

## MUSEUM TECHNIQUES : A REVIEW

BY

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The preservation of pathological material has never been of greater importance than at present, when the introduction of new, successful methods of therapy is changing the picture of disease out of all recognition. It is interesting to walk through any medical museum established a hundred years and to note how many specimens relate to conditions which are either never or rarely seen at necropsy to-day or which, if encountered, are greatly modified by treatment. On the other hand, surgical treatment, by prolonging life, often permits of conditions not previously encountered; it is a matter of interesting conjecture to consider what disorders may in future manifest themselves in increasing numbers. Obvious examples are the degenerative conditions of old age, as the treatment of acute infections becomes progressively more effective. Pathological museums are in part historical, representing the pioneer work of diagnosticians and therapists; in part they are becoming museums in the generally accepted sense of the word, presenting records of past states not now encountered, or conditions of great rarity; and finally they provide the student with the basic material of his current teaching. But no one can prophesy when the last class will be converted almost overnight into one of the former.

### Collection of Material

The great majority of museum specimens are collected from teaching hospitals. This is a great pity, since by their nature such specimens cover only a part of human disease, excluding, for example, chronic conditions, and in any case only a small part of the available material passes through the teaching hospitals. It is most desirable that a centralized clearing station for specimens should be established.

From every point of view, and in particular to ensure the best museum specimens, necropsies should be neither hurried nor perfunctory. They should be performed at leisure before a limited audience, and the demonstration of material should be the subject of a separate session. Necropsies should of course be performed as soon as possible after death, but in many cases, particularly in order to preserve the stomach, intestinal tract, or brain, 4% formol saline should be injected into the peritoneal cavity, the carotid arteries, or stomach as soon as possible after death.

One of the commonest causes of inferior specimens is contact with tap water. The resultant haemolysis greatly reduces their value. Specimens should be washed only with saline, and should be kept in saline while awaiting demonstration as

drying again ruins the surface appearances, but as autolysis quickly sets in they should not remain in saline for more than two hours.

Material for microscopical section should, if possible, be selected at the time of mounting the specimen, and it is often most desirable not to open hollow viscera or to dissect specimens until fixation is complete.

It is essential that the cut surface of a specimen should be flat. In many cases this can be ensured by passing a glass rod into a structure such as a large bronchus and cutting along the rod with a large broad knife. Many special methods have been devised. Lundy and Camp (1939) describe a technique for large anatomical specimens. Anderson (1929) has designed an apparatus comprising a flat board with glass rods, which is particularly valuable for brain slices. Gough and Wentworth (1949) have been particularly successful with preparations of lungs in cases of pneumoconiosis, when the lungs are inflated and immersed in fixative; thin slices are then cut with a knife by hand, and impregnated with increasing strengths of gelatine. After final embedding in formol gelatine, sections of 600  $\mu$  thickness are cut on a large microtome and the sections are laid on sheets of "perspex." Sheets of Whatman's No. 1 filter paper are

squeezed over the surface and left to dry; the sections adhere to the paper, and can be peeled off. The specimens can then be filed in book form.

Vowles (1942) describes a cheap method of mounting thin slices of organs in pure Trinidad Lake asphalt. Hall (1944) uses a rotary meat slicer, and mounts specimens stained or unstained between sheets of glass sealed with a paraffin wax mixture. Freezing and cutting with a hand-saw is useful, especially for bones prepared with soft tissues. A method has been described by Baker (1940).

Injection techniques, especially for the vascular system, are often necessary at the necropsy. Narat, Loef, and Narat (1936) have elaborated a vinyl resin technique, permitting of multicoloured effects, followed by corrosion with fuming hydrochloric acid; Leibow (1947) and Hill (1937) also suggested the use of latex

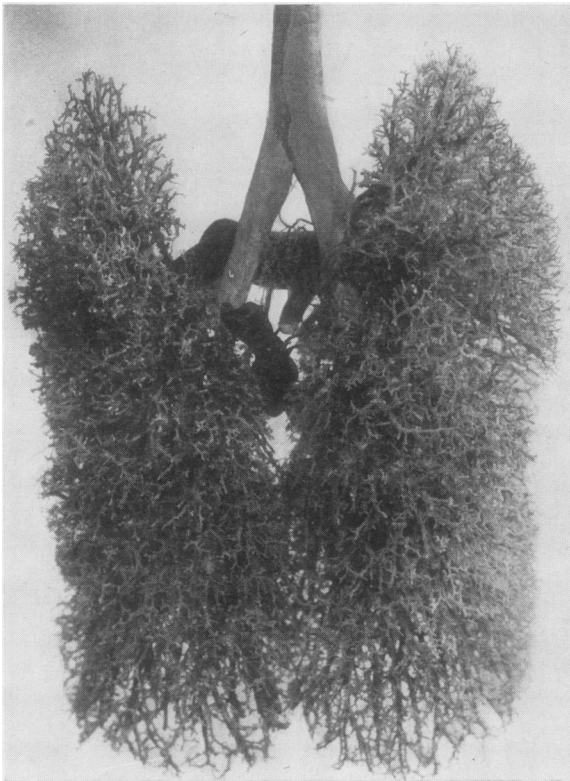


FIG. 1.—Demonstration of bronchial tree by plastics.  
(Photograph kindly lent by Dr. A. A. Liebow, U.S.A.)

(Fig. 1). We have followed the method of Lieb (1940), using liquid "neoprene," with excellent results. Further details of this valuable technique are given by Duff and More (1944), and for the coronary system by Smith and Henry (1945).

### Fixation

There are certain important general principles of fixation. Specimens containing bile or stained by bile must be fixed and stored apart from others, as they will stain them. Specimens undergoing fixation must not touch other specimens, or the sides of jars; they must either lie on washed fluffless lint or be suspended by linen thread. Specimens may be pressed out on cork, or stretched on glass or metal frames, but this causes distortion; it is often preferable to fill hollow viscera with cotton-wool soaked in fixative, tie off at both ends, and immerse them in a fixing tank. Unopened cystic cavities should be injected with fixative; if opened they should be packed with cotton-wool. Certain solid viscera should be fixed by vascular injection—e.g., the brain through the basilar artery. Dukes and McDonnell (1938) give particulars for the treatment of renal specimens, which should never be cut before fixation. Dukes and Bussey (1936) have described a method of preparing specimens of intestinal tumours which has become widely used and should be consulted by anyone who may not be familiar with it. The lungs and limbs are particularly suitable for fixation by vascular injection. The spleen should never be cut before fixation, but unfortunately, even after vascular injection, patchy fixation is often a nuisance. For diagnostic purposes specimens must be taken at the time of necropsy.

The preservation of the colour and form of specimens depends entirely on the fluids used to fix and to mount them. The original method of Kaiserling (1897) is still widely used. Three solutions are employed, with the following formulae:—

|                           |           |
|---------------------------|-----------|
| <i>Kaiserling No. I</i>   |           |
| Formalin (40%)            | 400 ml.   |
| Pot. nitrate              | 30 g.     |
| Pot. acetate              | 60 g.     |
| Tap water                 | 2,000 ml. |
| <i>Kaiserling No. II</i>  |           |
| Ethyl alcohol             | 80%       |
| <i>Kaiserling No. III</i> |           |
| Glycerine                 | 500 ml.   |
| Arsenious acid 1%         | 200 ml.   |
| Pot. acetate              | 250 g.    |
| Thymol                    | 2.5 g.    |

There are several important details to be observed if this method is employed. Fixation in formalin is almost universal, but the danger of formalin to technicians and pathologists is not adequately recognized. Not only does it cause dermatitis, but its effect on the nasal mucosa is also serious, and sinusitis is a common result. A museum mounting room must be thoroughly ventilated and an exhaust fan fitted.

Specimens should be fixed in Solution No. I for periods up to two weeks depending on their size, and larger specimens should always be injected. In this solution the colour contrasts disappear, and are to some extent restored by the

ethyl alcohol, a rather expensive reagent. Specimens should remain in alcohol for periods of up to one hour, but must be carefully watched to ensure that they are removed when the optimum stage is reached; if kept for longer periods the colour fades and cannot be restored.

Solution No. III is the mounting fluid, containing arsenious acid and thymol to inhibit moulds, and glycerine, again an expensive reagent, for its effect on the refractive index. It is difficult to get the arsenious acid into solution, but good results are obtained by making up 1% arsenic trioxide in water and steaming the solution for six hours in a steam sterilizer. The thymol is ground up and floated on the surface of the fluid (Kaiserling, 1897). In later modifications the glycerine content was raised to 30%, and 0.5% formalin was substituted for the arsenic and the thymol.

