam dissolved in xylene is 1.497 and dries to 1.532 (Loveland) and so the optical quality of the mountant changes from when the slide is first made to later inspection.

#### 3.2 Natural resinous media.

#### 3.2.1 Canada balsam (RI = 1.52-1.54).

Canada balsam was first described as a suitable mounting medium for the new science of transmitted light microscopy by Andrew Pritchard in the 1830s. It is the most widely used mountant because of its proven archival quality, with a track record of over 150 years, and does not crystallise or absorb moisture as do gum chloral mounts. Eastop (1984: 248) observed that Canada balsam slides of aphids made by Francis Walker in 1847 show no signs of deterioration except for yellowing (Fig. 20). This yellowing is demonstrated in Fig. 21 where one yellow Canada balsam slide is compared with 3 colourless Euparal slides of the same age (20 years). Mound & Pitkin (1972: 122) state that Canada balsam is the only mountant known not to deteriorate when kept for many years in a variety of climates. Noyes (1982: 329) states several million years as the longevity of Canada balsam, comparing it with natural fossilised amber. Because Canada balsam is similar to amber, it has been used to mount insects in amber on to microscope slides (Grimaldi, 1993, 46). Figure 22 is of the infamous 'fossil' latrine fly hoax discovered by Dr Andrew Ross and reported in the New Scientist (Palmer, 1993: 4) where the fly was mounted in Canada balsam within a manufactured pocket in a genuine piece of Baltic amber. Canada balsam can be acidic, which will erode calcium carbonate and fine structures on some organisms and minerals, so one should be certain to select a neutral balsam as described by the manufacturer for slide preparations of coccoliths and ostracods.

Hood (1940), who mounted over 50,000 microscope slides, came to the conclusion that Canada balsam was the only mountant to use. Similarly Cooper (1988: 228), after many years experience in the NHM Histology section, used only Canada balsam. Saito & Osakabe (1992: 427) considered Hoyer's to be only temporary and published (1993: 593) an improved method for mites using Canada balsam, fixing in methanol-acetic acid and with x-terpineol, but they do not mention the refractive index. Figure 7 shows a deep mount of a whole Trichopteran in Canada balsam which has darkened with age and has thus reduced the visibility of the specimen. Specimens as large as this are better preserved dry on pins or in spirit. Yellowing and darkening was not considered a serious problem by those commenting on balsam in the NHM survey. The tedious rinsing through three or four, more concentrated, alcohols to dehydrate specimens has been replaced by a single rinse in glacial acetic acid so that the Canada balsam technique is now less time consuming. Rawlins (1992: 56) relegates Canada balsam as having been widely used at one time as it is strongly "autofluorescent". He also points out the harmful solvents which constitute a health hazard such as xylene, might limit the choice of mountants in UK museums which

must conform with Control of Substances Hazardous to Health (COSHH) regulations. The use of non toxic solvents for Canada balsam instead of xylene (possibly Cellosolve or Histoclear) would alleviate the safety problem but might cause other problems such as slower hardening rates and premature darkening. Some workers have reported deterioration in Canada balsam such as "crazing" by Green (1995: 162) which might be due to incorrect preparation of specimens or a genuine problem in a minority of balsam mounts.

#### 3.2.2 Phenol balsam.

Advocated by dipterists working on small flies, this variant of the Canada balsam technique, replaces xylene with phenol as clearing agent and thinners (Wirth & Marston, 1968). It is more convenient and "produces the best slides". NHM dipterists have considered using this as the preferred mountant for Ceratopogonidae and, possibly, the Simuliidae. Phenol can be used as relaxant, clearing agent and dehydrating agent in one soak and was considered less dangerous than xylene. The author's discovery of black Canada balsam slides in the aphid collection (Fig. 23) may indicate future problems with the use of phenol balsam. Specimens cleared in chloral phenol and then mounted in Canada balsam have turned black with similar cuticular disruption as that found in gum chloral mounts, unlike the contemporary material cleared with KOH in balsam, so this suggests a link with phenol. These slides blacken from the edge inwards which may suggest atmospheric oxygen being involved so ringing may be required to prevent this from happening. Drs Richard Lane and John Boorman (pers. comm.) have suggested the wider use of phenol balsam as a mountant in the NHM Department of Entomology slide collections but this may be risky in the light of the author's experience.

#### 3.2.3 Euparal (RI = 1.48).

The Euparal recipe is now a trade secret but early papers list the possible ingredients as being eucalyptus oil, methyl salicylate, camsal, sandarac, and possibly paraldehyde. Euparal is manufactured in Germany and Britain (ASCO Laboratory, Manchester) and the two products have been found to differ. It has been reported that workers at the Smithsonian Institution have found that the German product does not match the standard of the ASCO Euparal.

Rawlins (1992: 56) lists Euparal as being more popular as a permanent mountant than Canada balsam. Euparal is widely used and in the NHM its use is second only to Canada balsam, being used for cytology mounts, plant mounts (also at The Royal Botanic Gardens at Kew) and for some insects. The virtues of Euparal were extolled by Imms (1929: 166) as not yellowing with age like Canada balsam and as having a lower refractive index of 1.48. Thus structures which are too transparent in Canada balsam can be seen in Euparal with bright field microscopy (Fig 24). This is the main argument used by lepidopterists at the NHM (see discussion on Canada balsam for comparisons). Euparal is regarded as being a good permanent preservative, proven over the passage of time (for example, over 30 years in the NHM Microlepidoptera collection), of consistent quality, safe, quick and easy to use, good optically with low

refractive index and drying quickly. In the NHM many slide preparators (as reported in the mountant survey part of the MA project), commented about Euparal being better than Canada balsam, "not requiring the use of carcinogenic xylene". Euparal seems to be the most popular alternative to balsam, especially if COSHH regulations preclude the use of Canada balsam in either xylene or phenol mixtures. Hood (1940) stated that Euparal was unsuitable because it quickly developed a meniscus which can damage fine structures when specimens were being arranged in the mountant, bubbles took longer to clear from the mount if at all, and that Euparal slides were prone to crystallisation although in the author's experience this does not occur. This might be due to incorrect preparation technique or possibly might involve the German product. Carolyn Lowry (pers. comm.) has experimented with Euparal and found that some fine structures of Simuliid flies become too fragile in the slide making process to make good mounts. Theresa Howard has found that using Cellosolve instead of Euparal Essence keeps such fine structures supple so that they can be arranged more easily. At the RBG at Kew, Histoclear is used instead of Cellosolve with the same benefits.

#### 3.3 Synthetic resinous media (plastics).

These are rarely used at the NHM due to the known shrinkage and crazing in plastic mountants such as DePeX and polyvinyl lactophenol.

## 3.3.1 Cellofas = Carboxy Methyl Cellulose (CMC) (RI = 1.428).

Used in the NHM Palaeontology Department for mounting fossil specimens. Stehr (1987: 16) questions whether CMCP-9 is suitable as a permanent mountant. Knudsen, (1966: 501) describes how Turtox CMC can be used to kill, clear, stain (CMC-S) and permanently mounts specimens from water or alcohol in one process. Hobson & Banse (1981: 6) refer to its use for polychaetes and mention that it contracts when hardening so that extra mountant must be added later before the slide is ringed. Clark & Morishita (1950: 789) used CMC for mites because of its low refractive index and because it did not crystallise.

#### 3.3.2 DePeX (Distrene plasticiser xylene) (RI = 1.53).

Rawlins (1992: 56) lists DePeX incorrectly as being a permanent mountant. Gurr (1956: 42) mentioned that DePeX suffers from "a considerable degree of shrinkage when drying" and suggests that it should be applied liberally to the slide to allow for this. What once seemed a perfect mountant with suitable refractive index has now proved not to be permanent because of continued shrinkage causing disruption of specimens and loss of coverslip with a change in refractive index and severe optical distortion within specimens. Only one collection of (aphid) chromosomes is still mounted in DePeX and this is of secondary importance to the collection of photomicrographs.

## 3.3.3 Dimethyl hydantoin formaldehyde (DHFM) (RI = 1.45-1.46).

This synthetic resin which is miscible with water has recently been suggested as a suitable mountant by Dr M.

Alonso-Zarazaga of the Museo Nacional de Ciencias Naturales, Madrid (pers. comm.). Trials may be carried out with mites as an alternative to Hoyer's mountant. Nematodes (Smith, 1966: 177), beetle genitalia (Cooter, 1991: 57) and turbellaria (Steedman, 1958: 451) have been mounted in DHFM. It is reported not to shrink but otherwise there is no record of longevity.

#### 3.3.4 Naphrax (RI = 1.71).

Of the naphthalene resins only the toluene-based Naphrax is still used in the NHM for mounting diatoms which need a large contrast in refractive index to make the surface detail of the diatom's silica (RI = 1.43) visible (Green, 1995: 163). McLaughlin (1986: 285) discusses the requirements for a good diatom mountant. Ragge (1955: 8) found that the tracheae, supported by chitinous taenidia, of orthopteran wings were almost invisible in Canada balsam, and consequently he changed to using Naphrax. After 50 years some darkening is evident (Fig. 25).

#### 3.3.5 Permount (RI = 1.51).

Used for mounting Porifera in the NHM and recommended together with Numount, for their clarity and non-shrinkability. Dr Eric Metzler (pers. comm.) reports that his twenty year old lepidoptera genitalia mounts in Permount have crystallised but that they can be recovered by soaking in xylene. Otherwise there is little in the literature reporting on the permanence of Permount.

#### 3.3.6 Histomount (RI = 1.49-1.50)

Although this mountant has been little used at the NHM, Donald Quicke has seen no deterioration over 10 years and the importers National Diagnostics report no cases of deterioration in 20 years, unlike with the less successful Omnimount. Histomount is at present being tested by the author. This mountant can be thinned with the unrelated Histoclear which is considered a safe solvent being based on orange oil.

