

Museum Techniques and Practices in Histopathology; A Compendium

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ABSTRACT

Medical Museums are institutions that store and exhibit objects of historical, scientific or conditions of great rarity that has a link to medicine or health. Medical museum specimens are used as a visual undergraduate and postgraduate teaching aid. This article is aimed at elucidating more on museum techniques and practices in Histopathology. In order to produce a permanent museum mount in histopathology, specimens are dealt with in the following order; Reception, preparation, fixation, restoration, preservation and presentation. Specimens in the older medical museums were mounted in glass jars which were originally cylindrical in shape. Special museum techniques reviewed in this study include maceration and calculi. There are a number of stains that are used to demonstrate the presence of normal or abnormal constituents in tissues to be mounted in a museum jars, examples of these stains are: iodine or Congo Red; Perl's Prussian Blue; Sudan IV or Scarlet R and Alizarin Red S. There is still no substitute for well dissected and mounted specimens that can be used for medical exhibitions, examination vivas, lecture demonstrations as well as self-teaching aid for both undergraduate and postgraduate students in Histopathology.

Keywords: Amyloid, Haemosiderin, Jars, Museums, Perspex.

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INTRODUCTION

Medical Museums are institutions that store and exhibit objects of historical, scientific or conditions of great rarity that has a link to medicine or health. They also provide an individual/student with the basic materials for research and a platform for personal teaching [1]. Collection of specimens for museum techniques in histopathology can be satisfactorily housed in any small well-lit room with suitable shelving. Medical museums tend to be used more assiduously if there is a small table and chair where students or individuals can sit to examine the specimens and related catalogue entries in comfort. It is also valuable to have a microscope, preferable robust and easy to use on the table, and to give students easy access to histological slides relating to gross specimens in the museum. Case histories, X-rays, Laboratory reports, clinical photographs, ECG tracings, etc, can all be displayed alongside pots and microscope slides [2]. When only limited space is available, much can be done to extend the range of the specimen by incorporating clinical photographs, photographs of X-rays etc into the specimen jar, and copies of liver scans, brain scans, ECGs etc into the descriptive catalogue entries [3]. It is important that a museum does not become a static unchanging collection of potted specimens; efforts should be made to regularly update catalogue entries, incorporating clinical follow-up data, any subsequent necropsy data. Old established specimens should be replaced by subsequent, more satisfactory, material and the older specimens stored in a cupboard out of sight for examination vivas, pathology spotter exams [4, 5].

A well-organised pathology museum serves many functions, and should aim to be:

- A permanent exhibition of common pathological conditions for undergraduate and post-graduate self-education
- A collection of specimens illustrating rare conditions, or specimens of historical interest;
- A collection of specimens which can be used as the basis of pathology quizzes, tape-slide programmes, medical exhibitions, examination vivas, lecture demonstrations etc
- A permanent source of histological material for teaching, research etc.
- A permanent source of photographic material, both gross and histological, for exhibitions, publications etc [5, 6].

Basic museum techniques

The way in which specimens are dealt with to produce a permanent museum mount can be summarised as follows:

- Reception
- Preparation
- Fixation
- Restoration
- Preservation
- Presentation [4].

Reception of specimen

Specimens that are received for permanent display can come from a number of sources: from hospital operating theatres, from the Post-Mortem room, or research laboratories. Although initially, there may be a small number of specimens received, it is essential that accurate records are kept from the outset [3]. This is best done by having a reception book in which all specimens are recorded, including all the relevant details. These consist of diagnosis, the name of the patient or donor, surgeon or pathologist, hospital and histological section number. On arrival, each specimen is given an accession number. This is followed by the year of

entry, e.g 1/1981. Continuing through that year and starting again as 1/1982 at the beginning of another year. The specimen will carry this number until it is given a final catalogue number according to its place in the collection. It cannot be over-emphasised that whereas a specimen may change its Museum catalogue number and place in a permanent collection, the initial accession number is final.

This initial number is then written on a tie-on type parcel label in indelible ink and the label is either firmly tied to the specimen, or each specimen is dealt with in a separate container with the label placed with it. If a number of specimens are in the same container with their respective numbers unattached, confusion will occur. Liver specimens must always be stored separately as the bile tends to discolour other specimens [4].

Preparation of the specimen

From whatever source the tissue is received, the best ultimate museum specimen is only possible if it arrives in a fresh unfixed condition so that the laboratorian can then deal with it with a final museum mount in mind. Whether the specimen is fresh or already in a fixative, any gross trimming and dissection

necessary should be carried out immediately, because when the final fixation of the specimen takes place, there is a limited penetration of fixative, and if any further extensive cutting or dissection is done, unfixed areas of the specimen will be revealed [7].