The colour of specimens in Kaiserling's fluid leaves much to be desired and fading sets in rather soon. Jores (1913) modified the method, which was further altered by Klotz and Maclachlan (1915), and this latest modification is intended whenever Kaiserling is referred to in this article.

#### Solution I (Colour Fixation Fluid)

|                             |           |
|-----------------------------|-----------|
| Chloral hydrate             | 50 g.     |
| Carlsbad salts (artificial) | 50 g.     |
| Formalin                    | 100 ml.   |
| Tap water                   | 1,000 ml. |

The composition of the Carlsbad salts is

|                  |       |
|------------------|-------|
| Sod. sulphate    | 22 g. |
| Sod. bicarbonate | 20 g. |
| Sod. chloride    | 18 g. |
| Pot. nitrate     | 38 g. |
| Pot. sulphate    | 2 g.  |

After fixation for periods of up to six weeks specimens are washing in running water for 12 hours and then mounted in Solution No. II.

#### Solution II

|              |           |
|--------------|-----------|
| Pot. acetate | 300 g.    |
| Glycerine    | 600 ml.   |
| Dist. water  | 1,000 ml. |

The advantages of this method were combined by Aegerter (1941) in the following formula:—

#### Colour Fixation Fluid

|                 |           |
|-----------------|-----------|
| Sod. chloride   | 140 g.    |
| Sod. bicarb.    | 80 g.     |
| Chloral hydrate | 625 g.    |
| Formalin (40%)  | 512 ml.   |
| Dist. water to  | 20 litres |

After immersion in this fluid for up to 20 hours specimens are placed in the original Kaiserling I solution for from one to five days without washing and then, again without washing, mounted in a modified Kaiserling III solution

|                 |           |
|-----------------|-----------|
| Pot. acetate    | 2,000 g.  |
| Glycerine       | 4,000 ml. |
| Thymol          | 5 g.      |
| Distilled water | 20 litres |

A very large number of other fixatives and mounting media have been described. For special purposes special fixatives are necessary. The method used for most purposes at the Westminster Medical School is based on a personal observation (Pulvertaft, 1936) that the colour of faded specimens of chloroma could be restored by the addition of sodium hydrosulphite to the medium; as a result all specimens mounted during the last 13 years have been so preserved. Further papers on this method have been provided by Wentworth (1938, 1942, 1947).

The method in use at the Westminster Medical School is as follows. Specimens are fixed in Kaiserling Solution No. I, and after fixation transferred to fresh Kaiserling Solution No. I as modified for storing. For mounting they are transferred to Kaiserling Solution No. III.

|                     |      |                  |
|---------------------|------|------------------|
| Glycerine           | 30%  | } adjust to pH 8 |
| Sod. acetate (B.P.) | 10%  |                  |
| Formalin            | 0.5% |                  |

If the solution is not crystal clear, impurities in the sod. acetate are usually to blame. Such solutions should be filtered through paper pulp under negative pressure. Immediately before sealing 0.4% sod. hydrosulphite is added. It is important that specimens should not be rinsed in tap water even after fixation.

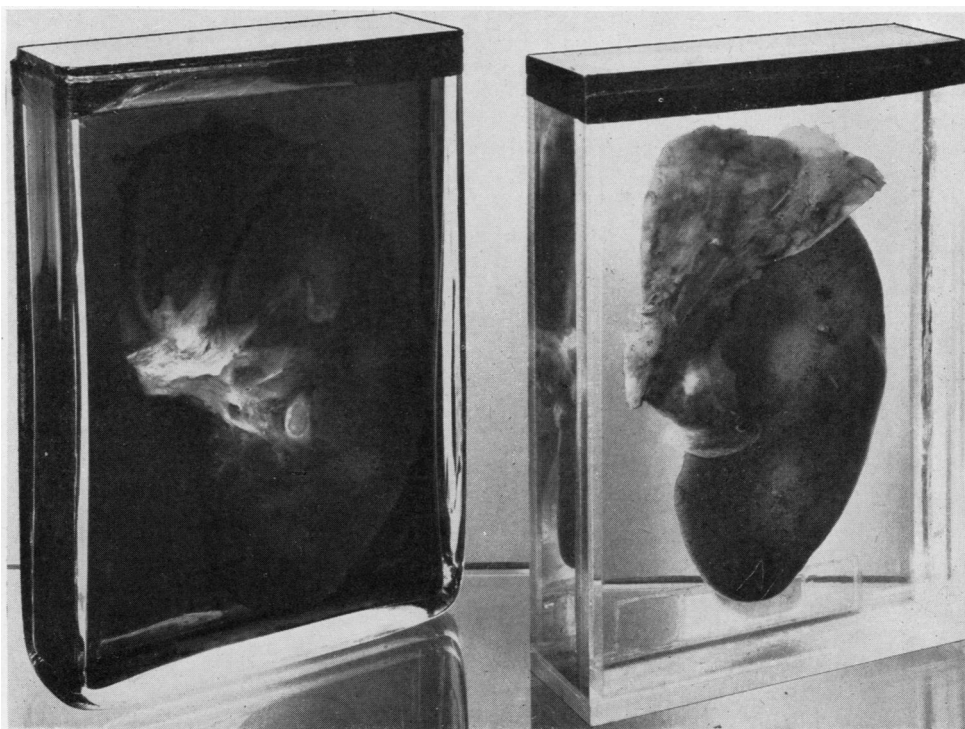


FIG. 2.—Two halves of the same kidney. The specimen on the left has been washed in running water for 12 hours, that on the right in normal saline.

Experiments performed by Dr. A. D. Morgan show that haemolysis later sets in (Fig. 2) if there is much blood when specimens are washed in running water. Saline or Kaiserling Solution No. I may be used for rinsing. The amount of hydrosulphite should not normally exceed 0.4%. If colour restoration must be rapid, 0.6% may be added, but this is to be avoided, as a white precipitate may form.

Jars must be sealed immediately after mounting, and of course the lid must not be perforated, a practice necessary with large glass jars to prevent cracking on cooling in winter but quite unnecessary with "perspex" jars. Unsatisfactory results are due to inadequate fixation, washing with tap water after fixation, excess hydrosulphite, delayed sealing of the jars, acidity of the mounting fluid (whose pH should be 8), or the use of stale formalin containing *para*-formaldehyde as a white precipitate. Fragile specimens, such as embryos, shrink in this mounting medium owing to osmosis, which may be prevented by lowering the glycerine content and by injecting gelatine into the cranial cavity. Wentworth (1942) omits glycerine in the mounting medium. This is an economy, but reduces the refractive index of the medium and therefore causes a loss of brilliancy.

Carbon monoxide has also been employed as a colour-retaining agent. Schultz (1931) introduced the technique, which gives brilliant colour contrast, but entails the risks of poisoning and explosion. These may be avoided by the technique of Robertson and Lundquist (1934). The method is also described by Lewis and Gaines (1936). Kaiserling I solution is used while the specimens are being gassed, and final mounting is in Kaiserling III.

Both the hydrosulphite and CO methods produce brilliant colour contrasts; by the hydrosulphite method colours are retained with little fading for 13 years at least; by the CO method no change has been noted to date. However, the colours are not natural in either case and for this reason some curators dislike the methods. There is probably no ideal mounting medium, as the taste and objectives of curators differ.

#### **Maceration of Bones**

The maceration technique is particularly useful in showing osteogenic sarcomata, but it has many other uses. Methods are reviewed by Wagoner and Nuckols (1935). For delicate specimens prolonged putrefaction gives the best results. Quick results for gross specimens are given by autoclaving them for 10 minutes at 10 lb. pressure with N/1 NaOH. As a routine, maceration in N/4 NaOH at a temperature of 90° C. gives excellent results. During maceration the specimen is removed at intervals, and, after washing in a stream of water, portions of soft tissue are removed with forceps. When all the soft tissues have been removed specimens are dried in an incubator, and grease is removed by immersion in chloroform for three hours. They are then bleached by H<sub>2</sub>O<sub>2</sub>, and are mounted dry in "perspex" jars (Figs. 3 and 4).