#### 3.3.7 Polyvinyl Lactophenol (PVLP) (RI = 1.43-1.44).

Huys & Boxshall (1991: 451) suggest that polyvinyl lactophenol "is not suitable for type collections because specimens (of copepods) are slowly over-cleared in the mount, the mountant is slowly replaced with rosettes of long thin crystals and it often dries out if not sealed". The NHM Zoology department uses PVLP for mounting copepods and reptile scales but otherwise it has fallen out of favour, although the Central Science Laboratory of the Ministry of Agriculture still uses the Heinze formula (Heinze, 1951: 177) for mounting thrips and mites. Bink (1979: 160) mentions that some 18 year old polyvinyl lactophenol preps were in good condition but others were crystallising and turbid. Increasingly, polyvinyl lactophenol is being considered as semi-permanent at best and thus not suitable for important material as it continues to shrink (Fig. 26).

#### 3.4 Gum chloral mountants (RI = 1.48).

In the NHM survey, "Berlese" and "Hoyer's" gum chloral mounts were stated as being the best optically for the groups concerned as well as traditionally used. This group of mountants consists of many closely related recipes including gum Arabic, phenol, glycerol, lactic acid, sugars and salts. Upton (1993) discusses these recipes in detail and discusses how many differing recipes have been confused and authors misquoted. Some describe "Berlese" as being only semi-permanent and advise the use of Canada balsam (Hodkinson & White, 1979: 3) and Euparal (Freeman, 1987: 196). Likewise, Kozarzhevskaya (1968: 147) lists "Berlese" only as a temporary mount for scale insects. Stehr (1987: 16) questions the permanence of "Hoyer's" and states that, under humid conditions, unringed "Hoyer's" slides will loosen and slip. Noyes (1982: 329) states that resinous mountants (such as Canada balsam) withstand a wider range of climatic conditions than water soluble "Hoyer's" and "Berlese" which are not only sometimes unstable but frequently cause distortion to such insects as thrips. Bink (1979: 160) mentions "Faure's" as becoming sticky in humid climates.

#### 3.4.1 Crystallisation.

Gum chloral mountants deteriorate by dehydration of the mountant with the resulting formation of small white and opaque chloral hydrate crystals (Fig. 27). These almost always form from the outer edge and advance towards the centre, overwhelming and obscuring the specimens. This occurs when the slides have not been ringed to avoid dehydration. Specimens can be rescued from a crystallised slide as the cuticle is not chemically eroded, although there can be some physical damage from the growing crystals. Crystallised slides can sometimes be saved by rehydrating the gum chloral mountant in a warm, moist atmosphere with Thymol to avoid fungal infection. Otherwise, specimens can be soaked out of the gum chloral in water, dehydrated in glacial acetic acid and remounted into Canada balsam (Martin, 1987). Disney (1988: 106) suggests an excess of glucose of over 8% of the mountant is the cause, but Freeman, (1987: 196) guotes Keith Harris who blames the poor quality of modern gum Arabic. It is more likely due to a higher percentage of chloral hydrate in the recipe such as in Imms' "Berlese". If glycerine is used in the recipe instead of glacial acetic acid and glucose syrup is present, a granular opalescence can develop in the mountant which obscures fine hairs and cuticular details (Eastop & van Emden, 1972: 8).

#### 3.4.2 Blackening.

Blackening was not listed as a problem with gum chloral mountants by Upton (1993) but this has become the most serious problem within the NHM Entomology department slide collections and can be irreversible (Figs. 8 & 28). Within the Aphidoidea collection, the first sign of deterioration is a pinkish tinge emanating from the specimens, which rapidly turns bluish and then opaque black across the whole mountant, within a period of as little as six months. The cuticle of the aphid becomes transparent and then disappears, sometimes before the slide has become opaque. The cause of this blackening has been attributed to inadequate rinsing of the macerating and clearing agents, potassium hydroxide and chloral phenol repsectively, from the specimens. The presence of light and high temperature have also been suggested as contributing factors. Other mounts made in the 1970's which were cleared with chloral

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phenol instead of KOH have distorted due to osmotic differences between mountant and body contents. Stuart Harbron (pers. comm.) suggests that the blackening may be a polymerative oxidation of phenol, started by a single molecule of an oxidant such as KOH and possibly also triggered by light. Blackening starts within the specimens where phenol has been entrapped when the specimens were mounted from chloral phenol to the gum chloral mountant. Womersley (1943: 181) uses phenol in the gum chloral mountant to try to prevent crystallisation but the presence of 25% phenol may be a sure invitation for blackening to occur. At the NHM, expressly because of blackening, Hemiptera are now mounted in Canada balsam after clearing in KOH with careful rinsing in 30% ethanol and dehydration using glacial acetic acid with another careful rinsing in clove oil as standard procedure. The problem of Canada balsam having the same refractive index as insect cuticle is overcome by the use of phase contrast microscopy instead of bright field. Specimens which have not been cleared in chloral phenol before mounting in Gum chloral may not turn black but this possibility has not been fully investigated.

Other gum chloral slides turn brown at a much slower rate with a slower rate of erosion of the insect cuticle which may be related to the above. Dr Victor Eastop has suggested (pers. comm.) that these specimens were initially cleared with lactic acid and not potassium hydroxide so that the chemical reaction may be a different one. A different form of blackening occurs in some of the Stroyan aphid slides where the mountant darkens from the Murrayite ringing medium. This blackening does not damage the specimens.

The Lewis collection of phlebotomine and simuliid dipteran flies consists of 20,000 "Berlese" slides, which are also turning black. This blackening (Fig. 8) has been attributed to a chemical reaction with the Euparal ringing mountant and has been noted as starting at the edge and working inwards (Lane, 1993: 112). Disney (1988: 106) suggests the blackening is attributed to residual preservatives such as phenolics and formaldehyde within specimens. This mountant dissolves in acetone and the specimens can be remounted successfully. There is no evidence of cuticular disruption, even in the most blackened slides. Lewis quotes the Puri recipe in Kirk & Lewis (1951: 500), which could be the problem mountant he used for his collection, and later in Smith (1973: 173) he also quotes the Stroyan (1949) recipe which he might have found to be better. With aphid Berlese slides, even if the blackened Berlese can be restored, the insect cuticle is still irrevocably damaged and a programme of remounting material not yet damaged is much better than restoring already blackened damaged specimens.

After the long discussion in print about the effectiveness of Canada balsam (Noyes) versus the permanence of "Berlese" (Disney), Disney (1994: 387) finally concedes, quoting Upton (1993) and admits that there are problems with the permanence of gum chloral mountants but advises the use of Euparal for a majority of mounts and a few in "Berlese" to illustrate the fine characters such as the Dufour's crop mechanism of many phorid fly females.

Disney (1989: 48) risks the loss of permanence for bright field, visual access to museum specimens. His comment that remounting type specimens every 50-100 years is a small

price to pay, might more likely be every 15-30 years and the cost of employing a trained worker to undertake the job could be much reduced by using Canada balsam and purchasing a phase contrast microscope! Each time fragile specimens are remounted, there is a high risk of inadvertent or other damage taking place. Some workers have used gum chloral mountants for many years and have not seen any such problems possibly because they do not use phenol as a clearing agent (Zhang, pers. comm.). However, in the face of the well known problems, only mites and some small dipteran groups are still mounted in gum chloral mountants at the NHM.

#### 3.5 Liquid mounts. (Fig. 17)

Some workers consider that liquid mounts can be permanent if ringed with a permanent ringing medium. But ringing media can shrink as they age and behave differently than when used as mounting media under a coverslip. A thin ring can crack and allow the liquid to evaporate. Gutierrez (1985: 352) considers liquid mounts as only temporary. Dieguez & Montero (1992: 315) found that 90% of the diatom liquid formaldehyde slides of E. Caballero Bellido made between 1891 and 1927, had survived in good condition. Formalin mounts are no longer used at the NHM due to risk of drying out (25% of formalin mounts have been lost in the Rotifera collection and no staff are available for conservation work) but Ealing Hospital uses formalin sealed in paraffin wax for permanent collection of tissue biopsies (J.D. Arnold pers comm.).

#### 3.5.1 Glycerine-glycerol (RI = 1.40-1.47).

Glycerine and glycerol variants are the most common liquid mounts at the NHM, being used to preserve pollen and spores in the Botany Department, Nematoda, Rotifera and Bryozoa in the Zoology Department and miospores and dinoflagellates in the Palaeontology Department. It is used in liquid mounts either on slides or in glass or gelatine capsules where the three dimensional nature of the structure must be fully preserved. Spence (1940: 144) reports that it prevents mould growth and does not dissolve calcium carbonate. Glycerol readily shrinks and expands with variation in relative humidity which could stress coverslip and specimens unless totally sealed. As with other water soluble mountants it was chosen because it was cheap, safe, quick and easy to use with little preparation needed for the specimens, and it is inert and good optically. Wiles (in manuscript) discusses his technique for double coverslip gelatine mounts using aluminium Cobb slides for water mites.

#### 3.5.2 Karo corn syrup (RI = 1.47).

This is used by the NHM Botany Department to mount plankton and parts of larger marine and freshwater algae. Karo corn syrup or dextrose is commonly available and the technique is described by Monk (1938: 174) and Knudsen (1972: 23). Taft (1978: 263) uses glucose in a similar technique. It is chosen because it is cheap, safe, quick and easy to use with little preparation needed for the specimens, "algae can be placed straight into Karo syrup from sea water", and it is inert and good optically. An effective seal is required to stop water loss from the mount.

#### 3.5.3 Lactophenol.

Huys and Boxshall (1991: 451) recommend lactophenol as the best mountant for Copepods as long as the slides are effectively sealed. In the NHM both Copepods and Crustacea are mounted in lactophenol.

#### 3.6 Ringing media.

The resin mountants do not need ringing, although White (1992: 29) states that "it is not advisable to store Canada balsam slides vertically unless they have been ringed with Glyceel or a similar ringing compound". Gray (1954) suggests that Canada balsam be ringed to stop it darkening by atmospheric oxidation. Disney (1988: 106) suggested that Euparal, when used to ring "Berlese" mounts, causes discolouration of "Berlese" over time. Within the Stroyan aphid collection, discolouration of the gum chloral mountant is probably due to direct contact with Murrayite and not Euparal. Disney advocates Glyceel, Trycolac and "ladies" nail varnish with or without colour". Paraffin wax is a secondary sealant which can be smeared over the first sealant to reinforce the seal. Canada balsam, red Glyptal insulating varnish and Zut have, with other shellac and asphalt based cements, been used successfully at the NHM.

