Two improved methods of mounting museum specimens / by C. W. Rowntree.

TWO IMPROVED METHODS OF MOUNTING MUSEUM SPECIMENS.

By C. W. ROWNTREE, M.B., B.S., F.R.C.S.

Methods of mounting and preserving museum specimens in their natural colours have long been the subject of investigation by pathologists, notably Jores and Kaiserling, and by their methods or modifications of them, it has now become a comparatively easy and certain matter to fix tissues and organs in such a way that the appearances present during life can be accurately preserved.

It is a more difficult matter, however, to ensure the permanence of these preparations, for the mounting fluid most generally used—glycerine and water—has among other drawbacks the disadvantage of allowing the growth of moulds, for the prevention of which the addition of formalin or other antiseptic is necessary. It is this which is probably responsible for the gradual bleaching of the specimen that so often occurs. Other solutions that have been suggested have the common drawback of either undergoing changes themselves or exerting a solvent action on the pigments of the specimen.

The ideal mounting medium in addition to being colourless and transparent, should have no action of any kind on the specimen, and should itself not be liable to chemical or putrefactive change. In searching for some substance which might be expected to fulfil these requirements, liquid paraffin (Paraffin. Liq. B.P.) suggested itself.

Liquid paraffin has many obvious advantages; it is colourless, perfectly transparent, absolutely non-volatile at all ordinary temperatures and has a low freezing point. Chemically speaking it appears to be almost entirely inert, the only substances upon which it has any solvent action being a few organic compounds of its own class, such as benzene, xylol, etc.; it is obvious, therefore, that changes due to any chemical action on the specimen mounted in this medium are not to be anticipated. While it possesses no active antiseptic properties, yet organisms are unable to grow in it, so that the

disadvantage of having to add an antiseptic to prevent the growth of moulds is done away with.*

And, lastly, a very great advantage of liquid paraffin is that its index of refraction is almost identical with that of the glass of which museum jars are manufactured; this combined with its transparency gives to specimens mounted in this medium a peculiar sharpness of outline and brilliance of colour contrasts, while there is the additional advantage that excellent photographs of the specimens can be obtained without removing them from the bottle.

In attempting to make use of this substance as a mounting medium one has to overcome the difficulty that liquid paraffin is not miscible with any of the solutions used in the preparation of this variety of specimens, and one rather anticipated that an emulsion would result from the admixture of liquid paraffin and glycerine and water, for instance. However, it was found that even if such a mixture were vigorously shaken, the resulting emulsion rapidly separated into two well-defined layers without turbidity or other change in either fluid.

It seemed possible, therefore, that if a specimen were taken straight from one of these watery fluids into paraffin, the latter might be expected to replace the water without damaging the appearance of the specimen. This was actually found to take place when a specimen was taken from watery glycerine to paraffin, the glycerine separating out in the course of 24 hours, and dropping to the bottom of the jar, where it formed a well-defined layer from which the paraffin was readily decanted.

The exact technique which I have found to give the best results is as follows :—

The organ or tissue to be preserved, as soon as possible after removal in the operating theatre or post-mortem room is placed in the following solution :—

Sodium Sulphate - - -	grms. 20
Sodium Chloride - - -	grms. 10
Magnesium Sulphate - - -	grms. 20
Formalin - - - - -	ccs. 50
Water - - - -	to 1000 ccs.

* One of the uses to which liquid paraffin has been put in this laboratory is as a protective layer over the gelatine or agar in the preparation of anaerobic culture tubes.

It is desirable that the specimen should not be washed or compressed prior to its immersion, in order to preserve as far as possible, the blood upon the presence of which so much of the ultimate appearance of the specimen depends. Any blood, etc., adhering to the outside of the specimen and spoiling its appearance can be removed readily, and with less damage later on when it is coagulated.

As in all methods in which formalin is used, the period for which the specimen should be left in the first solution depends upon its size; for anything up to the size of the kidney, 48 hours will be sufficient to harden and fix it throughout. Larger specimens should be left in a correspondingly longer time, but it is well to make it as short as possible, as the longer the specimen is in the formalin solution the more difficult is it to get the colour back again.

When it is judged that the specimen is thoroughly fixed, it is transferred to a 50 per cent. solution of Methylated Spirit, which must be free from Naphtha. This continues the hardening, and washes out the formalin and salts, which occasionally, when not thoroughly removed, crystallize out on the surface of the specimen.

After remaining in this solution for from 12 to 24 hours, the specimen is transferred to pure Naphtha free Methylated Spirit, in which the original colours of the specimen will return.

In about 4 to 6 hours the best result will have been attained and the specimen is then placed in the following solution, in which it may remain without harm for several days :—

Sodium Acetate	grms.	20
Glycerine	ccs.	500
Water	ccs.	500

The specimen at first floats in this solution owing to the spirit with which it is impregnated, and in order to ensure complete immersion, it is necessary to weight it for the first few hours until all the spirit has been displaced. From this last solution the specimen in the course of 2 or 3 days is placed in pure glycerine, where it remains for a similar period, when it is ready for permanent mounting in paraffin.

The two glycerine baths can be used repeatedly : a matter of considerable importance from the point of view of expense.