#### **Storage of Specimens before Mounting**

Labels are readily made from old x-ray films by removing the emulsion in hot water. They are then immersed in glacial acetic acid for 10 seconds, and placed in a solution of 5 g. sod. carbonate in a gallon of water until they become

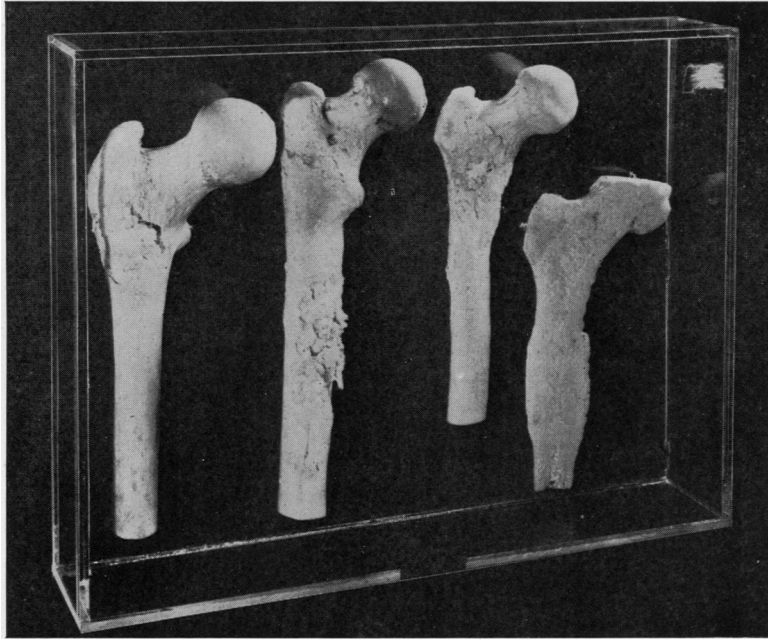


FIG. 3.—Macerated specimens of osteogenic sarcomata mounted on a centre-plate in a "perspex" box.

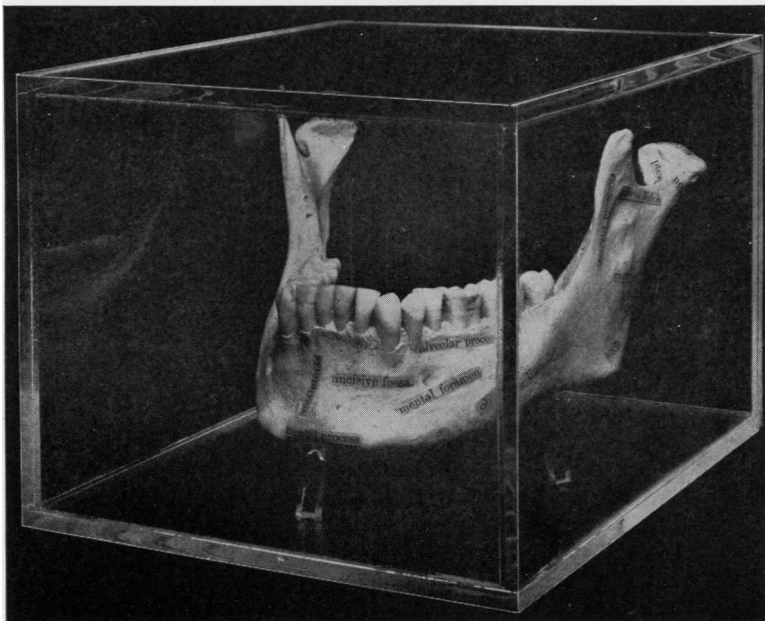


FIG. 4.—Macerated specimen of normal mandible demonstrating the use of "perspex" supports which have been cemented into position.

opalescent. They are next washed and dried, and a hole is punched to take a string. Identification details are written in indian ink. Immersion in acetone for a few seconds makes the label permanent. Two labels are prepared, and one is attached directly to a corner of a gauze square in which the specimen is wrapped by tying the corners of the square together. The other label is attached to a corner of the gauze square by a length of linen thread. Specimens are kept in Kaiserling I solution while awaiting mounting. Earthenware tanks of 25-gallon capacity measuring 34 in.  $\times$  22 in.  $\times$  19 in. may be procured, and surplus aluminium containers, unaffected by Kaiserling solution, can also be obtained. The tanks



FIG. 5.—Porcelain storage tank, showing method of keeping labels in order with wood strips. Necessary materials for wrapping and labelling specimens are displayed on the teak cover. One specimen is shown wrapped and labelled ready for storage.

are covered, preferably with teak. Strips of wood of the same length as the sides of the tank are provided and saw cuts  $\frac{1}{8}$  in. deep are made at intervals of 1 in. Through these notches the linen threads with attached labels hang, and thus specimens may quickly be selected (Fig. 5).

#### **Treatment of Specimens before Mounting**

Cavities, such as spermatocetes, should be filled with gelatine prepared as follows:

Using a reflux condenser 20 g. arsenious acid is boiled in 1 litre of water for two hours in a fume chamber. The solution is cooled and 120 g. of gelatine previously soaked in warm water added, excess water being removed by squeezing. It is then filtered through sand and paper pulp, and 10% glycerine and 0.5% Victoria blue added



drop by drop until the bulk is faintly blue; the gelatine should still appear colourless in a test-tube. The prepared gelatine is stored in the dark, and before use 0.4% formalin is added.

Anatomical details may be marked by "perspex" strips cut from  $\frac{1}{16}$  in. sheets ground to the desired shape by a carborundum wheel. "Perspex" rods and arrows can also be made with ease.

Friable specimens may be coated with gelatine as suggested by Wentworth (1947).

The bile discoloration from livers and gall-bladders can to a certain extent be prevented by soaking specimens in a saturated solution of calcium chloride for 24–48 hours before mounting.

#### Mounting in Jars

Plastic museum jars are now almost universally used in Britain, although in the U.S.A. watch-glass techniques are often preferred (Davis, 1942; Brown and Schroeder, 1937). Plastic watch-glasses can also be used (Larson and Levin, 1940). The advantages of plastic jars are numerous. Their inner and outer surfaces are parallel and free from ridges and there is no distortion. Their refractive index is 1.49, and light transmission is up to 92%. (The properties of plastic jars are fully discussed by Bartoe, 1940, and their use in museum work is reviewed by Kramer, 1940.) Plastic jars will stand severe strain. It is well known that large glass jars break with temperature changes if sealed, and it is not possible to use the hydrosulphite method with large specimens in glass jars. Plastic jars can be dropped 6 ft. without breaking. In a museum of 2,500 specimens an average of five breakages a week was found in glass-mounted specimens.

Fluids can be changed by drilling two holes in the base with a  $\frac{1}{16}$  in. drill, the holes being later filled with "perspex" soaked in chloroform; cement is finally applied.

The plastic jars scratch readily, although it is found that specimens in constant use have not needed attention for three years. Scratches are easily removed by metal polish.

**Apparatus for Making Plastic Jars.**—Boxes or jars can be made with no more than a tenon saw and sand-paper, but the following apparatus is desirable if numbers are to be made.

(1) A 6 in. bench grinder. This is an electric motor with spindles at each end, one end being fitted with a carborundum wheel, the other with a calico mop for polishing (cost, £15).

(2) A band facing machine, 36 in. × 4 in. This is a motor-driven sand-papering machine, with the advantages of precision and speed (cost, £24).

(3) A circular saw ("sylvan" No. 2). This has a rise-and-fall 8 in. blade, 16 teeth to the inch, with a slight set to avoid clogging. It has horizontal and vertical guides (cost, £40).

In making jars the sides must be cut absolutely square with finely ground edges to prevent bubbles. Welding is effected by immersion of the edges in chloroform or ethylene dichloride, or by "perspex" cement, No. 2 being fast-setting and No. 6 slow-setting. The front of the jar is cemented to the sides, supported in a former, the back is next cemented on, followed by the bottom and the top.

An alternative method of making jars has been devised by Professor Duguid, by whose courtesy the following method is presented.

A heating appliance for bending "perspex" is needed. This may be in the shape of two  $\frac{1}{2}$  in. copper pipes accurately fitted with a right angle guide so as to form a set square. Jets of steam are blown through the pipes and the strip of "perspex" laid between them for 12-15 minutes. It can then be easily bent and held at a right angle until cooled under water. A more convenient apparatus consists of two copper rod heating elements connected with a transformer so as to pass a high current of 700 amps at  $\frac{1}{2}$  volt. This heats the "perspex" to flexibility in about five minutes.