If the specimen has arrived in an unfixed state, it must not be allowed to dry, as irreversible discoloration occurs, and it must never be washed in water as the resulting haemolysis will cause permanent staining of the final mounting solution. If it is necessary to wash off excess blood, this should be done with fixative [7].

In an unfixed state, the specimen still retains its natural colour, and it is at this stage, after the initial dissection, that it should be photographed. If the specimen has already been fixed and shows the unnatural colour that results, photography should be delayed until the colour has been restored. Any blocks necessary for histological investigation are taken and if possible, these should be removed from a surface which is not important to the final mount. Some organs, e.g. kidney, can easily be bisected, using half for histological investigations and retaining the other half for mounting [7].

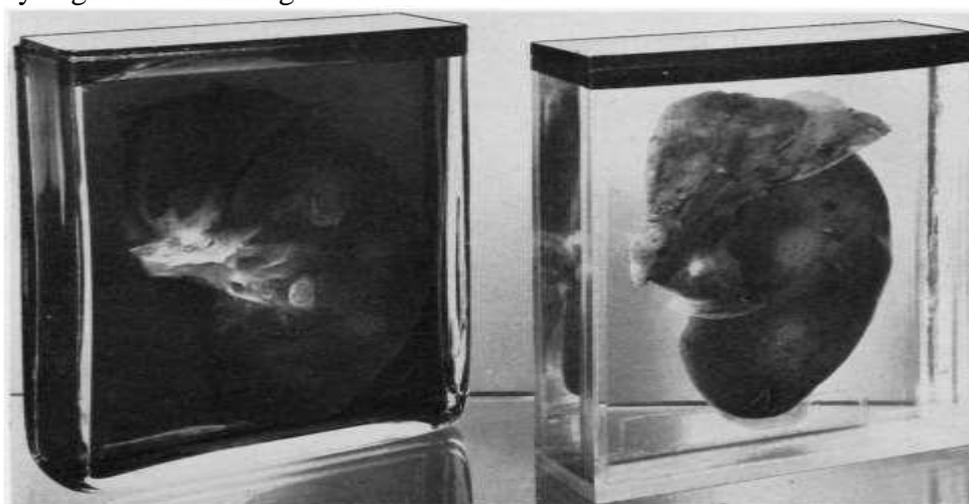


Fig 1. Two halves of the same kidney. The specimen on the left has been washed in running water for 12 hours, that on the right in normal saline [3].

Fixation of Specimen

When dealing with material for histological section, there are a number of fixatives that can be used but in order to preserve a specimen as a permanent museum mount and be able to restore it to its original colour, the only fixative which is acceptable is formalin. Usually specimens removed at operations or at necropsy have already been placed in a formal saline solution before being sent to the museum [8].

The fixative in use today in most medical museums is based on formalin fixation techniques which have been derived from [9], although, there are many modifications of his original technique. The method which Kaiserling recommended was based upon initial fixation in a formalin-based fixative which contained a number of salts to give an appropriately neutral pH to the solution. This solution contains 10% formalin, potassium acetate and potassium nitrate.

The following solution, which has been found satisfactory over a long period of time, is based on Kaiserling's original formula: Formalin (1 litre), Potassium acetate (85g), Potassium nitrate (45g), Water (Make up to ten litres). This solution is appropriately pH 7.0, and is known as Kaiserling I (K I) [9].

The specimen should be placed in an adequately overlarge container, with three or four times the volume of fixative. In most cases, one fixation solution will suffice, but with some larger specimens it may be necessary to change the fixative solution once or twice. The period the specimen should remain in the solution depends on its size, that is, from three days for a small specimen up to fourteen days for large specimens, e.g. whole lung, liver and limbs [9].

Due to the hardening action of formalin, the way in which the specimen will be ultimately presented depends upon maintaining its natural shape during

fixation. It is therefore important that a specimen should not rest on the bottom of its container, thus it will produce an artificial flat surface, and cause unfixed areas to persist at points of contact. All containers should be lined with fixative-soaked lint (non-furry side up) and where the specimen has an undulating surface it should be supported with fixative-soaked cotton wool.