#### 3.7 Adhesives.

The NHM Palaeontology and Mineralogy laboratories use a number of synthetic adhesives to glue sections and small specimens on to microscope slides. This specialist application is unrelated to the main requirements of natural history specimen mounting. Refractive index and permanency are important as also are the pH and the chemical constituents of the glue or mountant as such might erode or even destroy the specimen. The following media are presently used; Araldite epoxy resin, Cellofas, Crystalbond thermoplastic cement, Dymax ultra violet bonding resin, Elvacite, Epotek epoxy resin, Lakeside, Loctite ultra violet bonding resin and Petropoxy epoxy resin. Apart from Crystalbond which is a temporary glue, there has been no criticism in the survey regarding deterioration, and only the passage of time will show which are the most suitable.

#### 4. Literature survey & Bibliography.

A literature search was undertaken prior to the survey. Relevant papers on slide mountants in both microscopy and taxonomic journals were collected (and later augmented by the references given in the returned questionnaires). Many taxonomic papers had notes on mountants used for the organisms but such details were not reflected in the titles of the papers and so were not accessible to the computer searches which were carried out. A total of 250 books and papers were studied and included in the lengthy bibliography (of which only 28 were volunteered through the questionnaire). This bibliography and the list of 150 mountants and adhesives and published recipes gleaned from the papers (Appendix 1) are as important as the survey itself as few such lists exist. Gray (1954), Lillie et al. (1952) Barbosa (1974), Loveland & Centifanto (1986), and Upton (1993) study mountants in detail, Gray listing 243 mountants and adhesives of which many are archaic. Comprehensive works reviewing relevant mountants and slide mounting

techniques required for the study of specific, or for many different groups of organism included Hood, 1940 (insects), Johansen 1940, (plants), Wagstaffe & Fidler, 1970 (invertebrates), Knudsen, 1972 (plants and animals), Martin, 1977 (insects), Smithers, 1981 (insects), Steyskal, Murphy & Hoover, 1986 (insects) and Higgins & Thiel, 1988 (marine invertebrates). With the published information the author could then compare information from the returning questionnaires and draw conclusions from the collected data. Within this bibliography almost every organism is mentioned and the aim was for the reader of the dissertation to be able to choose a mountant and preparation technique suitable for their requirement.

## 5. Conclusion. Which are the best mountants and what future have slide collections?

How safe will the microscope slide collections be in the future? Legal obligations protect collections from disposal but what of the insidiously deteriorating mountants? The preparator must carefully consider all the risks involved and balance the current needs for optical quality with long term preservation.

With little guidance in the general museum literature on the merits of different mounting media, one must refer to the specialised literature covering the study of specific organisms (see the bibliography) to decide on the most suitable mountant. Descriptions of latest techniques are often difficult to find, as they are often appended to papers on taxonomy in specialist journals which are not widely available. Organisms react in different ways to different mounting methods. Some become too fragile or too rigid in the process to make good mounts.

Discussion will continue about the optical requirements of different organisms but the consensus of opinion is turning away from experimentation with new mounting media (except in the high powered experimental end of electron microscopy) as more and more slides are found to be deteriorating. It must be stressed that many mountants should not be considered permanent and that routine monitoring of collections should be standard procedure as with spirit and dry collections. Likewise, environmental conditions should be constant with regular monitoring as light, desiccation and freezing can have deleterious effects on many mountants. Other synthetic resin and gum chloral recipes which produce perfect slides both at present and in 20 years time, may deteriorate in 50 years time. The cost of employing staff to remount slides is expensive and may not be considered affordable now or in the future. This may also apply to the routine re-ringing of liquid or jelly mounts but some organisms demand the use of less permanent mounts, such as glycerol and karo syrup. The newer plastic adhesives used for plant and mineral sections are as yet untested by the passage of time and require staff to monitor them. The NHM has stopped the practice of clearing specimens in chloral phenol before mounting in gum chloral mountants and will continue and increase the use of Euparal and Canada balsam, especially if the latter can be thinned with the safe solvent Histoclear instead of xylene as they need relatively little future conservation monitoring. These two mountants should be used for primary types especially with the use of staining of specimens and phase contrast microscopy to overcome the

refractive index problem. Field tests of new mountants will continue with Histomount, dimethyl hydantoin formaldehyde and Entellan showing signs of being suitable preserving media.

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#### 7. Appendix 1: Mountant recipes

When faced with a set of deteriorating microscope slides one needs to find out which mountant was used and what active ingredients and environmental factors have caused the deterioration. Here follows a list of mountants with recipes when known. Some proprietary brands have "secret" recipes and are chemically unidentified. Recipes are taken from the liturature and the references are cited (within brackets). Mounting media which are underlined are presently in use at the NHM. Sources of supply are given [in square brackets].

#### NATURAL RESINS

<u>Canada balsam</u> = resin of *Abies balsamea* in Xylene or native.

(Barbosa, 1974: 86) (Baylak, 1986: 311) (Clastrier, 1984: 175) (Cooper, 1988: 228) (Cutler, 1978: 6) (Essig, 1948: 16) (Gates Clarke, 1941: 150) (Green, 1995: 161) (Hangey, 1985: 147) (Heming, 1969: 323) (Hood, 1940: 53) (Huys & Boxshall, 1991: 451) (Johansen, 1940: 110) (Knudsen, 1966: 501) (Kozarhevskaya, 1968: 146) (Martin, 1983: 411) (Moore, 1979: 494) (Mosely, 1943: 231) (Mound & Pitkin,

1972: 122) (Noyes, 1982: 329; 1989: 2) (Oldroyd, 1970: 137) (Palma, 1978: 432) (Purvis, 1964: 106) (Rattanarithikul, 1982: 148) (Rawlins, 1992: 56) (Remaudiere, 1992: 185) (Richards, 1964: 963) (Saito, et al., 1993: 593) (Smithers, 1981: 77) (Stehr, 1987: 16) (Wagstaffe, 1970: 174) (Wilkey, 1990: 349). [BDH (Gurr) and Hopkins & Williams].

<u>Phenol balsam</u> = Canada balsam thinned with phenol alcohol (without Xylene). (Barbosa, 1974: 86) (Boorman & Rowland, 1988: 65) (Lane & Crosskey, 1993: 113) (Wirth & Marston, 1968: 783) (Young & Duncan, 1994: 8).

**Gum dammar** (*Agathis dammara & Hopis shorea* resin, xylene soluble) = damar (Barbosa, 1974: 86) (Essig) (Hood, 1940: 54) (Lillie, 1953: 59).

**Diaphane** = juniper gum & phenols, (Batte, 1948: 524) (Hood, 1940: 56) (Lillie, 1953: 64).

**Euparal** (Colourless not green Euparal vert) = Eucalyptus oil & methyl salicylate, camsal, sandarac, paraldehyde.

(Batte, 1948: 524) (Bink, 1979: 160) (Cutler, 1978: 6) (Disney, 1994: 387) (Essig, 1948: 17) (Hangey, 1985: 147) (Herr, 1982: 164) (Hood, 1940: 55) (Knudsen, 1966: 501) (Purvis, 1964: 108) (Ramanna, 1973: 103) (Rattanarithikul, 1982: 148) (Rawlins, 1992: 56) (Robinson, 1976: 130) (Smithers, 1981: 78) (Steyskal, 1986: 36)) (Wagstaffe, 1970: 175) (Wahl, 1989: 181) (Westheide & Purschke, 1988: 152) (Wilkey, 1990: 349) [ASCO Lab and BDH (Merck)].

Lenzol = (Cedar oil), (Gurr, 1963: 88).

**Piperine balsam** = alkaloid of *Piper nigrum* in chloroform (Frison, 1955: 64).

#### SYNTHETIC RESINS

Araldite MY753 epoxy resin

(Monniot, 1988: 463) [ B & K Resins Ltd].

Aroclor chlorinated diphenyl polymer (Fidiam, 1993: 9) (Frison, 1955: 206) (McLaughlin, 1986: 287) (Wicks, 1946: 121).

B-72 (Morse, 1992: 4).

<u>Caedex</u> (cyclohexanol formaldehyde + plasticiser chlorinated diphenol + xylene) (Lillie, 1953: 69).

**Carbowax** = (Polyethylene glycol).

<u>Carboxy methyl cellulose</u> CMC = Cellofas & Turto		
(water based)		
Methyl cellulose	5 g.	
Carbowax (PEG)	2 g.	
Diethylene glycol	1 ml	

Diethylene glycol	1 ml.
Ethyl alcohol 95%	25 ml.
Lactic acid	100 ml.
Distilled water	75 ml.

(Barbosa, 1974: 90) (Becker & Heard, 1965: 234) (Beckett, 1982: 97) (Beer, 1954: 1111) (Clark & Morishita, 1950: 789) (Evans, 1961: 82) (Hobson & Banse, 1981: 6) (Holdich & Jones, 1983: 20) (Huryn & Perlmutter, 1988: 432) (Knudsen, 1966: 501) (Krantz, 1978: 89) (Martin, 1977: 109) (New, 1974: 23) (Singer, 1967: 483) (Stehr, 1987: 16) (Walker & Crosby, 1988: 23) [BDH].