**Method of Making Plastic Jars.**—A rectangular strip of  $\frac{1}{8}$  in. "perspex" is bent to right angles at two points so as to form the top and sides of the jar, and the edges are accurately trimmed with a circular saw. One edge is soaked in ethylene dichloride for 10 minutes. This can be done by propping the strip on three pieces of fine wire or nylon thread laid on a glass slab and running ethylene dichloride around the lower edge so as to form a pool of the fluid. When the edge is softened a roughly cut sheet of  $\frac{1}{8}$  in. "perspex" is applied to it so as to form one face of the jar, and gentle pressure applied by weights. In 10-15 minutes the joint is firm

enough to permit manipulation, and the other face can be fixed by the same process. Weights up to 14 lb. should then be applied overnight. Next day with the circular saw the redundant parts of the facing sheets are cut off flush with the sides of the jar and the edges polished on the buffing machine. The bottom of the jar is also squared off with the circular saw so as to leave an even rim to which the base is fixed. A rectangular slab of  $\frac{1}{4}$  in. "perspex" is cut and polished to form a base, and a  $\frac{1}{8}$  in. hole is bored in it through which the jar can finally be filled with mounting fluid. The specimen to be mounted is fixed to a  $\frac{1}{8}$  in. "perspex" sheet, which is cut to fit firmly in the jar. The lower rim of the jar is softened by ethylene dichloride and the jar inverted so that the specimen can be quickly lowered into it. Sufficient mounting fluid to cover the specimen is poured in and the base is then laid in position and weights applied overnight. Next day the jar is completely filled with fluid through the hole in the base so that all air is

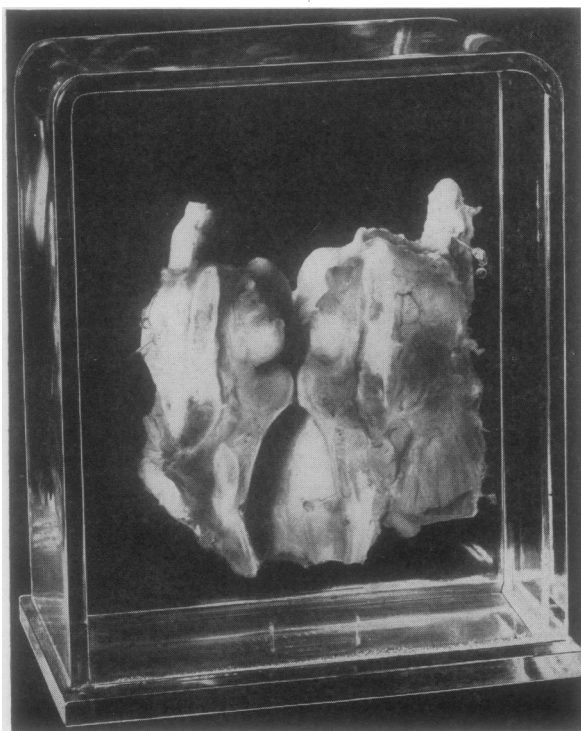


FIG. 6.—"Perspex" museum jar made by the method originated by Professor Duguid. The bending of the corners was effected with the flame from a pasteur pipette. The "perspex" stop to keep the centre-plate in position can be seen on the inside of the base of the jar; a similar one is cemented to the top.

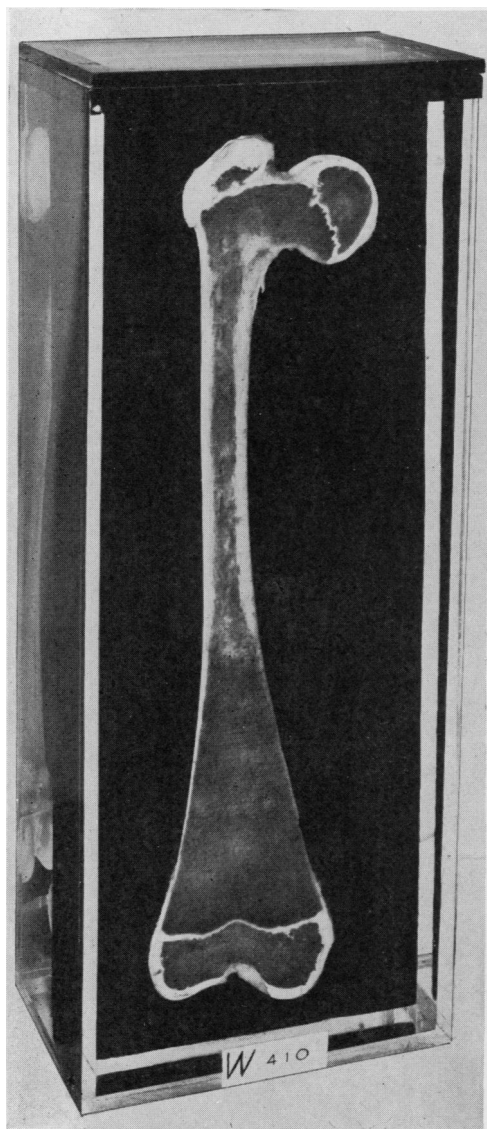
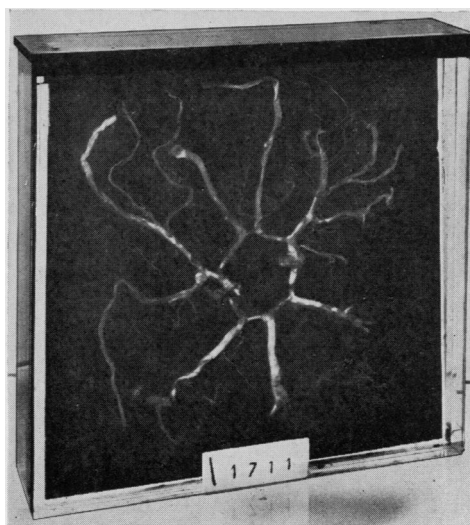
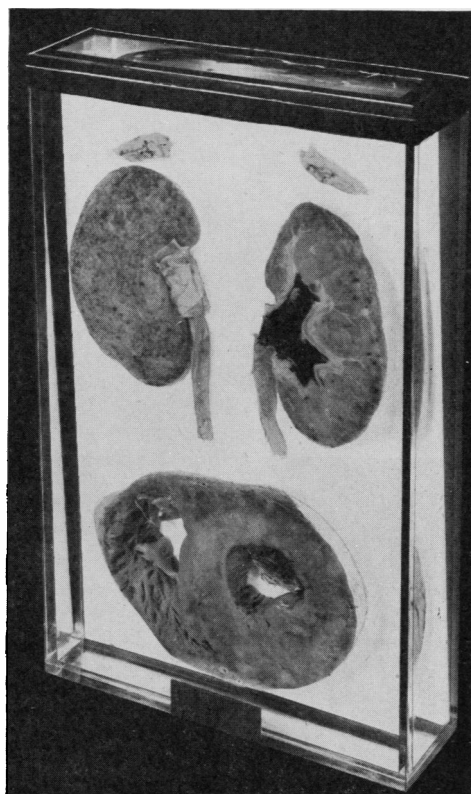


FIG. 7.



FIGS. 8 and 9.

FIG. 7.—The femur from a case of Gaucher's disease mounted on a black "perspex" centre-plate to contrast with the white bone and bright red bone marrow.

FIG. 8.—Kidneys, suprarenals, and heart from a case of acute malignant hypertension, mounted on a white centre-plate to contrast with the bright colour of the specimen.

FIG. 9.—An aneurysm of the circle of Willis fixed in position on a centre-plate of black "perspex" with a thin layer of gelatine. The jar with centre-plate in position was filled with mounting fluid before sealing.

expelled, and the hole finally plugged with a peg of "perspex" soaked in ethylene dichloride.

There are several modifications of the method of heating the "perspex" strip for bending. One consists of heating a length (6 in.) of copper tubing in a batswing burner flame, having previously marked with a scribe the place at which the bend is required. When the copper tube is sufficiently hot the scribe mark is laid along it; this will burn away the mark from the "perspex," also heating it right through. When it is pliable the strip is bent into a right angle, using a framework to ensure that it is square, and held or clamped in this position until cold.

Another simple method is to use a finely drawn pasteur pipette as a gas jet and draw it across the "perspex" strip on both sides at the desired place. When it is pliable it is treated as described above (Fig. 6). The principle is to heat as narrow a strip across the "perspex" as is consistent with bending.

**Method of Mounting.**—The specimen should be measured, and  $\frac{1}{2}$  in. clearance allowed at the sides and 1 in. at the top and bottom.

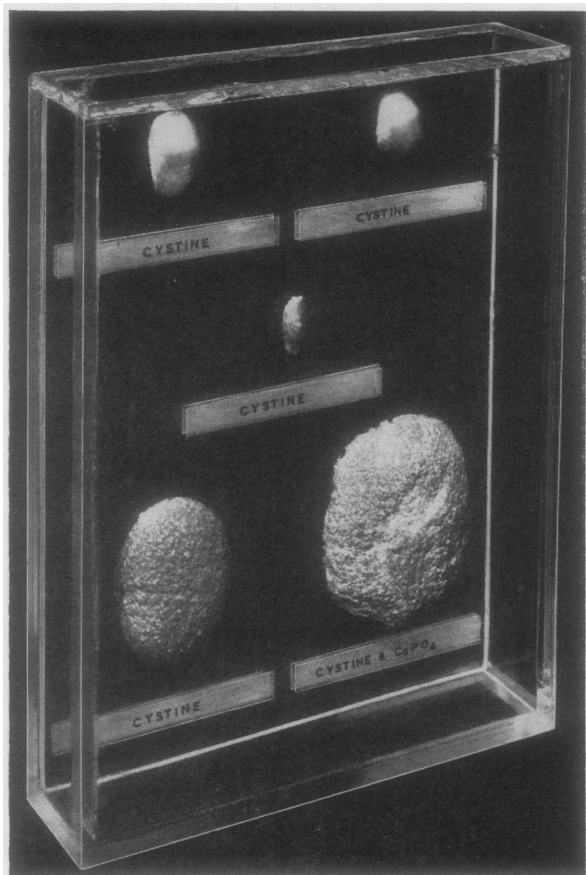


FIG. 10.—Calculi mounted by the method described by Culling.