Hollow Viscera

Cut hollow organs should be padded out with cotton wool, but if uncut, they can be pressure-inflated, e.g. through urethra into the bladder, through ureter into pelvicalyceal system, through trachea into lung, and by direct injection in the case of cysts. The fixative can be injected into such organs with a Higginson syringe or with a conventional hypodermic syringe, and the injection pressure required is usually obvious. It is important to avoid over-inflation and there should be more care when handling elastic organ such as lung, especially if it is affected by a disease such as emphysema. In this case, especially when dealing with a number of such specimens, a piece of apparatus using a re-cycling pump and calculated to produce a fixed pressure is recommended [4].

Solid organs

Solid organs such as liver and spleen may sometimes be perfused through the main artery, but if this is impossible due to blockage of the vessels, or because the organ has already been cut, it should be immediately sliced in the required plane to allow adequate fixation of the exposed surfaces. The slice is placed cut-face downwards on to the lint-covered base of the container. When such specimens are cut, this is done with a long, flat bladed knife (at least 30cms) in one cutting stroke, avoiding the serrated surface produced by a sawing action of the knife.

This cutting method is particularly important if it is desired to remove a thin slice from an already fixed surface, bearing in mind that the depth of penetration of the fixative may only be approximately 10mm, depending upon the tissue [4].

Limbs

The injection method of perfusion is also used for whole limb specimens, but because of problems arising from inadequate circulation or tumour formation, and if it is intended that the specimen is to be bisected, it will be found that cutting the specimen prior to fixation is advantageous. Because of the mixture of very soft and very hard tissues involved, the most satisfactory way of doing this is that of Baker [10], placing the unfixed limb in an adequate large container of 95% alcohol pre-cooled with solid carbon dioxide. Strings are attached to the limb to enable it to be suspended in, and ultimately removed from the cold alcohol (-60°C) without the hands coming into contact with the solution. It remains in this solution for a minimum of one hour, during which time the cooling is maintained by the addition of more solid carbon dioxide. When the limb is frozen, and with careful reference to X-rays previously taken, the limb can be cut with a band saw in the required plane. The two halves are then placed face down in a container of fixative to thaw and fix [10].

Heart

Specimens of heart have usually been cut before being sent to the museum and in order to maintain the natural shape, it is important to pad out all cavities and major vessels with cotton wool before fixation. If, however, a heart is received fresh and uncut, it is placed in an adequately large container of fixative and additional fixative perfused through the coronary ostia with a syringe. This will not only fix

the heart tissue, but as the coronary circulation fills, the heart will revert to its natural shape. This is the ideal method for fixing hearts for display [10].

Brain

Because of its soft consistency and the difficulty of handling in a fresh state, it is necessary to fix the brain before cutting [11]. Also because of the softness, if the specimen is allowed to rest on the base of the container, even if supported with cotton wool, distortion will still occur. It is therefore preferable to perfuse the brain through the basilar and cerebral arteries at its base and it should then be suspended by the basilar artery within the fixative. If left in this condition for at least a week, it can be easily bisected or sliced with a brain knife using a cutting device [11].

Factors affecting fixation

- Buffering
- Penetration
- Volume
- Temperature
- Concentration
- Time interval
- Position of tissue [12].

Buffering

- Fixation is best carried out close to neutral pH, in the range of 6-8.
- Hypoxia of tissues lowers the pH, so there must be buffering capacity in the fixative to prevent excessive acidity.
- Acidity favors formation of formalin-heme pigment that appears as black, polarizable deposits in tissue.
- Common buffers include phosphate, bicarbonate, cacodylate, and veronal.
- Commercial formalin is buffered with phosphate at a pH of 7.0 [12].

Penetration

- Penetration depends upon the diffusability of each individual fixative, which is a constant.

- Formalin and alcohol penetrate the best [13], and glutaraldehyde the worst.

Volume

- The minimal acceptable volume of fixation fluid is about 15 to 20 times the volume of the specimen.
- The use of small volumes of fixation fluids for larger specimens is the most frequent cause of poor tissue preservation [12].

Temperature

- Increasing the temperature will increase the speed of fixation.
- Hot formalin will fix tissues faster but concomitantly distort the architecture of the tissue and also denature the tissue protein [12].

Concentration of fixative

- Concentration of fixative should be adjusted down to the lowest level possible.
- High concentration may adversely affect the tissues [13], and produce artifact similar to excessive heat [12].

