



NatSCA

Natural Sciences Collections Association

<http://www.natsca.org>

NatSCA News

Title: Conserving Transparencies

Author(s): Moore, S.

Source: Moore, S. (2006). Conserving Transparencies. *NatSCA News, Issue 8*, 12 - 14.

URL: <http://www.natsca.org/article/262>

NatSCA supports open access publication as part of its mission is to promote and support natural science collections. NatSCA uses the Creative Commons Attribution License (CCAL) <http://creativecommons.org/licenses/by/2.5/> for all works we publish. Under CCAL authors retain ownership of the copyright for their article, but authors allow anyone to download, reuse, reprint, modify, distribute, and/or copy articles in NatSCA publications, so long as the original authors and source are cited.

Conserving transparencies - Simon Moore

Abstract

Fluid collections often contain at least a few specimens preserved in something other than formalin, IMS or phenoxy-ethanol-based preservative fluids. Some of these are transparencies, either traditional, alizarin-dyed, *in situ* endoskeletal preparations or anatomical vaso-injected preparations. This article reviews the preservatives and methods used to conserve these types of preparations and the solvent hazards involved.

Review

Transparency specimens are prepared to show some internal structure of part of an animal or plant or an entire endoskeleton of bone or cartilage. Alizarin dyes, whether red, blue or yellow (even green) are still currently used to stain bone, as they are calcium-specific. Victoria (dark blue) or Alcian blue (kingfisher blue) are used to stain cartilage. Older anatomy preparations often contain parts of organs or tracts where the associated blood vessels or lymph ducts or other vessels have either been injected with dyed starch/latex or mercury. Botanical specimens are simply placed into a transparency-inducing medium (such as glycerol) to show internal anatomical structure. Many botanical preservative media also contain glycerol as a humectant but this should not be confused with its use as a transparency preservative.

Outline of techniques

- For Alizarin staining (Figs 1-3), entire (small) animals are fixed in 70% IMS and then either skinned or de-scaled (scales often contain some calcium which will become stained with the dye, masking the skeleton) and sometimes the alimentary tract is removed to give a clearer result.

The fixed, skinned and/or eviscerated specimen is then macerated in a glass container with 2-5% aqueous potassium hydroxide (KOH), depending on specimen size and/or fragility, over a series of days or weeks until the tiniest bones have become visible inside the tissues. Ensure that the volume of KOH = at least twice the volume of the specimen, else yellow areas in the fluid may form indicating a gradual loss of fixation which may lead to specimen collapse.



Fig. 1 Sterlets stained by double-staining alizarin red/alcian blue technique for bone and cartilage. The darker areas are blue (cartilage) and the grey areas are red (bone).
Specimen preparation, courtesy of Ralf Britz, Natural History Museum, London.

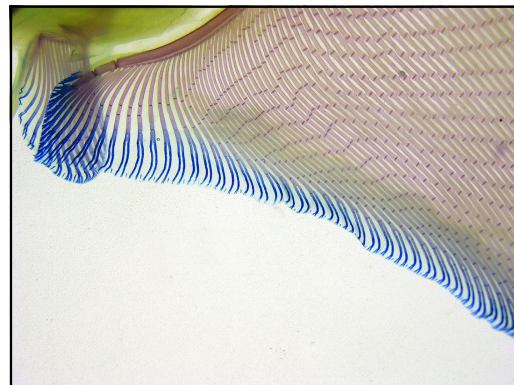


Fig. 2 Edge of ray wing stained with blue and red dyes, as before, to show cartilage and semi-calcified areas of cartilage. *Specimen preparation, courtesy of Ralf Britz, Natural History Museum, London.*

The specimen is then stained in fresh KOH mixed with some saturated solution of Alizarin dye in alcohol until the KOH is deeply coloured with the dye. It is then transferred into 5% to 10% glycerol, depending on its fragility, especially if a fragile (semi-embryonic) skull is involved [see Osmotic Pressure section]. The specimen gradually starts to become transparent and the slightly acidic nature of the glycerol differentiates the excess dye, removing it from non-calcium positive areas that have become stained. After several weeks, the specimen can be transferred into 70% or (eventually, if preferred) pure glycerol, as desired, and the preparation is complete. The dense fluid also supports the frail specimen. A crystal of thymol is often floated on the surface to prevent mould growth.

Some preparators prefer to use propylene glycol since it is less dense and less exudatory than glycerol and should not require any fungus preventative. The result should also show any internal organs as unstained but predatory organisms can be shown to have ingested smaller animals and, if as yet undigested, these can be shown as stained skeletons in the gut of the predator, or, as unborn in the uterus of a pregnant animal.

Cartilaginous skeletons are stained with either Victoria or Alcian (Kingfisher) blue dyes. Bony and cartilaginous endoskeletons can be stained with a combination of the blue and red dyes to show both types together (Figs 2-3).

Conservation note: Most preparations require little conservation. Fungal-infested or low level preservative can be carefully changed or topped up. If fungus has affected the staining then the specimen must be returned, gradually, to KOH and re-stained.