**Clarite** (naphthalene polymer) (Essig, 1948: 19) (Lillie, 1953: 66) (Newell, 1947: 12) (Wicks, 1946: 122).

Clark's Clearcol (Chapman, 1985: 117). Crystalbond 509 thermoplastic cement [Meclec Co.]. Coumarone naphthalene polymer (Frison, 1955: 40) (Lillie, 1953: 67). **DePeX** = (Distrene plasticiser xylene) (polystyrene) (Chapman, 1985: 117) (Gagne, 1994: 35) (Lillie, 1953: 65) (Rawlins, 1992: 56) (Westheide & Purschke, 1988: 152) [BDH (Gurr)]. Distrene in xylene + dibutyl phthalate (plasticiser) (polystyrene). Dymax 304 u.v resin [Intertronics]. Elvacite [ BDH]. Elvanol = Polyvinyl alcohol. Entellan synthetic mounting medium by Merck (Rawlins, 1992: 57). Epon/Araldite epoxy resin Dodecenvl succinic anhydride 10 ml. 6.2 ml. Epon 812 Araldite 506 8.1 ml. Dibutylpthalate 0.75 ml. 2,4.6-tri (dimethyl-aminomethyl phenol) 25 ml. (Rieger & Ruppert, 1978: 226) (Smith & Tyler, 1984: 261). Epon epoxy resin Dodecenyl succinic anhydride 100 ml. Epon 812 62 ml. +7 parts of solution Nadic 89 ml. methyl anhydrite Epon 812 100 ml. +3 parts of solution 2,4,6-tri (dimethyl-aminomethyl phenol) 1.5% (Cavey & Cloney, 1973: 150). Epotek 301 epoxy resin [Intertronics]. Epoxy resins (Loveland: 1986). Eukitt salicylic acid imbalance caused precipitation of crystals (personal observation K.R.C. Tuck). Gurr's neutral mounting medium (coumarone and other resins in eucalyptol) = Clearmount (Hockin, 1981: 222) (Huys & Boxshall, 1991: 451) (Lillie, 1953: 68). Hymount Hyrax naphthalene resin (Barr, 1973: 16) (Bartsch, 1988: 419) (Beer, 1954: 1111) (Evans, 1961: 82) (Frison, 1955: 64) (McLaughlin, 1986: 287) (Newell, 1947: 7-9) (Singer, 1967: 475) (Wicks, 1946: 121). Impruv Potting Compound 363 (Loctite) ultra-violet light polymerising plastic (Silverman, 1986: 135). Impruv Sealant/adhesive 365 (Loctite) ultra-violet light polymerising plastic (Silverman, 1986: 135). Klearmount epoxy resin (Knudsen, 1966: 501) (Lane, 1974: 7) (Lillie, 1953: 69) (Loveland, 1986: 187). Lakeside 70C thermoplastic cement (Murray, 1979: 11) [BDH]. Loctite 358 u.v resin [BDH and BSL Ltd]. Lucite (44 & 46) methyl methacrylate plastic (Frison, 1955: 209) (Wicks, 1946: 121). Lactic acid

Malinol (Westheide & Purschke, 1988: 152) (Westheide, 1990: 43). Meltmount (DeForest, 1987: 154). Mulford EX-80 polyester resin (Blackburn & Christophel, 1976: 126). Naphrax glacial acetic acid 300 ml. sulphuric acid 100 ml. Naphthalene 100 g. Formaldehyde (37% solution) 100 ml. (Fleming, 1943: 35; 1954: 42) (McLaughlin, 1986: 287) (Ragge, 1955: 8) [NBS]. Novolacs (Union Carbide BRPB 5215) = Phenolic polymer resin (Crumpton, 1980: 347). Numount (Barton, 1991, 17) (DeForest, 1987: 253) [R.A.Lamb]. Permount (hydrogenated terpene naphthalene polymer) (Herr, 1982: 164) (Knudsen, 1966: 501) (Lillie, 1953: 66) (Stehr, 1987: 16) (Wicks, 1946: 122) [R.A.Lamb]. Petropoxy 154 epoxy resin [ BDH and Production Techniques]. Piccolyte (polyterpene polymer resin, xylene soluble) (Frison, 1955: 66) (Herr, 1982: 164) (Knudsen, 1966: 501) (Wicks, 1946: 121). Pleurax Phenol crystals 100 g. sulphur 40 g. sodium sulphide crystals 2 g. isopropyl alcohol 100 ml. (Hepworth, 1994: 21). Polybed 812 (Ruppert, 1988: 306). Polyethylene glycol (Carbowax) (PEG) epoxy resin (Walker & Crosby, 1988: 23) (New, 1974: 23.) Polystyrene & Methylene iodide Polystyrene 15.75 g. Toluene 50 ml. Methylene iodide 200 g. (Czarnecki, 1972: 73). **Polyvinyl** acetate Cellosolve 68% Diamyl phthalate 12% Polyvinyl acetate 20% (Walker & Crosby, 1988) (Ried, 1994) Polyvinyl alcohol (Danielsson) PVA Mowiol N 4-982 5 g. PVA Mowiol N 56-98 5 g. 30 ml. Ethanol 105 ml. Lactic acid Distilled water 105 ml. (Danielsson, 1985: 383) (Heikinheimo, 1988: 36). Polyvinyl alcohol (=Elvanol ex DuPont) Polyvinyl alcohol (low viscosity) 2 g. 70% acetone 7 ml. Glycerine 5 ml.

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5 ml.

Distilled water	10 ml.
(Brown, 1951: 263) (Essig, 1948: 18) (Gray, 1	950: 290)
(Jones, 1946: 85) (Ribiero, 1967: 158).	
Polyvinyl alcohol Ribeiro	
Polyvinyl alcohol	130 g.
Formic acid	40 ml.
Distilled water	40 ml.
	50 mi.
(Barbosa, 1974: 96) (Ribeiro, 1967: 159).	
Polyvinyl alcohol Ribeiro II	
Polyvinyl alcohol	6 g.
Formic acid	10 ml.
Chloral hydrate	72 g.
Phenol	12 g.
(Barbosa, 1974: 97).	C
Polyvinyl alcohol Salmon	
	5 0
Polyvinyl alcohol (Elvanol 71/24)	5 g.
Lactic acid	10 ml.
glycerine	1 ml.
distilled water	30 ml.
(Salmon, 1954: 66).	
Polyvinyl lacto glycerol	
Polyvinyl alcohol	16.6g.
Lactic acid	100ml.
Glycerol	10ml.
distilled water	100ml.
(Halliday, 1994: 12)	room.
Polyvinyl lactophenol	
Polyvinyl alcohol (Elvonol A)	2.5 g.
Lactic acid	45 ml.
Phenol crystals	45 g.
Distilled water	10 ml.
(Athersuch et al. 1989: 38) (Barbosa, 1974: 98	) (Bellinger,
1953: 170) (Bink, 1979: 160) (Henderson, 199	
(Hodkinson & White, 1979: 3; semi-permanen	
1977: 171) (New, 1974: 23) (Nosek, 1973: 84)	
1977: 163, [Chroma Gesellschaft Schmid & C	
1961: 377) (Salmon, 1949: 250) (Wagstaffe, 19	
[Gurr].	//0.1/0)
Polyvinyl lactophenol Downs	
Elvanol PVA solution	56 ml.
Lactic acid	22 ml.
Phenol crystals	22 ml.
(Barbosa, 1974: 96) (Evans, 1961: 81) (Essig,	1948: 19)
(Beer, 1954: 1111) (Downs, 1943: 539) (Lipov	sky, 1953:
43).	And a state of the state of the
Polyvinyl lactophenol Jones	
	63 0
Polyvinyl alcohol	6.3 g.
100% alcohol saturated picric acid	18 ml.
Lacto phenol	45 ml.
(Beer, 1954: 1111) (Jones, 1946: 85).	
Polyvinyl lactophenol Heinze (used at Harper	nden)
Polyvinyl alcohol	10 g.
Chloral hydrate	20 g.
Glycerol	20 g.
	20 g. 10 ml.
Phenol 1.5% solution	
Phenol 1.5% solution Lactic acid	10 ml. 25 ml.
Lactic acid	10 ml. 25 ml. 35 ml.
Lactic acid Distilled water	10 ml. 25 ml.
Lactic acid Distilled water (Heinze, 1953: 177) (MacFarlane, 1991: 80).	10 ml. 25 ml. 35 ml.
Lactic acid Distilled water	10 ml. 25 ml. 35 ml.

Letter a state of the second s	2 RAPIT
Polyvinyl pyrrolidone	50 g.
glycerol	2 g.
Distilled water (Steedman, 1976: 191).	50 ml.
<b>Pro-Texx</b> (DeForest, 1987: 253).	
<b>Realgar</b> (McLaughlin, 1986: 287) (Spence, 19	40. 270)
Sira (Wagstaffe, 1970: 176).	40.270).
Spurr low viscosity embedding medium epo	xy resin
Vinylcyclohexane	10 ml.
Diglycidyl ether of polypropyleneglycol	6 ml. 26 ml.
Nonenyl succinic anhydride (Herr, 1982: 164).	20 mi.
Steedman's post-fixation preservative, PFP	
Propylene phenoxetol	5 ml.
Propylene glycol	50 ml.
Distilled water (Moore, 1979: 494).	445 ml.
Styrax (Fidiam, 1993: 9) (Frison, 1955: 63) (N	AcI aughlin
1986: 287) (Spence, 1940: 266).	neLauginin,
Sucrose benzoate	
Sucrose benzoate	60 g.
Polyethylene glycol 600 benzoate	0.25 ml. 1.75 ml.
Benzyl benzoate Xylene	1.75 ml. 38 ml.
(Steedman, 1976: 193).	50 m.
<b>Technicon mounting medium</b> (Westheide & 1988: 152).	Purschke,
<b>Tissue-Tek</b> = Polyvinyl alcohol and polyethyl	ene glycol mix
(Rawlins, 1992: 51).	6,
Xam maleic polymer terpene resin in xylene + (Gurr, 1963: 87) (Lillie, 1953: 69) (DeForest,	
Zeiss W15 (Huys & Boxshall, 1991: 451) (We Purschke, 1988: 152).	estheide &
GUM CHLORAL MOUNTANTS (Gray, 19: 1993).	54) (Upton,
"Andre's" fluid	
Gum Arabic	50 g.
Chloral hydrate	125 g.
Glacial acetic acid Distilled water	50 ml. 50 ml.
(Cunningham, 1972: 906) (Krantz, 1978: 86) (	
169).	
Baylis & Munro	
Gum Arabic Chloral hydrate	15 g. 16 g.
Glucose syrup	10 g. 10 g.
Glacial acetic acid	5 ml.
Distilled water	20 ml.
(Smart, 1965: 287).	
<u>"Berlese"</u> = Imms medium = Lee 1921= Day	
Gum Arabic Chloral hydrate	15 g. 160 g.
Glucose	100 g.
Acetic acid	5 ml.
Distilled water	20 ml.
(Barbosa, 1974: 93) (Essig, 1948: 14) (Gater, (Hood, 1940: 44) (Martin, 1977: 169) (Upton,	