Restoration of Specimen

Kaiserling's Method of Colour Restoration

After fixation of specimen, its natural colour is lost; it is therefore necessary to restore the colour to as near its natural colour as possible. There are many ways in which this can be achieved and the one recommended is the second stage of Kaiserling's method (KII). This involves removing the specimen from the fixative, washing in running water and transferring to 95% alcohol. The specimen is placed in alcohol between 30 minutes to 10 hours (depending on the size of tissue) during which it is watched carefully as the colour develops throughout the specimen. If not already done, it is at this stage that the specimen is photographed. When the

specimen is removed from the alcohol, it is blotted dry, to avoid highlights in the photograph due to reflection from a wet surface [14].

When the colour restoration is satisfactory, the specimen is removed and placed in a preserving or mounting solution. It should be noted that if the specimen is left too long in alcohol, the colour will fade and this effect is irreversible. This can be controlled by examining specimens mounted and displayed in older museums [14].

Rejuvenator Solution (Pulvertaft's Modification) for Colour Restoration

Pulvertaft [15] described a method of restoring the colour by adding reducing agent (sodium hydrosulphite) to the mounting fluid: Pyridine (100ml), Sodium hydrosulphite (100gm), Distilled water (4 litres) [15]. Meanwhile rejuvenator solution restores and maintains the colour but this solution (rejuvenator) can show remarkably little fading even after 35 years.

Schultz's Method of Colour Restoration

Carbon monoxide has also been employed as colour retaining agent. This technique gives brilliant colour contrast, but has a risk of poisoning and explosion and also, colours are unrealistic [16].

Preservation of Specimen

The final preserving solution is the one in which the specimen will be mounted for display. It is recommended that the third solution of Kaiserling (III) is used. This is a glycerine solution containing sodium acetate and although in his original solution, Kaiserling recommended a 25% solution, when a 40% glycerine solution is used, the refractive index is similar to that of the Perspex used in modern containers. This produces a more solid effect to the final mount [14]. This solution is as follows: Sodium acetate (1416g),

Glycerine (4 litres) and water (Make up to 10 litres)[14]. The addition of a little formalin or thymol will prevent the formation of moulds.

The specimen remains in this solution until it is well permeated; initially it will float and should be covered with soaked lint, but will eventually sink to the base of the container. It is almost impossible to preserve a specimen showing its original fresh colour and any technique which will help achieve this effect is obviously worth trying, provided there are no adverse effects to the specimen.

There have been many modification of Kaiserling's technique; some still in use and some have been rejected. These have ranged from the hazardous technique of Schultz [16] of bubbling coal gas through the final preserving solution so producing the pink carboxy-haemoglobin coloration to the specimen, to the more recent method, of Wentworth [17] in which he used only sodium hydrosulphite and omitted glycerol from the final mountant [17].

Romhanyi [18] method is based on the formation of bright red complexes of haemochromogen with various nitrogen bases such as pyridine and nicotine. The reaction is carried out in aqueous solution using pyridine and/or nicotine in the presence of sodium dithionite. This solution also contains formalin. In the original technique, the fixation and storage takes place in the same solution [18].

This was made up as follows: Formalin (120cm³), Pyridine (10cm³), Nicotine curdum 5% in water (10cm³), Sodium Dithionite (20g) and water (make up to 1 litre) [12].

After making up this solution, there is slight turbidity which settles as a dark brown deposit within 24 hours. If the solution is made up in a large aspirator this deposit will rest below the outlet and a clear solution can be run off.

Originally, the specimen would be fixed in the solution and then transferred to the museum jar and preserve in this same solution. Specimens that have been fixed in formalin for a long period regained their colour. There are three objections to using this fluid in this way. Firstly, nicotine is poisonous. Secondly, pyridine has an obnoxious smell, and thirdly, as the specimens are now mounted in Perspex, it is far better to retain the optical effect of using an approximately 40% glycerine. The method of choice is the original Kaiserling technique of fixing the specimen in K I, restoring the colour in K II and mounting the specimen in K III [19].

By replacing some of the K III with Romhanyi's solution to approximately 25% by volume, an improved colour to the specimen can be produced. One of the minor problems with Romhanyi's solution is the occasional formation of a heavy black deposit on some specimens, especially bones. This reveals itself within a few days and can be removed by washing the specimen inside the container with 10% hydrochloric acid. The specimen is then remounted in Kaiserling III. Romhanyi solution is not recommended for any specimen which has been previously preserved in liquid paraffin or alcohol, or has been perfused with one of the phenol-based solutions used in the dissecting room [19].

Pulvertaft-Kaiserling mounting fluid III

This include: Glycerine (300ml), Sodium acetate (100g), Formalin (5ml), Tap water (1 litre) [3].