The specimen should be mounted on a centre-plate; if more than one specimen is mounted in the same container opaque "perspex" should be used (Figs. 7, 8, 9). (This practice is often invaluable for demonstrating allied lesions.) The specimen is laid on the centre-plate, and scratches made to indicate the position of ties. Two  $\frac{1}{16}$  in. holes are drilled with a dentist's drill about  $\frac{1}{8}$  in. apart and the specimens stitched on with nylon thread, which is practically invisible in the fluid. Nylon is difficult to tie, and a surgeon's knot is necessary. The tie should be on the face of the specimen. Centre-plates can be bent to any required shape by heating in an oven to  $78^{\circ}$  C. if plasticized, or  $105^{\circ}$  C. if unplasticized. They are then immersed in cold water. Over-heating causes bubbling. The centre-plates are held in position by "perspex" strips cemented in place (Fig. 6).

When the specimen has been placed in the jar mounting medium and hydrosulphite is run in to within  $\frac{1}{4}$  in. of the top, care being taken that trapped bubbles are released.

"Perspex" (5%) in chloroform or "perspex" cement No. 2 or 6 is applied to the top edges with a pasteur pipette, and after 30 seconds the lid is applied lightly; if any "perspex" exudes it must immediately be removed. A light weight is then applied for one hour. The lid must have a hole bored in it to introduce the remaining mounting medium with which the jar is entirely filled. After 48 hours residual bubbles are removed. A "perspex" plug is made by cutting a pasteur pipette so that its internal diameter is slightly larger than that of the hole in the lid. A piece of "perspex" is heated until pliable, and the pipette is then used like a cork borer to remove a plug. This can be readily expressed from the pipette and naturally tapers; alternatively,  $\frac{1}{8}$  in. diameter "perspex" rod is now available for this purpose. The plug is then tapped into position. A drop of cement may be added but is not usually necessary.

Calculi may be mounted by a method described by Culling (1949) (Fig. 10).

Macerated bones are mounted dry but need a firm support. Nylon "monomer" filament 252 is desirable for fixation on the centre-plate, and "perspex" cement should be applied to the ties and portions of the specimen touching the centre-plate (Figs. 3 and 4).

Membranes may be demonstrated with a "perspex" lens (Fig. 11).

**Gelatine Embedding.**—This method was formerly much used with glass containers, as breakages were fewer and did not cause trouble from fluid loss. Colour preservation after treatment with Kaiserling Solutions I and II is good.

Gelatine is prepared as previously described in this paper, and formalin added just before use. With face uppermost the specimen is coated with gelatine, and when the gelatine has set it is placed face downwards in the jar. A piece of adhesive tape is fixed across the top of the jar and gelatine is later added to a depth of  $\frac{1}{4}$  in. to hold the specimen in place. The technique up to this point may be used to hold delicate specimens in position with the hydrosulphite method (Fig. 9). After setting in a refrigerator the jar is filled with prepared gelatine, bubbles being burst with a hot needle (Sahasrabudhe, 1934). Water miscible plastics which set are also now obtainable.

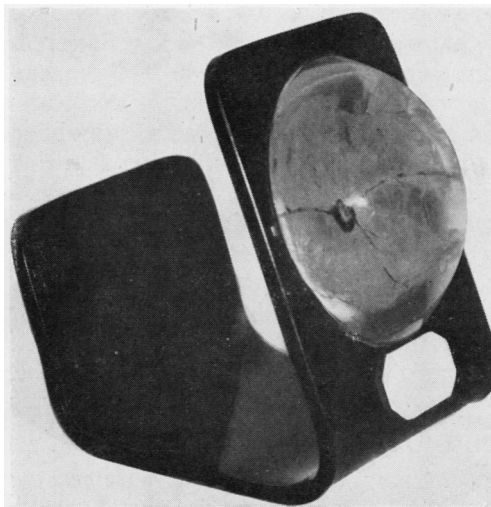


FIG. 11.—Method of demonstrating a membrane by means of a "perspex" cup which when filled with fluid acts as a low power lens. (Photograph kindly lent by Mr. D. Martin, Hospital for Sick Children, Great Ormond Street, London.)

### Mounting in Solid "Perspex"

The ideal museum specimen should be mounted in a transparent, unbreakable solid mass, and should retain its natural colour and shape indefinitely. The embedding of biological material in transparent plastics is still in the experimental stage and the ideal plastic has yet to be discovered. Unless great care is taken to prevent an undue temperature being reached during polymerization, especially

when thick layers or blocks are being handled, internal stresses may result in damage to the specimens or in fissures in the plastic. This difficulty may be avoided by lowering the temperature for polymerization or reducing the quantities of catalyst and accelerator used which will slow the rate of polymerization. An early advance towards the ideal objective was made by Carroll and Neidhoefer (1936), who accidentally embedded specimens in celluloid, after immersing them in oil of wintergreen which dissolved the celluloid. Plastics would seem to be ideally suited to this technique, but many difficulties have been encountered, owing to bubble formation and clouding of the plastic; the relevant problems are discussed by Hibben (1937) and Kramer (1940), who remind us that the bee in amber is an early example of this technique.

A very elaborate method, with certain unique features, has been designed by Strumia and Hershey (1948). Specimens are not fixed or dehydrated by any of the traditional methods, but frozen to a temperature of  $-20^{\circ}$  C. and then dehydrated from the frozen state by sublimation of water vapour. They are then impregnated with a liquid monomer, and subsequently polymerized with benzoyl peroxide. The colours are said to remain unchanged for at least five years, and their coloured illustrations show a remarkable degree of preservation of the shape, texture, and colour of a xanthoma. It is clear that a large initial outlay on equipment is necessary for this method.

Biologists will all agree that any method of by-passing the traditional methods of fixation and dehydration deserve serious consideration, for it is to these that we must ascribe the unlikelike appearance of tissues.

**Three Alternative Methods for Opaque Specimens.**—(1) A method supplied by I.C.I. Plastics, Ltd., is at present under trial, and the following materials are required:

Methyl methacrylate monomer  
Benzoyl peroxide  
Dibutyl phthalate  
Sheet "perspex"  $\frac{1}{16}$  in. or  $\frac{1}{8}$  in. thickness  
Caustic soda  
"Perspex" cement No. 6

*Washing of Monomer.*—During manufacture the methyl methacrylate monomer is stabilized by the addition of hydro quinone and is then known as "kallodoc" liquid. Before thickening the stabilizer must be removed by washing with 5% caustic soda solution in the following manner. Equal quantities of monomer and caustic soda should be used, and washing is best carried out in a separation funnel, where the discoloured washings, which form a layer below the monomer, can easily be run off. Two washings with the 5% caustic soda solution are sufficient, and should be followed by washing with water, until the liquid run off is no longer alkaline (test with phenolphthalein). As before, the monomer forms the top layer in the separating funnel. The methyl methacrylate is then run off and allowed to stand for 24 hours with slaked calcium chloride for its complete dehydration. After filtering through filter paper it is ready for thickening, but if it is not required for use immediately the monomer should be stored in a refrigerator.

*Syrup Preparation.*—A mixture containing 85 parts by volume of washed methyl methacrylate monomer containing 0.02% benzoyl peroxide (by weight), and 15 parts dibutyl phthalate, is placed in a flask, which should only be half filled, fitted with an air condenser. Thickening takes place in approximately 15 minutes using an oil

bath at 130° C., or in 30 minutes using a boiling water bath. Three or four shakings are necessary in each case during the process. As a precautionary measure a sink full of cold water should be at hand, so that if the reaction becomes violent during a shaking the flask can be immediately cooled. The monomer is thickened to the maximum convenient viscosity (the syrup thickens in cooling), and stored in a refrigerator until it is required for use.