(Wagstaffe, 1970: 175) (Walker & Crosby, 198	38: 79)	Ho
(Womersley, 1943: 181)[ASCO Lab].		Gui
Doetschman		Chl Gly
Gum Arabic	20 g.	Dis
Chloral hydrate	20 g.	(Ba
Glycerin	20 ml.	(Fa
Glucose syrup Distilled water	3 ml. 35 ml.	352
(Barbosa, 1974: 94) (Doetschman, 1944: 177)		199
(Doctsennian, 1944, 177) 268).	(Opton, 1995.	199
Eastop & Van Emden		198
Gum Arabic	48 g.	(Wa
Chloral hydrate	48 g. 80 g.	Jep
Glacial acetic acid	20 ml.	Gui
glucose syrup (50%)	20 ml.	Chl
Distilled water	120 ml.	Gly
Eastop, 1972: 8) (Upton, 1993: 268).		Sor
Ewing		Dis
Gum Arabic	20 g.	(Jep
Chloral hydrate	30 g.	Pu
Glycerin	12 ml.	Gui
Glucose syrup	3 ml.	Chl Gly
Distilled water	35 ml.	Gla
(Barbosa, 1974: 96) (Doetschman, 1944: 176)	(Upton, 1993:	Dis
268).		(Hc
Faure's		196
Gum Arabic	30 g.	Rey
Chloral hydrate	50 g.	Gui
Chloralhydrate of cocaine (optional)	0.5 g.	Chl
Glycerine	20 ml.	Gly
Distilled water (Berhause, 1074, 02) (Birth, 1070, 160.) (Europe	50 ml.	Dis
(Barbosa, 1974: 92) (Bink, 1979: 160) (Evans (Hood, 1940: 46) (Krantz, 1978: 89) (MacFarla		(Ha
(Martin, 1977: 170) (Morgan & King, 1976: 24		Roo
1993: 268) (Wagstaffe, 1970: 176) (Walker & C	Crosby 1988	Gui
80).	010009, 19000.	Chl
Foulkes (Foulkes, 1983: 211)		Gly
Gum Arabic	16 g.	Dis
Chloral hydrate	140 g.	(Hi
Glycerine	10 ml.	192
Acetic acid	6 ml.	Sin
Distilled water	16 ml.	Gui
Higgins' 1983		Chl
Gum Arabic (crystals)	30 g.	Gly Dis
Chloral hydrate	100 g.	(Br
Glycerine	20 g.	196
Iodine crystals	2 g.	Str
Potassium iodide	1 g.	Gur
Distilled water	50 ml.	Chl
(Higgins, 1983) (Huys & Boxshall, 1991: 451)		Glu
& Higgins, 1984: 3) (Westheide & Purschke, 1	700: 139).	Ace
Hoyer's "125"	20	Dis
Gum Arabic	30 g.	(Bla
Chloral hydrate	125 g.	11)
Glycerine Distilled water	20 ml. 50 ml.	194
		Swa Gur
Macquitty, 1987: 15) (Higgins 1983) (Westheid Purschke, 1988: 152).		Chl Glu
1 urselike, 1900. 132).		Giù

Hoyer's Gum Arabic (crystals) Chloral hydrate Glycerine Distilled water (Barbosa, 1974: 86) (Beer, 1954: 1111) (Evans (Fain 198X: 169, repairing old mounts) (Gutien 352) (Jeppson, 1975: 116) (Krantz, 1978: 88) ( 1991: 78) (Martin, 1977: 169) (Pritchard, 1955 1992: 427, temporary) (Soriguer et al., 1993: 1 1987: 16, questionably permanent) (Upton, 199	rrez, 1985: MacFarlane, : 2) (Saito, 13) (Stehr,
(Wahl, 1984: 228) (Walker & Crosby, 1988: 80 Jeppson et al. Gum Arabic Chloral hydrate Glycerine Sorbitol Distilled water (Jeppson, 1975: 388) (Upton, 1993: 268). Puri's	20 g. 125 g. 30 ml. 30 g. 50 ml.
Gum Arabic Chloral hydrate Glycerine Glacial acetic acid Distilled water (Hopkins, 1952: 31) (Kirk & Lewis, 1951: 500 1965: 287) (Upton, 1993: 268).	8 g. 70 g. 5 ml. 3 ml. 10 ml. ) (Smart,
Reyne Gum Arabic Chloral hydrate Glycerine Distilled water (Hamond, 1969: 145) (Reyne, 1950: 40) (Wells Roepke	60 g. 100 g. 25 g. 100 ml. 5, 1988: 383).
Gum Arabic Chloral hydrate Glycerine Distilled water (Hille Ris Lambers, 1949: 57) (Heie, 1980: 70) 1929: 918).	12 g. 20 g. 6 ml. 20 ml. (Roepke,
Singer ("Andre's fluid" ?) Gum Arabic Chloral hydrate Glycerine Distilled water (Bretfield, 1991: 218) (Conde, B.V.N., 1988: 42 1967: 483) (Upton, 1993: 268).	50 g. 125 g. 30 ml. 50 ml. 26) (Singer,
Stroyan (Berlese) Gum Arabic Chloral hydrate Glucose syrup Acetic Acid Distilled water (Blackman, 1974: 123) (Disney, 1983: 8) (Free 11) (Henshaw, 1981: 206) (Smith, 1973: 173) ( 1949: 6) (Upton, 1993: 268) (Withers, 1989: 12	Stroyan,
Swan's (Berlese) Gum Arabic Chloral hydrate Glucose syrup	15 g. 60 g. 10 g.

Glacial acetic acid	5 ml.		
Distilled water	20 ml.		
(Barbosa, 1974: 95) (Nosek, 1973: 84) (Smart, 1965: 287) (Upton, 1993: 268).			
Womersley 1939 (Berlese)	10 ~		
Gum Arabic	12 g.		
Chloral hydrate	20 g. 4 ml.		
Glucose syrup	9		
Acetic acid Distilled water	10 ml. 30 ml.		
	50 mi.		
(Womersley, 1939) (Upton, 1993: 268).	3		
Womersley 1943	10		
Gum Arabic	40 g.		
Chloral hydrate	50 g.		
Phenol	50 g.		
Glucose syrup	10 g.		
Glacial acetic acid	20 ml.		
Distilled water	100 ml.		
(Womersley, 1943: 181).	j.		
Celochloral			
Celodal 1(Beyer mount)	100 g.		
Chloral hydrate	120 g.		
Glucose	20 g.		
Glacial acetic acid	20 ml.		
Distilled water	100 ml.		
(Muller, 1961: 70) (Ossiannilsson, 1958: 2).			
Keifer's Formaldehyde medium			
Gum Arabic	1 g.		
Chloral hydrate	14 g.		
Glycerine	l ml.		
Sorbitol	3 g.		
Formaldehyde 4% solution	5 ml.		
Potassium iodide	2 ml.		
Iodine	2 ml.		
(MacFarlane, 1991: 80).			
OTHER AQUEOUS MOUNTAN	NTS		
Apathy's fluid			
Gum Arabic	50 g.		
Sucrose	50 g.		
Distilled water	50 ml.		
Thymol	0.05 g.		
(Grimstone, 1972: 58) (Pantin, 1954: 20) (Stee	edman. 1976:		
189).	8		
Aquamount (Oliver & Meechan, 1993: 17)	8 E		
[BDH (Gurr) and R.A.Lamb].			
Dextrose (Monk, 1938: 174).			
Dimethyl hydantoin formaldehyde (DMHF) resin.	synthetic		
Dimethyl hydantoin formaldehyde	70 g.		
Ethyl alcohol	70 ml.		
Distilled water	30 ml.		
(Bameul, 1990: 233) (Barbosa, 1974: 99) (Coc	oter, 1991: 57)		
(Smith, 1966: 177) (Steedman, 1958: 451; 197	6: 189)		
[Chemical Intermediates Co Ltd,].	·- 8		
Farrant's medium			
Gum Arabic	40 g.		
Glycerol	20 g.		
Distilled water	40 ml.		

LITTLE CHART FOR THE REAL PROPERTY OF THE PARTY OF THE PA	
(Grimstone, 1972: 58) (Hood, 1940: 45).	
Giovacchini's Gelatine	
Glycerol	50 ml.
Gelatine	15 g.
Phenol Distilled water	0.5 g. 55 ml.
(Purvis, 1964: 105).	55 mi.
Glycerine Jelly (Singer)	
Glycerine	80 g.
Gelatine	30 g.
Arsenic trioxide saturated solution	150 ml.
(Curry, 1982: 44) (Evans, 1961: 83) (Even (Garner & Horie, 1984: 93) (Hooper, 1970 501) (Pantin, 1964: 21) (Rawlins, 1992: 50 483) (Spence, 1940: 143) (Taft, 1983: 399	)) (Knudsen, 1966: 6) (Singer, 1967:
Glycerine with Ammonium picrate.	
Glycerine Jelly (Kaiser)	
Glycerine	100 ml.
Gelatine Phenol	15 g. 0.25 g.
Distilled water	100 mL
(Frison, 1955: 169) (Hooper, 1970) (Joha (Pantin, 1964: 21) (Steedman, 1976: 190).	
Glycerine jelly	
Glycerine	50 ml.
Gelatine	6 ml.
Phenol crystals Distilled water	2 g. 42 ml.
(Barbosa, 1974: 92) (Grimstone, 1972: 58)	
Glycerine jelly	
Glycerine	54g
Gelatine	10g
Phenol	0.5g
Distilled water (Wiles, 1989: 245)	50 ml
PROFESSION AND AND AND AND AND AND AND AND AND AN	
Glycerine, anhydrous Glycerine	5 ml.
absolute ethyl alcohol	5 ml.
Distilled water	90 ml.
(Chapman, 1985: 113) (Collinson, M.E., 1 (Erdtman, 1960: 563) (Green, 1995: 161) (Hayward, 1985: 30) (Huys & Boxshall, 1 (Maybury et al, 1991: 16) (Moore et al., 1 Warwick, 1983: 20) (Pleijel, 1991: 21) (Pr (Ward, 1944: 200) (Wilson, 1971: 139).[F Williams, Gurr (BDH)].	(Hood, 1940: 46) 991: 451) 991: 45) (Platt & rescott, 1970: 13)
Glycerine/10% formalin (Pleijel, 1991: 2	21).
Hydramount (Chapman, 1985: 117) (Gur	т, 1963: 89).
Karo syrup = dextrose	
White Karo corn syrup	10 ml.
Thymol (1% alcohol solution)	1 ml.
Certo (Knudsen 1072: 23) (Loveland 1086: 213	2 ml. 3) (Taft 1978)
(Knudsen, 1972: 23) (Loveland, 1986: 213 263).	9) (1an, 1978:
Keifer's Karo mount Starch-free white Karo syrup	12 ml.
Chloral hydrate crystals	60 g.
Potassium iodide crystals	0.5 g.
an <b>2</b>	255