- Camphor / Thymol can be added to prevent growth of moulds.
- Immediately before sealing, 0.4% sodium hydrosulphite is added. The amount of hydrosulphite should not normally exceed 0.4%.
- If color restoration must be rapid, 0.6% may be added, but this is to be

avoided, as a white precipitate may form.

- If the solution is not crystal clear, it is usually due to impurities in the sodium acetate.
- Such solutions should be filtered through paper pulp under negative pressure.
- If this fails, 50ml of saturated solution of camphor in alcohol should be added to 1 liter of the solution, re-filter as before [3].

Israel and Young

Used pure liquid paraffin as the final mountant after colour restoration with alcohol. This procedure reduces chances of discoloration of the mounting fluid by pigments in the specimen [20].

Presentation of Specimen

Specimens in the older medical museums were mounted in glass jars. These were originally cylindrical in shape, with a lip. These specimens were suspended by strings, the jar filled nearly to the top with preserving solution and a piece of lead covered the top [3]. Over this, wet sheep's bladder was stretched, tied around the lip of the jar and, when dry, painted. When rectangular jars were manufactured early in the 19th century, these gradually replaced the cylindrical ones. They had the advantage that the polished flat faces avoided the magnification and distortion of the specimen that the cylindrical jars produced. Specimens still had to be suspended by cords and these jars could not be completely filled with fluid. The tops of these rectangular jars were of glass and were attached with either putty or cement consisting of pitch, gutta-percha and wax [21]. The disadvantage of both of these types of jars was the limited range of sizes, their heaviness and fragility, and as they could not be filled completely, the suspended specimens were easily damaged during handling. With the introduction of

Perspex, museum jars can be constructed to any specification [21]. These plastic jars have the advantage that they can be completely filled with mounting solution, the specimen can be attached to rigid supporting plates within the jar, and they are light and strong. Plastics are, however, attacked by absolute alcohol and dissolved by methyl salicylate. It is therefore necessary to mount any specimen preserved in these solutions in glass jars [23].

Measuring of Specimen

Whether jars are ordered from manufacturers or made on the premises, it is necessary to specify the dimensions of the jar required [22]. As the effect of a well-dissected, fixed and preserved specimen can be completely spoilt by having it crammed into a container that is too small, or lost in an over-large one, it is essential that the jar suits the specimen. Before the required measurement can be calculated, the specimen should be oriented into its correct anatomical position where feasible, even though this may produce an uneven distribution of the specimen with the jar. The height, width and then depth (thickness) of the specimen is measured. If an extra 3.75cm is allowed on the height and an extra 2.5cm allowed on the width and depth, the final jar made to these dimensions will ideally accommodate the specimen. These are, of course, the inside dimensions of the jar and are quoted as: height X width X depth [24].

Mounting of Specimen

Tissues to be mounted are trimmed to the desired size and shape so that it fits into the jar. All unwanted and non-representative tissues should be removed by careful dissection.

If after removing cotton wool from tissues cavities and the specimen does not remain in a natural position after a normal

mounting methods; such cavities should be filled with arsenious acid gelatin [1, 24]. Specimens which are friable may be covered with a thin layer of arsenious acid-gelatin [25] and it may also be used locally to hold fragments such as blood clot in position. Bile stained specimens are soaked in saturated solution of calcium chloride for 24hrs to avoid discoloration of mounting fluid. This will only reduce the degree of colouring, and frequent changes of fluid are necessary to keep the discoloration at the minimum [2].

Routine Mounting Procedure

- Museum jars or boxes
- Centre plates
- Stitching specimens to centre plate
- Fixing the centre plate
- Filling and sealing [2].

Museum Jars or Boxes

Perspex boxes are used almost universally. They are available commercially or may be made in the laboratory. The method employed commercially to join the sides is far superior to the cementing process that is done in the laboratory. The tissue to be mounted should be laid on a flat, waterproof bench. The position at which tissue to be mounted should be anatomically correct. Perspex sheet can be moulded or bent to satisfy the requirements of individual specimens [2].

Centre plates

Advantage of perspex sheet is its flexibility. When heated; specimens are stitched to a flat sheet of perspex. Commercial boxes may be available with already fitted center plates.

Coloured opaque plates may be used to enhance the colour of the specimen or to attach specimens on both sides [2].

Stitching specimens to centre plate

- The specimen is arranged in the desired position, and crosses are

made on the centre plate with a scribe where stitches are to be placed.