*Preparation of Specimens.*—The fixed specimens must be thoroughly dehydrated as any residual moisture causes clouding of the “perspex.” Experiments involving a final soaking in unthickened monomer before embedding, or in a 10% solution of benzoyl peroxide in chloroform, have been performed. Overnight soaking in chloroform has given best results to date. Soaking in unthickened monomer tends to retard polymerization, whereas prolonged immersion in the solution of benzoyl peroxide may cause certain gaseous reactions to take place during polymerization if this is carried out at a temperature of 37° C. or over.

*Mounting.*—Embedding-cells may be made from sheets of glass held together by cellulose tape. Hollow specimens and soft tissues are impregnated with monomer of slightly less viscosity than that used in embedding. A mould can be quickly and easily made by joining suitably shaped pieces of glass with “semmentum” to form a box. “Semmentum” sets quickly and will hold the glass in place in a few minutes. The mould is either left overnight or may be completely dried and hardened in a 40° C. oven in a few hours.

*Embedding.*—Embedding is best done in layers. First, a layer of syrup, about  $\frac{1}{4}$  in. thick, is poured into the mould and polymerized at 40° C. for a few days to form a supporting layer. (Time may be saved by pouring a thinner first layer on to a sheet of  $\frac{1}{4}$  in. thick “perspex” on the bottom of the mould.) Polymerization at room temperature may be carried out by ultra-violet light. Second, another layer of syrup is poured into the mould and the specimen is placed in position in it. Further layers may be needed to cover the specimen adequately if it is thick and to allow for evaporation and incomplete polymerization. A glass lid should be bound on to the mould with cellophane tape to form an air-tight cover during polymerization to prevent evaporation. When polymerization is complete the block can be shaken out of the mould or the glass broken away. The block is cut and trimmed to size and shape and finally polished by hand or on a finishing machine with the finest possible emery papers and finished with “perspex” polish (or a good metal polish).

(2) Embedding in Ward’s “bio-plastic”\* requires the following materials:—

Ward’s “bio-plastic” (“selectron”)

Tertiary butyl hydroperoxide (Ward’s catalyst)

Opaque specimens are fixed and preserved in a formaldehyde fixative. Catalysed “bio-plastic” is prepared by adding 0.1%–0.5% of Ward’s catalyst to the monomer. The amount of catalyst used is proportional to the size or thickness of the layer. The supporting layer is poured into the mould and allowed to gel at room temperature for one to three hours. While any bubbles are rising from the catalysed monomer, the specimen is dried by blotting with absorbent material and then air-dried, just short of shrivelling and darkening. A layer of catalysed monomer, just sufficient to cover the specimen, is then poured. The specimen is carefully placed in position so that no air bubbles are trapped. Gelling is allowed to occur at room temperature. The final layer is poured and allowed to gel at room temperature. Final polymerization is accomplished in an oven starting at 37° C. and gradually rising to, but not exceeding,

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\*Ward’s Natural Science Establishment, Inc., Rochester 9, New York.

60° C. (The mould may be left in an incubator at 37° C. overnight.) The block is removed from the mould and shaped and polished by hand or on a finishing machine.

To get a cleared specimen it should be dehydrated with alcohol and then placed in anhydrous ether; from this it is transferred to uncatalysed monomer and the ether is slowly removed in a desiccator by slowly reducing the pressure. (It should be remembered that too rapid evacuation can cause the ether to boil and damage the specimen.) The specimen is placed into catalysed monomer and the above technique then followed.

(3) Embedding in "marco S.B.26C" resin requires the following materials:—

- "Marco S.B.26C" resin
- "Monomer C"
- H.C.H. catalyst (1-hydroxycyclohexyl hydroperoxide-1)
- "Accelerator E" (solution of cobalt naphthnate in "monomer C")
- Diethyl phthalate (plasticizer)

The plastic is prepared by mixing

|   |                       |           |                      |
|---|-----------------------|-----------|----------------------|
| A | "Marco S.B.26C" resin | 100 parts |                      |
|   | "Monomer C"           | 20 parts  |                      |
| B | Catalyst              | 2 parts   | } warmed to dissolve |
|   | "Monomer C"           | 20 parts  |                      |

and adding to the mixture 10 parts of plasticizer. This mixture is then filtered through glass wool. It is stable for 1 to 2 days.

"Accelerator E" (1 part) is added and the plastic is ready for polymerization.\*

The remainder of the technique for opaque and clear specimens is the same as that when using Ward's "bio-plastic."

**Transparent Specimens.**—The following methods of mounting transparent specimens depend on the replacement of tissue fluids by fluids of a high refractive index. Transparent specimens have a certain value in demonstrating injected vessels and the bones of embryos, but are not in general very instructive. They were purchased before the second world war at high prices in Germany, but can be fairly cheaply though rather tediously prepared. Oil of wintergreen, glycerine, and paraffin have been used in their preparation. The essential features are that dehydration must be very slow and quite complete, and the longer the process the better the results.

The method which we have employed successfully is that of Spalholz (1911) as follows. Specimens should be fixed in 10% formol saline very thoroughly, bleached in 50% H<sub>2</sub>O<sub>2</sub> (but this is not essential), and dehydrated very gradually before final mounting in benzyl benzoate.

|             |         |
|-------------|---------|
| 50% Alcohol | 2 weeks |
| 60%    "    | 2   "   |
| 70%    "    | 2   "   |
| 80%    "    | 2   "   |
| 95%    "    | 2   "   |

The final alcohol is absolute alcohol, and two changes are made each of two weeks, the second change with a layer of anhydrous copper sulphate on the bottom of the container covered by five layers of filter paper. Two changes of benzol are required, each

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\*These details were kindly supplied by Mr. W. A. Norman, Wellcome Museum of Medical Science.



of two weeks, and the same procedure is followed with benzyl benzoate. The specimen is now mounted in equal parts of benzyl benzoate and oil of wintergreen. This fluid is a "perspex" solvent, and therefore glass jars must be used. Most cement seals are also dissolved, but the best seal is made of

|                     |       |
|---------------------|-------|
| Powdered gum arabic | 50 g. |
| Sugar               | 50 g. |
| Sod. silicate       | 2 g.  |
| Formalin            | 1 ml. |

These ingredients are made into a thick paste with water, and after affixing the lid the jar is left undisturbed for 48 hours.

Methods using glycerine and alizarin (Fig. 12) are described by Richmond and Bennett (1938) and further reference may be made to Lee (1937) and Mall (1906).

We find that 5% gelatine with finely ground chrome yellow promotes a good injection mass for vessels in transparencies; 0.4% formalin is added just before use. Vessels should first be perfused with warm isotonic saline containing amyl nitrate to prevent vasoconstriction. Before fixation the vessels are ligated and the specimen placed in the refrigerator to set the gelatine (Fig. 13).

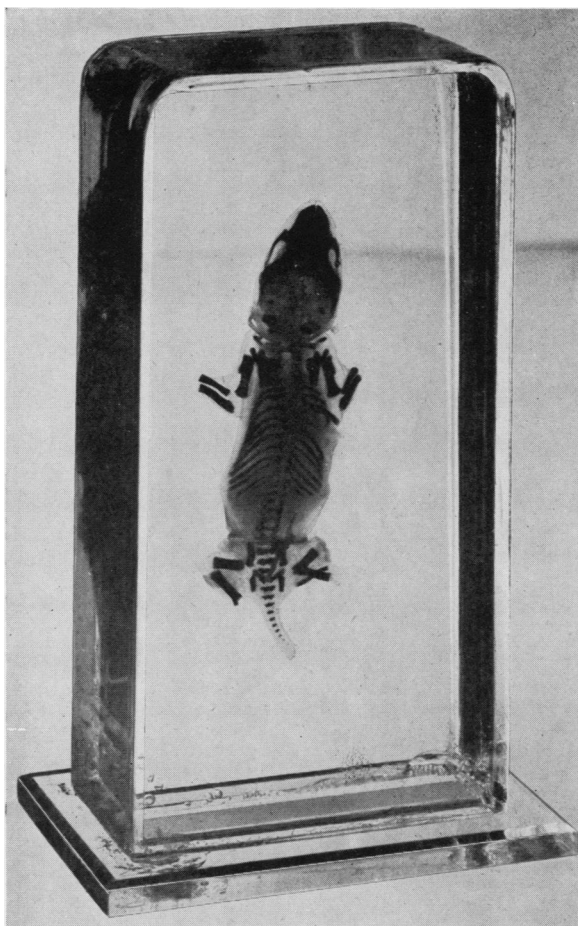


FIG. 12.—Transparency of a rabbit foetus prepared by Dawson's method using glycerine and alizarin. (Preparation kindly lent by Mr. J. King, London Hospital.)