Microscope	Slides —	Paul A	Brown
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Iodine crystals	2 g.			
Formaldehyde solution	10 ml.			
(Evans, 1961: 84).	1000			
Sodium silicate (water glass) (Bridson & Forman, 1992) (Gerakaris, 1984: 262) (Quisumbing, 1931: 45) (Spence, 1940: 236).				
FLUID MOUNTS (Gisin, 1968: 1) (Spence, 1 Essig's fluid	940: 114).			
Lactic acid	20 parts			
Phenol (saturated in distilled water)	2 parts			
Glacial acetic acid	4 parts			
Distilled water (Walker & Crosby, 1988: 80).	1 part			
Formaldehyde (Diegues & Montero, 1992: 31 (Farrington, 1989: 22).	5)			
Formalin Prescott, 1970				
Formalin	10 ml.			
Ethyl alcohol 95%	30 ml.			
Distilled water	60 ml.			
(Prescott, 1970: 12).				
2% Formalin + 1% cupric acetate solution.	NATION HARDEN HAR			
Formalin-acetic acid-alcohol (Prescott, 1970: 1978).	12) (Wells,			
Gisin's fluid				
Lactic acid	179 ml.			
Glycerol Glycerol + saturated picric acid	36 ml. 28 ml.			
Formaldehyde (40%)	28 ml. 7 ml.			
(Fjellberg, 1980: 9) (Nosek, 1973: 84).	<i>i</i> m.			
Lactophenol				
Phenol	20 ml.			
Lactic acid	20 ml.			
Glycerine	40 ml.			
Distilled water (Hooper 1970: 44) (Smart 1965: 287) (Wells	20 ml.			
(Hooper, 1970: 44) (Smart, 1965: 287) (Wells, 1978) [Hopkins & Williams].				
Lactophenol				
Phenol	30 ml.			
Lactic acid	10 ml.			
Glycerol	20 ml.			
Distilled water	10 ml.			
(Huys & Boxshall, 1991: 451).				
Monobromonaphthalene (Frison, 1955: 204).				
Potassium hydrargiodide (Frison, 1955: 205)				
<b>Soft paraffin</b> = Vaseline etc. (Spence, 1940: 1	15).			
Ripart & Petit's fluid				
Camphor water	75 ml.			
Acetic acid	1 ml. 0.3 g.			
Cupric acetate Cupric chloride	0.3 g.			
Distilled water	75 ml.			
(Pantin, 1964: 23).	in al particular in			
Silicone oil (Chapman, 1985: 118) (Loveland a 1986: 224).	& Centifanto,			
DRY MOUNTS				
(Barton, 1991: 17) (Curry, 1982: 42) (Green, 1	995: 162)			
(TI 1 1040 40)				

Black well slides (Athersuch et al. 1989: 39) (Hayward & Ryland, 1979: 34; 1985: 29). **RINGING MOUNTANTS & ADHESIVES** (Disney, 1988: 06) (Gerakaris, 1984: 259) (Gisin, 1968: 1). **APTES = y-aminopropyl triethoxysilane** Sigma tissue section bonding agent. (Rawlins, 1992: 55). Araldite (Huys & Boxshall, 1991: 451). Asphalt (Farrington, 1989: 22) (Garner & Horie, 1984: 93) (Johansen, 1940: 120) (Spence, 1940: 141). **Baker's** albumin Aqueous sodium chloride 1% 100 ml. Sodium p-hydroxybenzoate 0.2 g. Fresh egg white 100 ml. Bell's cement = black nitrocellulose lacquer (Garner & Horie, 1984: 93). Bioseal (Huys & Boxshall, 1991: 451). Bitumen in toluene (Moore) (Spence, 1940: 141). Canada balsam (Eastop & van Emden, 1972: 8) (Fjellberg, 1980: 9, unsuitable as it becomes brittle) (Johansen, 1940: 120) (Martin, 1977: 109) (Morgan & King, 1976: 24). Cellulose nitrate = Celloidin 2-10 % solution in ethyl acetate, plant section adhesive for cryostat, paraffin and glycol methacrylate sections. (Fink, 1987: 98). Clarite (Evans, 1961: 83). Cyanoacrylate adhesives (Rawlins, 1992: 51). Dammar (Garner & Horie, 1984: 98) (Henshaw, 1981: 206). Eukitt (Fjellberg, 1980: 9). Euparal (Freeman, 1983: 11) (Gutierrez, 1985: 353) (MacFarlane, 1991: 81) (Martin, 1977: 109). **Glyceel** = linseed oil, alcohol, nitrocellulose, butyl acetate & toluol = Zut. (Disney, 1983: 8) (Gutierrez, 1985: 353) (Hooper, 1970: 51) (Huys & Boxshall, 1991: 451) (MacFarlane, 1991: 81) (Martin, 1977: 109). Glyptal (red alkyd enamel resin insulation paint made by General Electric) (Foulkes, 1983: 211) (MacFarlane, 1991: 81) (Martin, 1977: 109) (Travis, 1968: 24) (Wahl, 1984: 228) (Wu, 1986: 87). Haupt's adhesive (for plant sections) Glycerol 15 ml. Gelatine 1 g. 2 g. Phenol crystals Distilled water 100 ml. (Johansen, 1940: 150) (Purvis, 1964: 105). Johansen plant section adhesive Acetone 2 ml. Methyl benzoate 1 ml. Distilled water 8 ml. (Johansen, 1940: 125). Laktoseal (Morgan & King, 1976: 24).

Laktoseal (Morgan & King, 1976: 24).Mayer's adhesive (for plant sections)Fresh egg white50 ml.Glycerol50 ml.Thymol1 g.(Johansen, 1940: 125) (Purvis, 1964: 105).

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(Hood, 1940: 42).

#### Murrayite

(Eastop & van Emden, 1972: 8) (Essig, 1948: 20) (Hille Ris Lambers, 1949: 57) (Huys & Boxshall, 1991: 451).

**Nail varnish** thinned with butyl acetate-acetone (typical formulation ex Wells)

Nitrocellulose	10 g.
Resin	10 g.
Plasticiser	5 g.
Ethyl alcohol	10 ml.
Ethyl acetate	20 ml.
Butyl acetate	20 ml.
Toluene	25 ml.

(Disney, 1994: 388) (Fjellberg, 1980: 9) (Wells, 1978).

#### Norland optical adhesive

Polyisobutylene 5-10% in petroleum ether adhesive for carbowax plant sections. (Fink, 1987: 98).

#### Polyvinyl Alcohol plant section adhesive

Polyvinyl alcohol	2 g.
Vinyl-triethoxysilane	0.2 g.
Distilled water	100 ml.
Methyl benzoate	0.2 g.

Dilute to 110 to 150 with distilled water for working solution (Fink, 1987: 31).

Shellac (dewaxed in alcohol and plasticised with 1% of castor oil)

(Barton, 1991: 17) (Frison, 1955: 204) (Garner & Horie, 1984: 93) (Gerakaris, 1984: 259) (Gray, 1954: 651) (Johansen, 1940: 120).

#### Thorn ringing compound thinned with acetone

Nitrocellulose solution	2 parts
Polymerised linseed oil	1 part
(Essig, 1948: 20).	

Trycolac (Disney, 1988: 106).

Vaseline (temporary) (Prescott, 1970: 14).

**Vinyl-triethoxysilane** 0.1% solution, water soluble plant section adhesive. (Fink, 1987: 30).

Paraffin Wax	
White vaseline	12 parts
paraffin wax	10 parts
Anhydrous lanolin	10 parts
(G) : 10(0 () 01 1 1070 0	

(Gisin, 1968: 1) (Nosek, 1973: 84).

Xam (Fjellberg, 1980: 9).

#### STAINS

Stains were not part of the survey but here is a list of a few of the stains known to the author.

Acid Fuchsin (New, 1974: 22) (Saito et al., 1993: 597).

Fast Green (New, 1974: 23).

Lignin Pink (Evans, 1955: 632) (New, 1974: 23).

<u>Chlorazol Black E</u> (New, 1974, 23 This stain requires Polyvinyl lactophenol or Methyl Cellulose / Carbowax unlike the other 3) (Robinson, 1976: 129).

Evans Blue (Saito, 1993: 597).

Aniline Blue (Ramanna, 1973: 103).

Orange G (Lee, 1921) (Gray, 1954)

benzenazo-beta-naphthol-disulphonate of soda, water soluble and acidic.