- With solid specimens the number of stitches will depend on the weight and consistency of the tissue: for example, half a kidney is adequately supported with a stitch at each pole.
- Hollow or cystic organs, or organs with attached structures, may require stitches to hold the specimen in the correct position in addition to providing support: for example, the oesophagus and stomach may require up to 12 stitches.
- Attached structures may need to be stitched to the main organ or to each other to hold them in position.
- Stitches must not be placed through pathological lesions.
- When the centre plate has been marked, holes 1/16 inch in diameter are drilled at those points.
- If linen thread is to be used, one hole is drilled at each point; if nylon thread is used two holes are necessary.
- Nylon thread has the advantage of being almost unbreakable but is so hard, it tends to cut through specimens and for this reason linen thread should be used for all specimens except bone.
- Lengths of linen thread are cut and a small clear glass bead is threaded on and tied in the centre; the bead should be slightly larger than the hole in the centre plate since it acts as a retainer for the tie.
- The centre plate is thoroughly washed in a detergent, and dried on a fluff less cloth.
- The specimen is stitched on by passing first one end of a tie and then the other through the centre plate and the specimen, pulling on

both ends until the glass bead is tight against the centre plate [2].

Fixing the centre plate

- i. The centre plate, with specimen attached, is put into the box and marks are made with a grease pencil if stops are required to hold the centre plate in position.
- ii. If the box is of the correct depth, there will be no movement of the specimen, but if a deeper box has been used, two rectangles with polished edges, are cemented to the wall of the box to keep the centre plate in position [2].

Filling and sealing

- When the specimen is in position, museum fluid, to which 0.4% sodium hydrogen sulphite has been added, is run in to within 1/2 inch of the top.
- Air-bubbles trapped between the specimen and centre plate are released with a broad bladed spatula.
- A hole of 1/8 inch diameter is drilled in one corner of the lid.
- The top of the box is wiped dry and Perspex cement applied with a Pasteur pipette.
- After 30seconds the lid is laid lightly in position.
- After a further 30 seconds, a lead weight is applied and left for at least 1 hour, preferably 2-3 hours
- A short length of Perspex rod, 1 inch in diameter, is tapped lightly into the hole in the lid and the specimen left for 24-48 hours to remove residual air bubbles.
- When the last bubble is removed, the Perspex plug is placed and tapped firmly into position and, when dried, a small amount of Perspex cement is applied [2].

Note: It will be found that large specimens develop air-bubbles over a period of 2-3 weeks after mounting; these may be removed by drilling a fresh hole, filling up and re-plugging.

Mounting in Glass Jars

- The specimens are mounted as described above except that holes are drilled with an engravers tool (a metal rod with a diamond-shaped end) in a hand drill, using camphor dissolved in turpentine as a lubricant.
- Glass jars are sealed with an asphaltum-rubber compound (Picein).
- The jars look neater if, after sealing the edges are painted black enamel or asphaltum varnish [26].

Note: If the glass jars are completely filled with mounting fluid, they will crack with atmospheric changes.

Special Museum Techniques

Maceration

Maceration is used to demonstrate bony lesions such as osteogenic sarcomas, chronic osteomyelitis, osteomas and tuberculosis. The technique employed depends on how delicate the tissue is, the degree and the type of the lesion. For the preservation of the finest spicules of bone, putrefaction methods will be employed. Boil in tap water or very dilute sodium hydroxide, at the interval during boiling; the softened tissue should be removed from the specimen [7].

A gross method for hard compact bone involves autoclaving the hard compact bone in sodium hydroxide for 5minutes. But this method will also remove the fine bony spicules along with the soft tissues [2].

Degreasing and Bleaching

After the removal of soft tissue by any of the above methods, bone is immersed in chloroform for 3 to 4 hours in order to remove fat. Specimens are dried in an incubator and bleached in hydrogen peroxide [7].

Mounting

Macerated bones are mounted dry, either on a central plate or on Perspex supports designed for individual specimens. The specimen is fixed with nylon wires [7].