### Special Methods

**Amyloid.**—Stained amyloid tissue often fades after mounting, and to prevent fading we find the following technique valuable. The specimen is immersed in the following solution:

|                |         |
|----------------|---------|
| Iodine         | 1 g.    |
| Pot. iod.      | 2 g.    |
| Water          | 100 ml. |
| Sulphuric acid | 1 ml.   |

After washing in tap water the specimen is mounted in liquid paraffin.

We have found that specimens stained with Congo Red retain their colour. After fixation in Kaiserling I specimens are immersed in 1% Congo Red for one hour and

are then transferred to a saturated solution of lithium carbonate for two minutes. They are differentiated in 80% alcohol. Normal arteries and veins tend to retain the colour. Specimens are mounted in Kaiserling III without sodium hydrosulphite

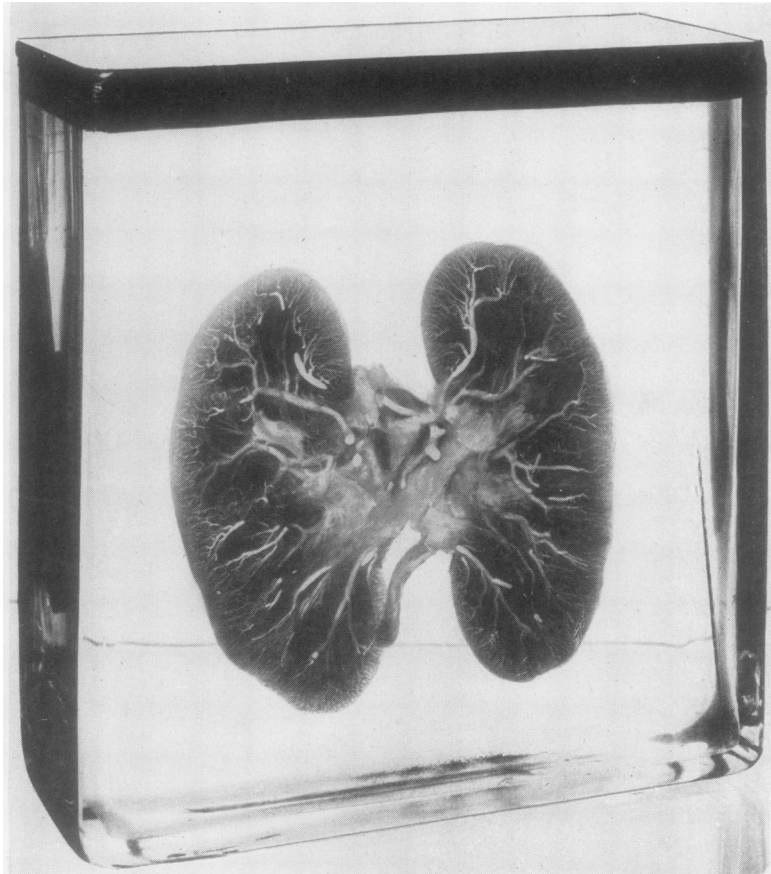


FIG. 13.—Specimen of kidney injected with chrome-yellow gelatine and cleared by Spalteholz technique to demonstrate circulatory system.

**Haemosiderin.**—Specimens are fixed in Kaiserling I, precautions being taken to prevent contact with iron rust. They are readily stained with a solution of equal parts of 10% HCl and 5% aqueous pot. ferrocyanide, and are then washed in running water for 12 hours. Old museum specimens of this type were mounted in 80% alcohol, as the iron diffuses in Kaiserling III. The alcohol is dangerous if specimens are opened by using a bunsen flame; and all museum curators should remember that alcohol was once a popular mounting medium.

Freshly prepared specimens should be mounted in 5% formol saline, which prevents colour diffusion except with specimens of haemochromatosis, where for some reason the colour diffuses and the fluid becomes milky within three months.

Fat may be demonstrated by staining Sudan III, and masses of malignant or other tissue by Ehrlich's haematoxylin, often an excellent way of demonstrating the limits of lesions.

### Labelling of Museum Jars

In museums where specimens are used for teaching and examination it is undesirable that they should be labelled with a diagnosis. However, since a student is faced with thousands of specimens he should be enabled rapidly to determine which specimens are typical examples of common disorders, which are duplicates, and which are rare, unusual, or historical specimens.

Our practice is to label each jar with a rectangle of cellulose paint in the centre at the bottom, the label being red for common disorders, white for duplicates of common disorders, and yellow for rarities. Thus a student may cover the salient features of disease by a study of jars with red labels. The number of the specimen, preceded by a letter indicating the section of the museum to which the specimen belongs, is painted in black (Figs. 7, 8, and 9).

If "perspex" jars are used labels may be made by cutting rectangles of coloured "perspex"  $\frac{1}{8}$  in. thick, and the numbers are stencilled on with "uno" stencils. "Perspex" cement is used to cover the number, and the rectangle is sealed in position with cement. If it is desired to label a specimen with its diagnosis, a "perspex" strip may be thus stencilled, covered with cement, and cemented to the centre-plate.

### Catalogues

It is essential that a plan of the museum should be visible to the visitor on entering; each section should be clearly labelled. Some curators differentiate conditions in each section by a distinguishing coloured band, e.g., red for malignancy, and yellow for inflammatory processes. Two different methods of cataloguing are given by Boyd (1934) and Mundell (1935). Our own preference is for the "shannon" catalogue which holds 96 cards on wires. The edges of the cards, and thus the diagnosis of the specimen, are all simultaneously visible. Cards are easily re-arranged. We have found this catalogue to stand up to daily use for ten years. On the descriptive card a reference to the patient's case or post-mortem number is added, and in certain cases to photomicrographs of the tissue. Several duplicates of each catalogue should be available.

### Lighting

A poorly lighted museum is useless, and in any museum the best lighted exhibits are the ones most frequently examined. Fluorescent lighting (Karsner, 1941) is the best available, giving at least fifty per cent better results than were previously available.

### Methods of Presentation

We are still far from the ideal museum of pathology. In every museum there are specimens of great value, mounted with technical precision, and fully apt to illustrate a lecture or inform a visitor. But the ideal pathological museum should present a student with the full picture of human disease; it should show, by photographs, the appearance of the patient, and the aetiological and environmental factors that lead to disease. Some attempt should also be made to impress upon students the statistical significance of each disorder, and the changes which

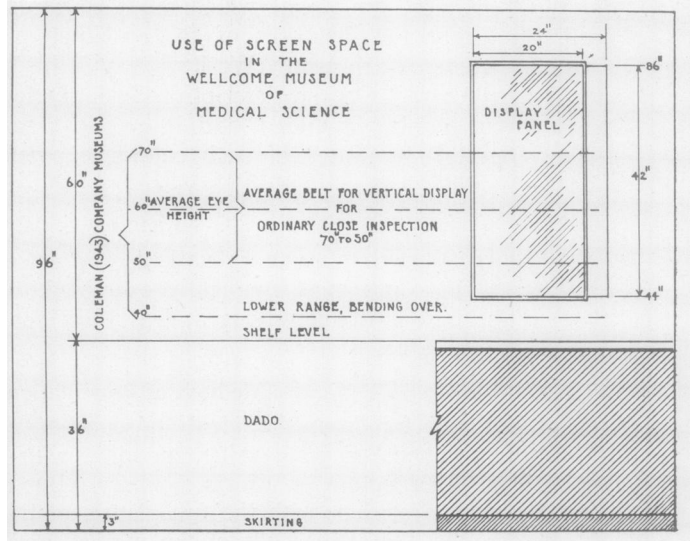
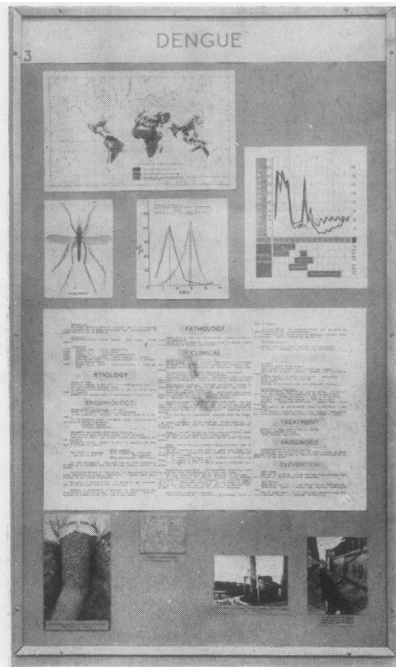
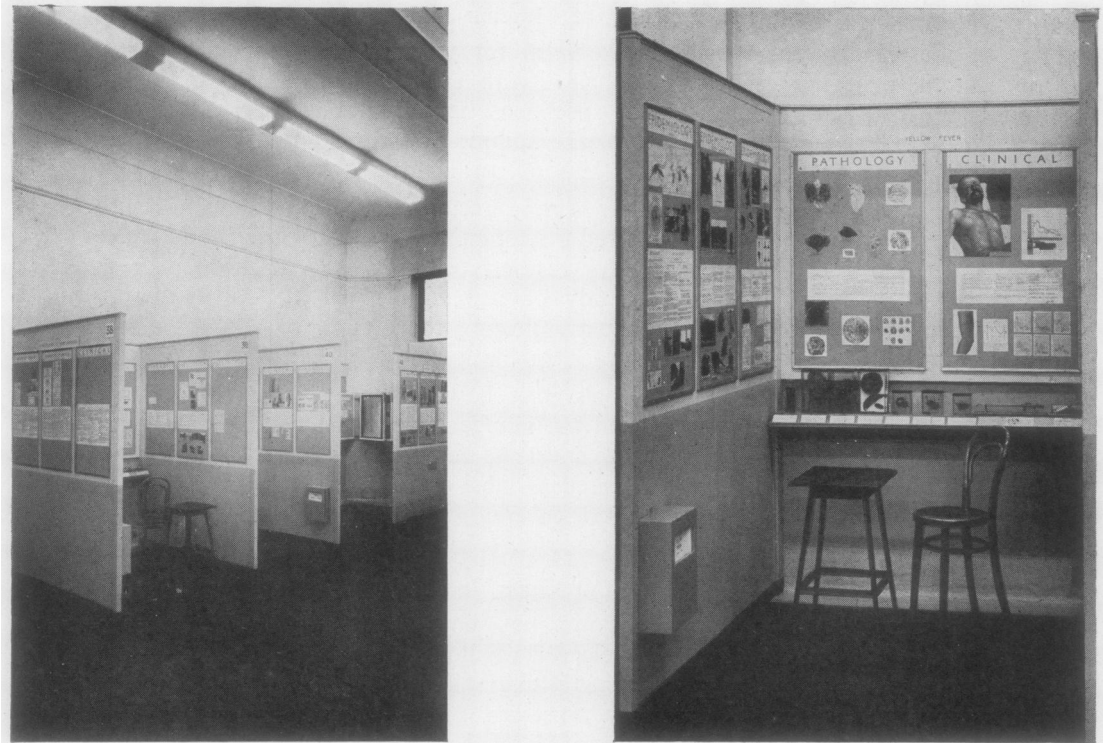