#### 8. Appendix 2: Refractive Indices

8. Appendix 2: Refractive Indices				
MOUNTING MEDIUM	R.I.	Author		
air	1.00	(McLaughlin)		
water Polyvinyl alcohol	1.33 1.4	(Rawlins) (Heikinheimo)		
Glycerine 50% solution	1.4	(Reyne)		
Doetschman	1.415	(Gray)		
Hoyer	1.419	(Gray)		
Methyl cellulose fluid	1.428	(Clark)		
(CMC) Glycerol jelly	1.428	(Clark) (Loveland)		
Polyvinyl pyrrolidine		(250 / Finite)		
(PVP)	1.43	(Steedman)		
Diatom silica	1.434	(McLaughlin)		
Faure Polyvinyl Lactophenol	1.437	(Gray)		
HA 1	1.438	(Wagstaffe)		
Glycerine jelly	1.443	(Steedman)		
Polyvinyl Lactophenol MA 4	1.448	(Wagstaffe)		
DMHF (in water) DMHF (in alcohol)	1.457 1.466	(Barmeul) (Barmeul)		
Polyvinyl acetate	1.466	(Loveland)		
Borosilicate glas		·		
(perspex)	1.47	(Heikinheimo)		
Karo syrup	1.47	(Bennett)		
Swan gum chloral Glycerol	1.470 1.47	(Gray) (Rawlins)		
Cellulose acetate	1.48-1.50	(Fink)		
Euparal	1.48	(Rawlins)		
Berlese	1.485	(Gray)		
Pro-Texx	1.49	(DeForest)		
Formvar Histoclear	1.49 1.49-1.5	(Fink)		
Entellan	1.49-1.50	(Rawlins)		
Cellulose nitrate	1.49-1.51	(Fink)		
Polyvinyl alcohol	1 101 1 70			
(when dry) Lucite	1.491-1.53 1.50	(Loveland) (Bennett)		
Silicone	1.50-1.51	(Lillie)		
Piccolite	1.50-1.52	(Loveland)		
Polyisobutylene	1.50-1.51	(Fink)		
Permount	1.51	(Loveland)		
Clearmount = neutral mounting medium	1.51	(Gurr)		
Immersion oil (Zeiss)	1.51	(Rawlins)		
crown glass	1.51	(Doetschman)		
Zeiss W15	1.515	(Westheide)		
Apathy's Piccolyte	1.52 1.52-1.54	(Steedman) (Wicks)		
Gum dammar	1.52-1.55	(Lillie)		
Canada balsam	1.52-1.53	(Rawlins)		
DePeX	1.529	(Rawlins)		
Xam (terpene resin) Meltmount	1.52-1.54 1.539	(Lillie) (DeForest)		
fixed & clear cell	1.559	(Der orest)		
constituents	1.54	(Pantin)		
DMHF (pure resin)	1.54	(Steedman)		
Hydramount Clarite	1.54 1.544	(Gurr) ??? (Loveland)		
Diaphane	1.548	(Loveland)		
Sira	1.55	(Wagstaffe)		
Styrax	1.56-1.63	(Loveland)		
Caedax	1.57 1.57	(Loveland)		
Sucrose benzoate Coumarone	1.57	(Steedman) (Frison)		
Distrene	1.60	(Wicks)		
Coumarone + cinnamic aldehyde	1.63	(Frison)		
Novolacs	1.65	(Crumpton)		
Polystyrene Aroclor	1.60 1.63	(Loveland) (Wicks)		
Hyrax	1.65	(McLaughlin)		
Aroclor 5442	1.66	(McLaughlin)		
Clearax	1.66	(Gurr)		
Pleurax Piperine balcam	1.67 1.68	(Hepworth)		
Piperine balsam Naphrax (naphthalene formaldehyde)	1.08	(Frison) (Fleming)		
Polystyrene methylene iodide	1.75	(Czarnecki)		
Hyrax	1.82	(Pantin)		
Realgar	2.40	(McLaughlin)		

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N.B. Refractive index changes with temperature of the mountant and with the percentage loss of solvent from it. Canada Balsam as used by biologists dissolved in Xylene is 1.497 and dries to 1.532 (Loveland). Thus a specimen can become less visible after it was initially clear when the slide was first made.

#### 9. Appendix 3: Materials and suppliers

Addresses of suppliers to the Natural History Museum. Inclusion in this list does not indicate that the NHM endorses these particular suppliers or products.

Aquamount, Canada balsam, Cellofas, DePeX, Elvacite, Eukitt, Flourmount, glycerol jelly, lactophenol, Lakeside 70C, Loctite, Petropoxy, polyvinyl lactophenol, Xam neutral medium. Entellen, Euparal BDH Merck Hunter Boulevard Magna Park Lutterworth Leics. LE17 4XN. Tel. 0800 223344 Fax 01455 558586

Aquamount, Numount, Permount, Canada balsam R.A.Lamb Laboratory Supplies 6 Sunbeam Road LONDON NW10 6JL Araldite 753 & 951 B & K Resins

B & K Resins Unit 2, Ashgrove Estate Bromley Kent BR1 4TH

Bisley (15) multidrawer paper storage cabinets Niceday business supplies Pondtail Close Horsham West Sussex RH12 5HW

Euparal, Berlese ASCO Laboratories 52 Levenshulme Road Gorton Manchester M18 7NN

Crystalbond Meclec Co 5-6 Towerfield Close Shoeburyness Essex SS3 9QP

Dimethyl hidantoin formaldehyde Chemical Intermediates Co Ltd Barnfield Industrial Estate Leek Staffs ST13 5QG Dymax 304 & Epotek 301 Intertronics

Unit 9

Station Field Industrial Estate Banbury Road Kidlington Oxon OX5 1JD Histomount, Histoclear, Hydromount National Diagnostics (UK) Ltd Unit 4 Fleet Business Park **Itlings** Lane Hessle, Hull HU13 9XL Tel. 01482 646022 Fax. 01482 646013 Hill Unit Cabinets Stephenson Blake 199 Upper Allen Street SHEFFIELD S3 7GW Tel. 0114 2728325 Fax 0114 2720065 Loctite 358 BSL Ltd 1120 London Road Norbury LONDON SW16 4DT Petropoxy 154 **Production Techniques** 13 Kings Road Fleet Hants GU13 9AV Plastic slide covers Preservation Equipment Ltd Church Road Shelfanger Diss Norfolk **IP22 2DG** Tel 01379 657527 Fax 01379 650582

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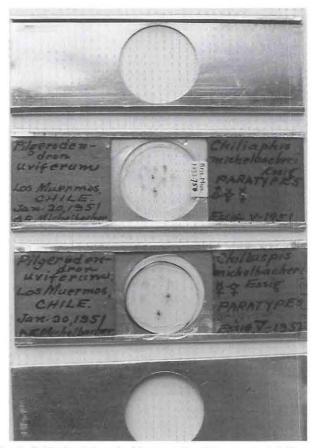


Fig. 1 Cobb aluminium double coverslip slide mounts, Aphid collection, Entomology dept, NHM.

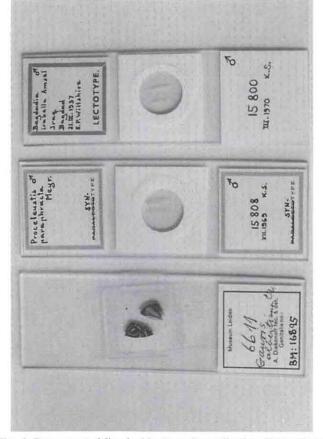


Fig. 3 Dry mounts, Microlepidoptera wing collection, Entomology dept, NHM

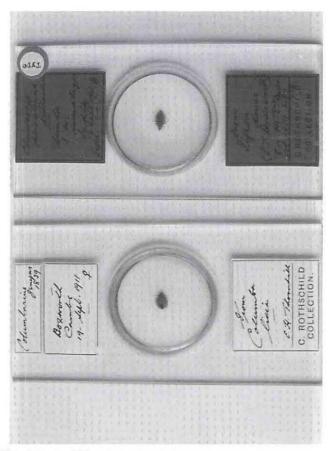


Fig. 2 Rothschild perspex well mounts, Heteroptera collection, Entomology dept, NHM.

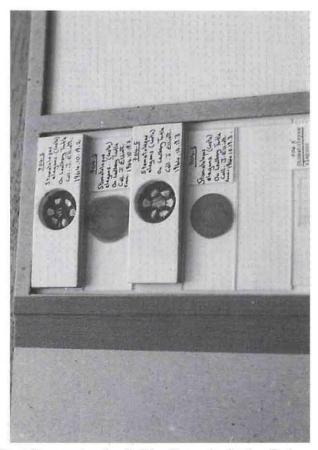


Fig. 4 Dry mount card well slides, Copepod collection, Zoology dept. NHM.

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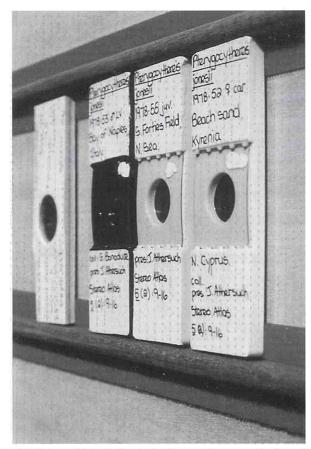


Fig. 5 White and black plastic dry Ostracod mounts, Zoology dept, NHM.



Fig. 7 Thick Canada balsam mount with celluloid reinforcement, on non-standard sized slide, Trichoptera collection, Entomology dept. NHM.

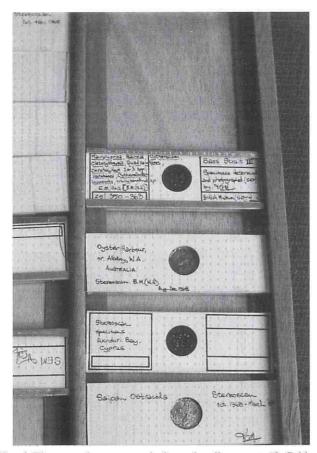


Fig. 6 Electron microscope stubs in card well amounts (& Cobb slide), Ostracod collection, Zoology dept. NHM



Fig. 8 Blackening 'Hoyer's Diptera mounts made by Lewis, Entomology dept. NHM.

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Fig. 9 Diptera slide with multiple cover slips, Entomology dept. NHM.



Fig. 11 Slide ringing stage with Euparal and Murrayite ringing media, aphid collection, Entomology dept. NHM.

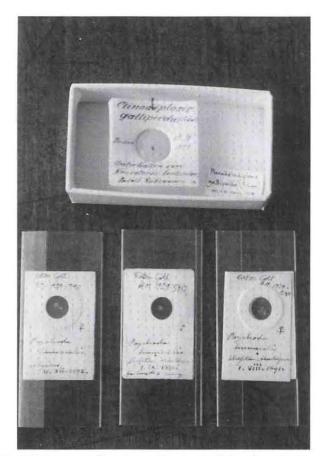


Fig. 10 Diptera cardboard coverlip mounts, Entomology dept. NHM.

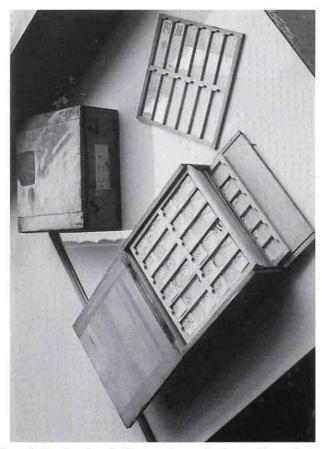


Fig. 12 Wood and card slide trays in wooden boxes, Entomology dept. NHM.