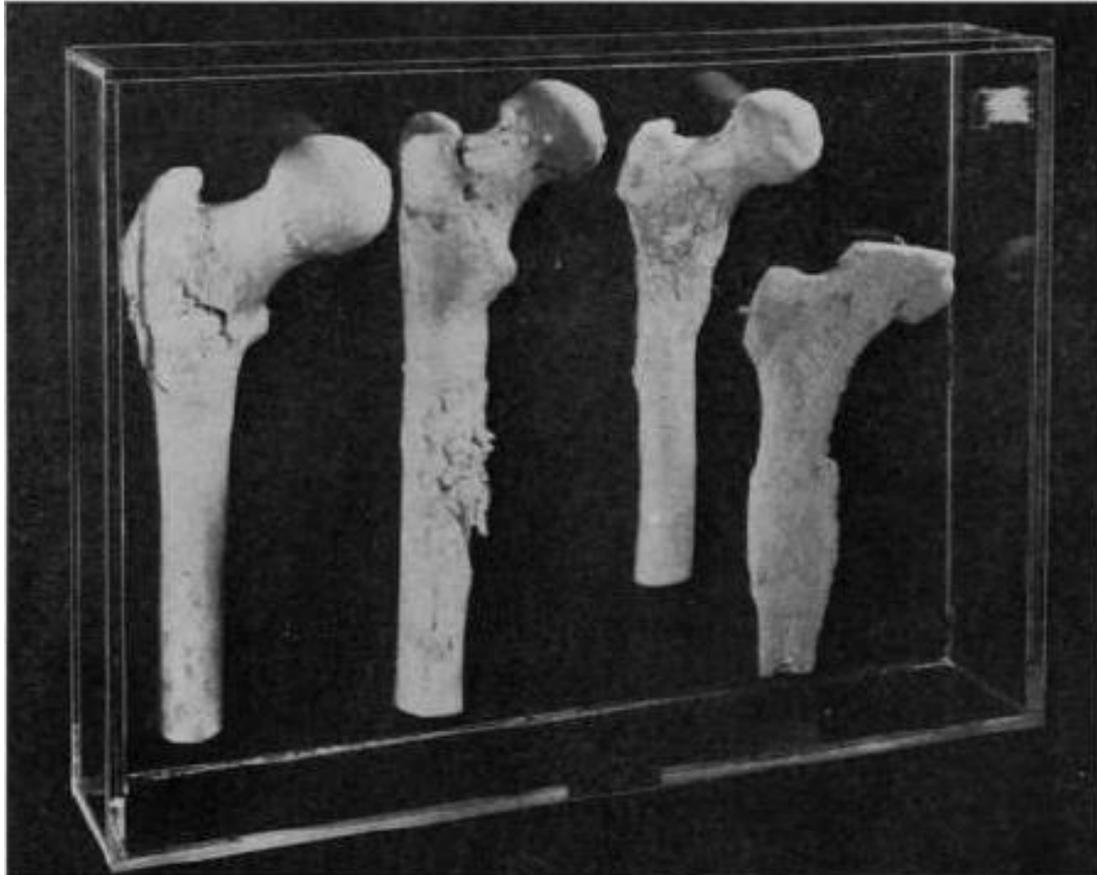


Fig. 2. Macerated specimens of osteogenic sarcomata, mounted on a centre plate in a Perspex box [3].