FIG. 14.—Series of photographs illustrating the method of presentation of material at the Wellcome Museum of Medical Science, London. (Photographs kindly lent by Dr. C. J. Hackett.)

are taking place in the incidence of each. X-ray and laboratory findings should be illustrated. Not least there should be photographs of the men and the women who have, by their labours, established clinical entities and evolved methods of treatment and diagnosis. Somewhere also there should be room for a few sentences from the writings of those who were inspired to make original and fundamental observations. Perhaps if each museum exhibited, as some few do, a memorable saying of a great writer publicly displayed as a motto, a step might be taken to make museums more important in the education of students. In the writings of Sir Thomas Browne and of Montaigne are to be found innumerable sentences which seem to define the spirit of medicine.

In Great Britain the Wellcome Museum of Medical Science, under the direction of Daukes (1929, 1936), has set a standard which few have approached and none excelled. It is not possible to give full references to American trends, but a discussion of these is given by Aegerter (1948).

In the Wellcome Museum the basic principle is that the belt of vision lies between 40 in. and 70 in. from the ground. There is a colour code throughout for aetiology, epidemiology, pathology, clinical treatment, prognosis, and prevention. Displays, in the form of a very large book with hinged frames, are available and frequently changed (Fig. 14). Methods of display are discussed by Greenwood (1944).

The mounting of lung specimens on transparent sheets introduced by Gough (1947) has inspired Benians (1948) and his colleagues to develop a miniature museum in portfolios. After fixation, specimens are cut by Anderson's method and mounted in gelatine in "perspex" frames. Folios are preserved in covers with histories and all the relevant clinical data, references for further reading, and photomicrographs and tracings of the specimen with explanatory notes. This method offers an enormous saving of space, and although there is some sacrifice of reality in losing the three dimensional presentation of a mounted specimen, hollow viscera not being suitable for this technique, there is little doubt that most museums will in future present such exhibits as ancillaries. We have found that stained giant sections mounted in the ordinary way in Canada balsam and other histological media are very valuable, and are more easily prepared than is generally recognized.

The scope of the medical illustration has been discussed by Treadgold (1949), where the practice of Guy's Hospital Medical School is given. The use of cartoons, and what was called by a later correspondent "education by terror," are innovations not likely to commend themselves to traditionalists, but they have long been in use in Vienna and in the German schools. Much attention is rightly given to the type of script, the use of colour, and the making of models. Moulage has in fact a great value in museums, which cannot for obvious reasons present specimens of hands or face. Material for this purpose was previously readily available in Germany, and we have not found the technique laborious; there is a need for its reintroduction in this country. The older museums present admirable models in wax. In certain American hospitals, e.g., Rochester General Hospital, specimens have been replaced by "kodachrome" transparencies, a practice not likely to be widely followed.

Some compromise between a collection of specimens for teaching purposes and a display intended to present the picture of disease as a whole is possible by the selection of certain disorders for special attention. For example, at Westminster Medical School we have built display cabinets in tiers with illuminated panels. In these a condition such as syphilis is illustrated, its history briefly shown, the statistics of its incidence graphically outlined, photographs of patients and specimens of lesions given, and letters from patients suffering from G.P.I. are exhibited (Fig. 15). Similar exhibits are offered for tuberculosis and malignant

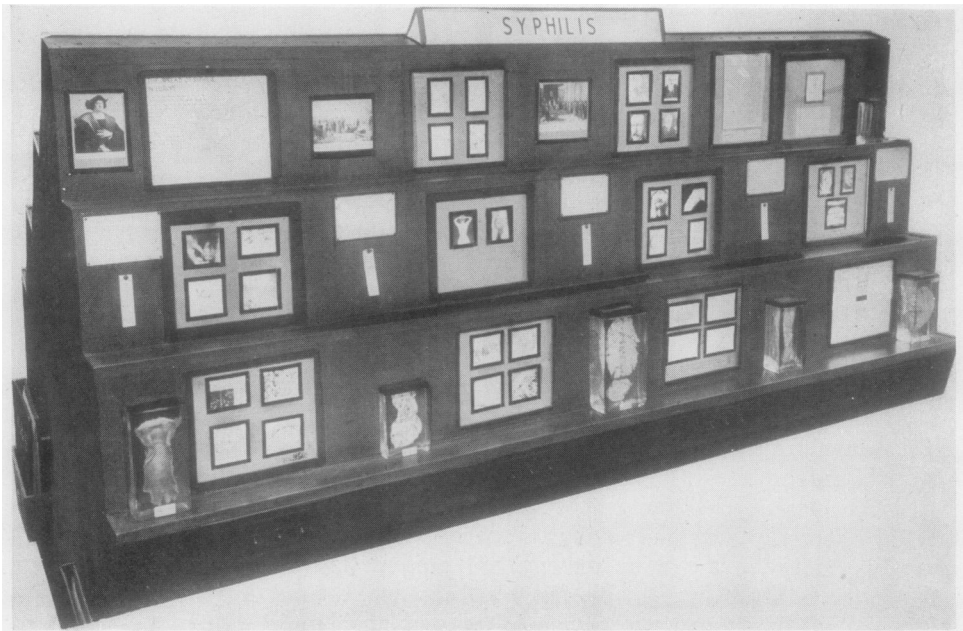


FIG. 15.—Central display cabinet in Westminster Medical School museum showing semi-permanent display on syphilis.

disease. Exhibits of this kind make great demands on the imagination and the time of curators. Much may be learned from commercial exhibitors. The art of advertising (Symes, 1948) has much to teach the medical teacher. A department of medical photography is essential, and its director must be prepared to study the technique of instantaneous impressiveness. Vivid colours, geometrical forms, epigrammatic script may seem childish devices, but material exhibited with their help may summarize once and for all a basic principle.

Finally, who should direct the policy of a museum and determine its content? It is usually the function of a pathologist, who must in any case have the last word. But a pathologist is often more interested in curiosities than in the everyday conditions, and in any case is only one of the teachers who use the material. His colleagues only too often wish him to include specimens whose removal was a personal triumph, or may believe that even a hundred specimens of their own

favourite lesion are not excessive. Consequently it is essential that there should be for each section of the museum a clinician with particular knowledge of the system studied, and the advice of clinicians should always be sought. Financial support for museums of pathology in Great Britain has been liberal, and only the initiative of curators and the limitations of space may impair their quality.

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