The best way in which to carry out the final mounting is to remove the excess of glycerine from the specimen by allowing it to drain on a perforated dish for a few minutes, and then to suspend it by a thread in a tall cylinder filled with liquid paraffin. At first the specimen is allowed to rest on the bottom of the cylinder, but during the course of the next few days the suspending thread is gradually shortened, so as to lift the specimen into the upper layers of perfectly clean paraffin and leave the glycerine displaced from the specimen at the bottom.

The specimen may then be transferred to the museum jar in which it is intended to mount it.

From the point of view of the permanence of specimens mounted in this way, I may here state that I have had specimens now for considerably over a year, exposed to sunlight and to considerable variations of temperature, and that no alteration in the appearance of the specimens has so far manifested itself.

In order to demonstrate the more intimate structure of tissues, the following method by which they are rendered transparent or relatively so, may be utilised. The process was introduced by Mr. Sampson Handley, who, when studying the process of lymphatic permeation in cases of carcinoma of the breast, took thin strips of skin with the attached subcutaneous tissues and after fixing and hardening in Müller's fluid, dehydrated them and cleared in cedar oil, when he found that the more deeply stained nodules of carcinoma showed up with great distinctness in the transparent subcutaneous tissues. Subsequently he greatly improved the method by using acetone as a dehydrating and xylol as a clearing agent, with the result that a much greater degree of transparency was obtained than by the original method.

I have since made use of the method for a variety of purposes ; in such cases as carcinoma of the breast I have found it unnecessary to use any staining agent, for if a slice through the whole of a carcinomatous breast be rendered

translucent, it will be found that the carcinomatous part remains opaque, the fat and subcutaneous tissue become quite transparent, while the fibrous tissue due to any co-existing mastitis becomes relatively transparent, and is quite easily distinguished from the opacity of growth on the one hand and the complete transparency of fat on the other.

This method can be applied to all organs and tissues, although of course the contrasts in tissues containing but little fat are not so great, while in organs of dark colour like liver it is necessary to use very thin slices indeed to get useful results.

Where the method has its most useful field is in the study of the spread of malignant growths. This can best be studied by cutting the affected organ into slices and while alternate slices are preserved to show the natural appearances the remaining slices are rendered transparent and compared with the others. A picture of the spread of malignant disease will be presented which cannot otherwise be obtained except by microscopic study.

Many other methods of applying this process will suggest themselves.

I have found that the developing bones and centres of ossification in human embryoes may be readily displayed by treating in this manner either individual limbs or the whole body in the case of embryoes up to the third month. Tape-worms, round-worms, and other parasites may be treated similarly, and very striking demonstrations of their ovaries and other internal organs obtained.

Interesting specimens may also be prepared by injecting the blood-vessels or lymphatics of such a structure as the intestine or mesentery and then making it transparent. Care must be taken, however, that the injection mass used is not soluble in any of the reagents to be employed in the preparation.

The exact procedure to be adopted is as follows:—

The organ or tissue to be prepared should be placed in the ice-house for a few hours until quite firm, but not frozen hard; slices about $\frac{1}{8}$-inch thick should then be cut with a brain knife and placed in 50 per cent. methylated spirit for 48 hours. Glass vessels of cylindrical shape should be

employed and weights attached to the lower ends of the strips to prevent shrinkage.

The material is then transferred to pure spirit in which it should remain 24 hours, when it should be placed in acetone for 4 hours to ensure complete dehydration. It is then put into xylol until quite clear (about 4–8 hours).

The specimen may then be permanently mounted in liquid paraffin, with which xylol readily mixes and in which the clearness of the specimen is not impaired.

In the case of worms, embryoes, and other specimens which it is desired to mount whole, it will be found that dehydration is more difficult, and that one or more changes of absolute alcohol should be used before the final dehydration in acetone.

I may add here particulars of a method of affixing the lids of museum jars which has been found to give satisfactory results.

The non-volatile nature of liquid paraffin permits the sealing of the jars with practically no fear of breakage in the hottest weather, and the material employed for this purpose is celluloid.

A thin sheet, 1–2 mm. thick, is cut the exact size of the mouth of the museum jar, and is then moistened along the edges on both surfaces with the following mixture :—

Acetone
Amyl Acetate } equal parts.
Acetic Acid

It is then placed in position, the glass lid fitted over it, and firm pressure maintained for several hours.

For an air-tight joint to be effected it is necessary that the surfaces of both jar and lid be ground, as the celluloid will not hold on a polished surface.

A sheet of celluloid so arranged may be utilised as a means of support for the specimen, which may be suspended from it by threads or by a very thin glass rod, made with a hook below to hold the specimen and a short rectangular arm above to attach to the celluloid.

The label describing the specimen may be placed between

the celluloid and the glass lid, where it will remain indefinitely, protected from dust and moisture.

In the case of very small jars it is unnecessary to use a glass lid in addition to the layer of celluloid, but in large jars it is necessary, and in any case it gives the preparation a more finished appearance.

Jars filled with liquid paraffin and sealed in this manner have been subjected to a temperature of 120° F. for several hours, the paraffin being in contact with the celluloid, without appreciable alteration.