- For dyed or resin vaso-injections, the required tissues are dissected so that they can be easily seen *in situ*. They are then flushed with saline to remove blood masses and clots. Air bubbles are removed in fluid by careful manipulation.

Older specimens may have been injected with dyed starch paste, gelatine or by chemically induced (coloured precipitation) methods.

Resin or dyed latex (normally red or blue for blood vessels and cream for lymph) is injected into the vascular system (ref. Mahoney, 1978) and allowed to harden (resin) or fixed (latex). Resin injections tend to be used for corrosion preparations where the tissues outside of the injected mass are subsequently dissolved away in hydrochloric acid.

Dyed organs for transparency are then moved up a dehydration ladder of graded alcohols (normally in 10% or 20% steps depending on the fragility of the specimen) until they can be stored in a suitable organic transparency medium such as methyl benzoate, turpentine (even turpentine substitute but not other paint thinners – as yet untested). If there is any opacity or cloudiness this will be due to contained water forming an emulsion with the storage medium: the specimen will have to be moved to isopropanol to remove the water content before returning the specimen to the transparency storage medium.

Conservation note: Where the dye has fallen out of the vessels it has often fragmented. If the specimen is important enough, then it will need to be taken down a dehydration ladder and re-injected. For specimens stored in alcohol or solvents, celloidin (Moore, 1999: p. 111) will have to be used to consolidate the dyed medium. For formalin-preserved specimens, gelatine (Moore, 1999: pp. 111-112) may be used as a consolidant.

- For mercury-injected specimens (Fig. 4), treat with great care. The mercury is obviously hazardous (see below) and the specimens are usually more than 100 years old. Mercury can easily pool at the bottom of an organ whose finer vessels have ruptured through careless or rough handling over time. Regrettably, the organ itself can become ruptured so that the mercury spills out into the jar. Even by tipping a jar, the mercury can become displaced making fluid changes difficult.



Fig. 3 Mercury injected portion of alimentary canal to show allied blood vessels; mid-18th

Conservation note: Most mercury preparations are stored either in methyl benzoate or turpentine and over time, this will become increasingly yellow, even orange. Although this discolouration will not damage the specimen, it will not enhance its appearance. Draining a mercury injection jar needs to be carried out very

slowly so that tissues bearing an increased weight of mercury will not rupture. Ensure that the jar is tilted at an angle of about 20-25° from the vertical and ensure that it is well-supported. This will prevent the mercury from pressing on the areas that have supported it all these years. Using a fine translucent tubing so that the fluid will flow slowly, drain the jar by siphoning. Refill the jar by doing the reverse with clean solvent.

Turpentine substitute has been tested to replace turpentine but not longer than five years. The author has tried cleaning the contaminated fluid using charcoal but this has not worked due to the chemical changes in the fluid.

Problems with Osmotic Pressure

Owing to the high osmotic pressure of dense fluids such as glycerol, care must be taken when transferring up the glycerol ladder, not to rush the process. Vulnerable tissues such as semi-embryonic mouse or bird skulls can become dented if the osmotic pressure change is too great and some can even become invaginated (and difficult to reverse!). By transferring the specimen back to a more dilute stage, this undesirable effect is reversed without having to resort to fluid injection to inflate the skull, which can also lead

to distortion or rupture of the fragile tissue. Most transparencies are stored in 70% glycerol (diluted with deionised water).



Fig. 4 Jar containing turpentine with a semi-dissolved plastic backing plate – subsequently replaced by glass.

The reverse and inverse can also occur when transferring up a dehydration ladder, leading to a solvent storage medium but the same rule applies – if the specimen shows signs of osmotic swelling or shrinkage, return it to the previous stage of dehydration for a longer period.

Hazards

As a normal part of good practice, jars should always be labelled to identify the fluids that they contain and, ideally, a history of fluids used in the process (fixative, IMS ladder &c). This is necessary both to conform to health and safety regulations and to give an idea of how the specimen was processed should any-

thing go wrong with it in the future.

Discreet hazard labels as to solvents, flammables and irritants should also be present on the jar, taking care that these do not become contaminated with solvent, upon opening a jar, or they will need replacing. Always bear in mind the solvent action of these preservatives. The author has found on several occasions coloured plastic backing plates being used to mount transparency specimens preserved in solvents. The resulting opaque solution may (Fig. 5) look alarming but provided the specimen is still, more or less, supported by it then no damage should have occurred. The specimen will obviously require a glass or ceramic backing plate and a renewal of preserving fluid.

Mercury injected specimens should be treated with great care, not only to prevent organ rupture and mercury-into-jar seepage (as above) but to prevent mercury from escaping from the jar and becoming a hazard in itself. Always bear in mind that even the tiniest amounts of spilled mercury are a serious hazard and can give rise to cumulative heavy metal toxemia if ingested.

Hampshire County Council Museums, Libraries and Archives Service,
 Chilcomb House, Chilcomb Lane,
 Winchester SO23 8RD.