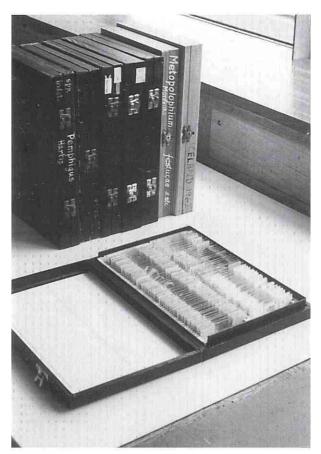


Fig. 13 Wooden slotted slide boxes (stroyan aphid collection), Entomology dept, NHM.

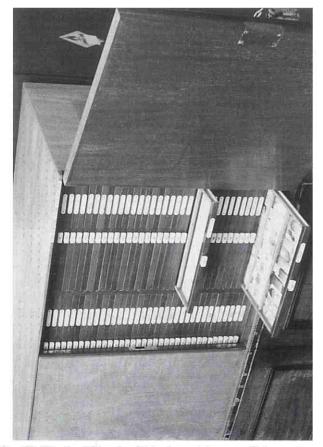


Fig. 15 Wooden Hill unit with horizontal drawers, Orthoptera collection, Entomology dept. NHM.

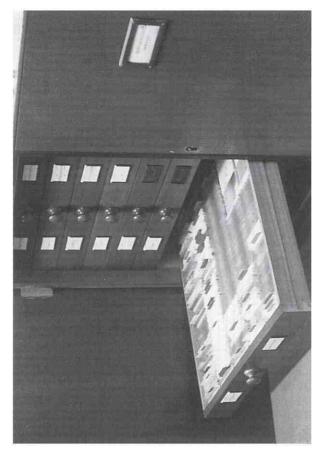


Fig. 14 Wooden Hill unit with vertical drawers, Thysanoptera collection, Entomology dept. NHM.

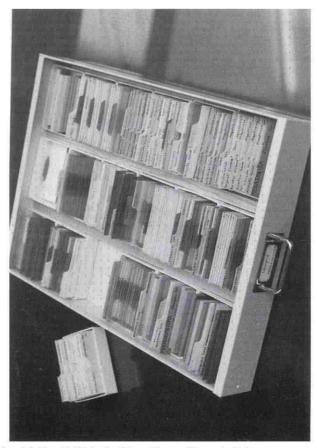


Fig. 16 Metal "Bisley" slide cabinet with vertical drawers, Psyliidae collection, Entomology dept. NHM.

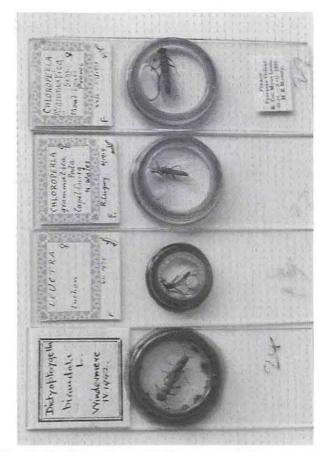


Fig. 17 Liquid formalin mounts, Megoptera collection, Entomology dept. NHM.

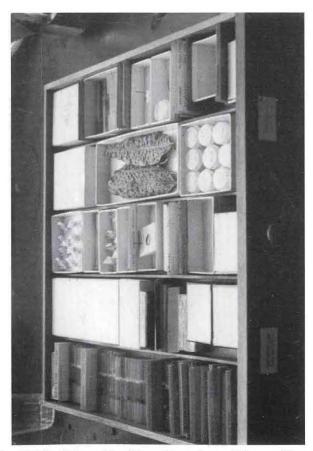


Fig. 19 Mixed dry, spirit, slide and host plant gall drawer, Diptera collection, Entomology dept. NHM.

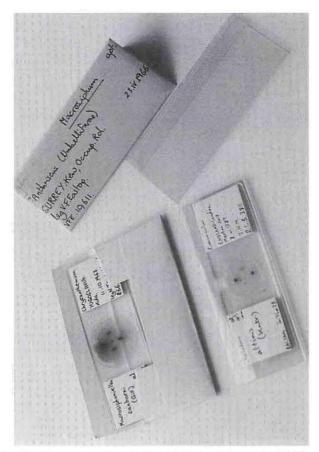


Fig. 18 Microscope slide plastic and card envelopes, Aphidoidea collection, Entomology dept. NHM.

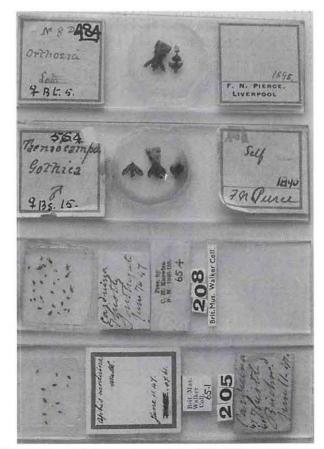


Fig. 20 Canada balsam slides made in 1847, Walker aphid collection and 1898, Pierce Lepidoptera genitalia stained with green ink, Entomology dept. NHM.

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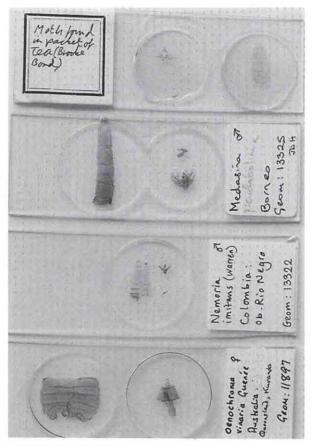


Fig. 21 Three Euparal and one Canada balsam mounts, Lepidoptera genitalia collection, Entomology dept. NHM

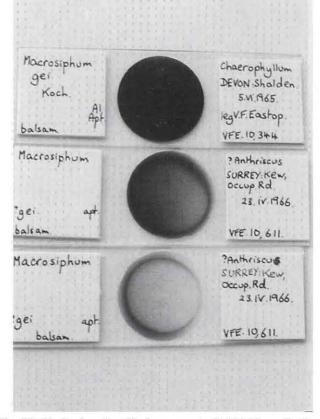


Fig. 23 Blackening phenol balsam mounts, Aphidoidea collection, Entomology dept. NHM.



Fig. 22 insect in amber, slide mounted with Canada balsam, the fossil latrine fly hoax, Paleontology dept. NHM.

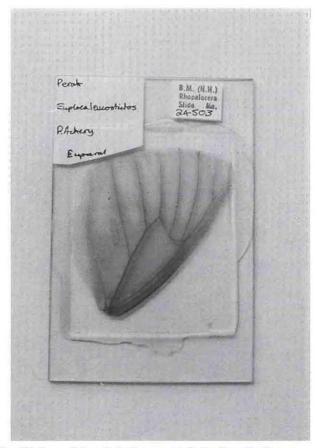


Fig. 24 Euparal descaled wing mount, Butterfly collection, Entomology dept. NHM.

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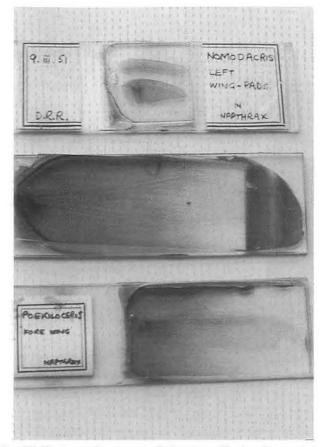


Fig. 25 Naphrax wing mounts, Orthoptera collection, Entomology dept. NHM.

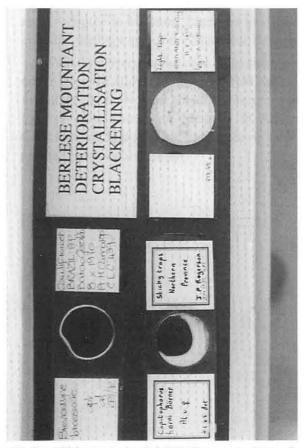


Fig. 27 Crystallising gum choral Aphidoidea mounts, Entomology dept. NHM.



Fig. 26 D.H.R. Lambers slide deterioration, Aphidoidea collection, Entomology dept. NHM.

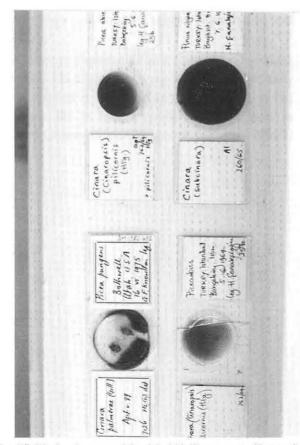


Fig. 28 Blackening gum chloral Aphidoidea mounts, Entomology dept. NHM.

NOVEMBER 1997



#### **Curriculum Vitae**



Paul A. Brown

After being awarded a BSc Honours degree in Forestry & Applied Zoology at the University College of North Wales, Bangor in 1977, employment was found at the Natural History Museum, London in the Lepidoptera section, dealing with pinned specimens and with microscope slide preparations of lepidoptera genitaslia using Euparal (1977-1979). Since 1980, he has curated and conserved the Sternorrhyncha (aphid) microscope slide collection, learning techniques in mounting whole specimens with gum chloral and Canada balsam mountants. From 1983 he has been author and assistant author to Dr Roger Blackman of papers on aphid taxonomy and became a Fellow of the Royal Entomological Society of London in 1981 and a Member of the Institute of Biology in 1982. In 1994 Paul was awarded an MA degree in Museum Studies at the Institute of Archaeology, University College, London and was awarded the Diploma of the Museums Association in 1995.

Paul now oversees the slide collections within the Entomology Department and consults and lectures on microscope slides within the NHM and to outside bodies. He organised a day conference on slide mountants at the Natural History Museum sponsored by Merck Ltd in 1996 and lectures on the Imperial College MSc course on Methods in Advanced Taxonomy. He is now playing an active role in Conservation, being on the committee of the Natural Sciences Conservation Group and represents NSCG on the Conservation Forum.

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