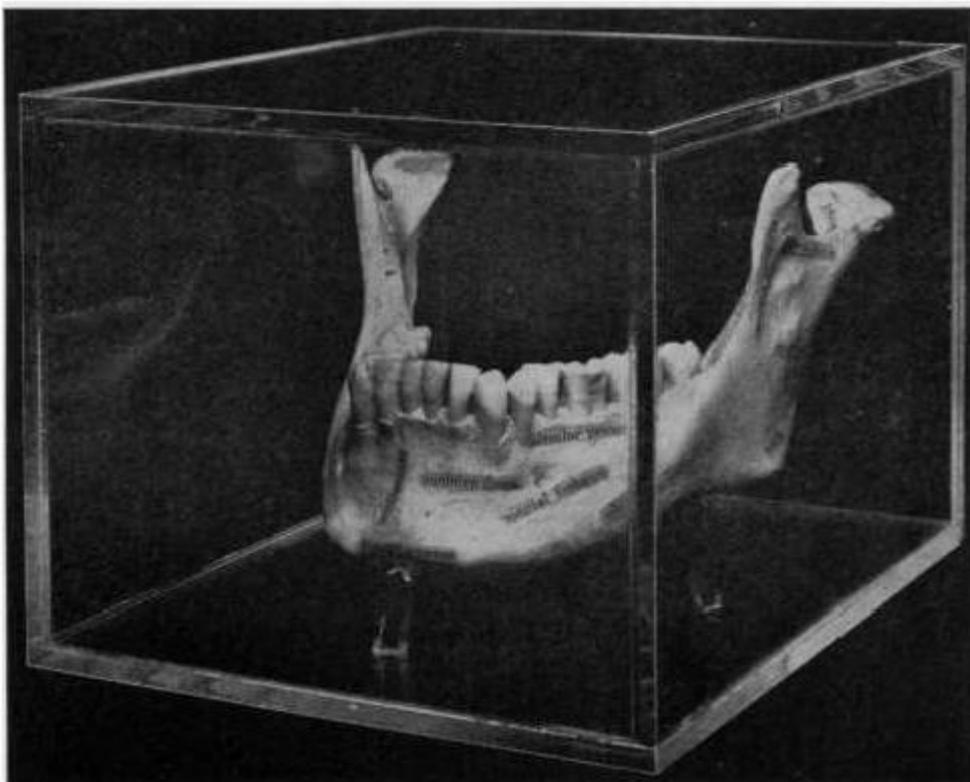


Fig 3. Macerated specimen of normal mandible demonstrating the use of “perspex” supports which have been cemented into position [3].

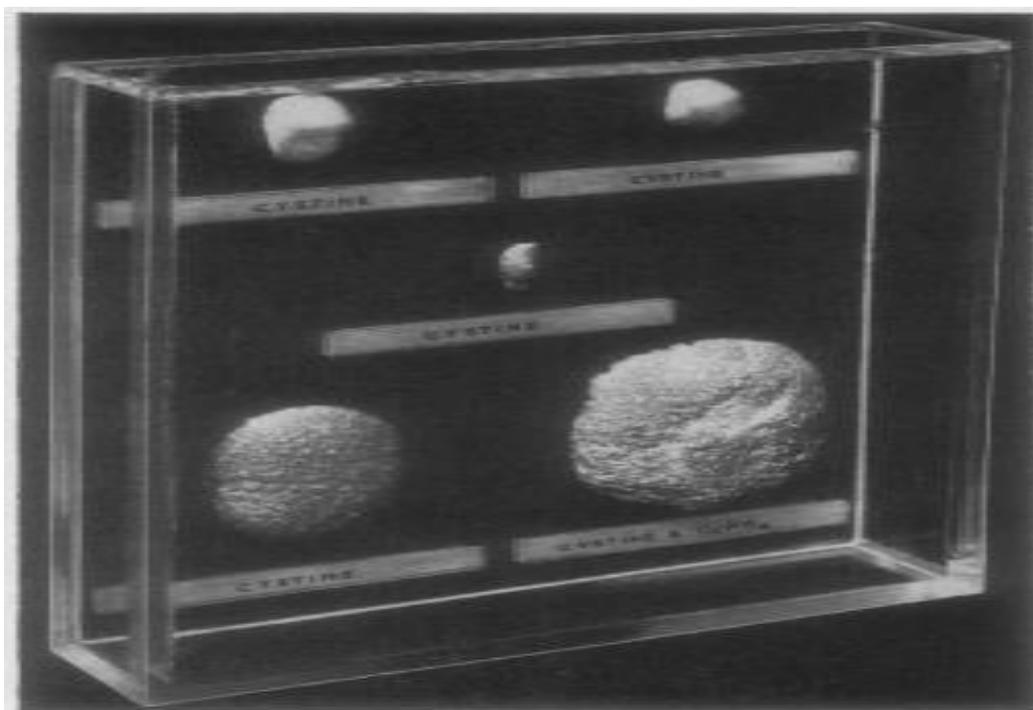


Fig. 4. Calculi mounted by the method described by Culling, [1].

Calculi

When calculi are mounted, they are bisected and the cut surface smoothed with fine sandpaper. As these stones are often very fragile and do not withstand drilling, they are best glued to a plate. The container should be of 0.4 cm Perspex to enable a groove to be put in the sides [7].

Gross Staining of Specimens

There are a number of stains used to demonstrate the presence of normal or abnormal constituents; where relevant a normal negative control should always be mounted alongside.

Amyloid

This is usually demonstrated on fixed, thin or slices of tissue. Permanent results are obtained using the iodine or Congo red techniques. In the iodine technique, the fixed tissue is placed in Lugol's iodine overnight, differentiated in 80% alcohol, air dried and mounted in liquid paraffin [1].

For the Congo Red technique, the fixed tissue is placed in 1% congo red for one to two hours, transferred to a saturated solution of lithium carbonate for two minutes, differentiated in 80% alcohol and mounted in Kaiserling III [1].

Haemosiderin and Free Iron

This is demonstrated by Perls' Prussian blue reaction. Tissue should be fixed in formal-saline or in Kaiserling solution 1 (KI). Rust or iron containing fluids must not contaminate the specimen or the fixing fluids. Wash tissue in running water and rinse in distilled water.

Place the specimen in a mixture of equal parts of 5% potassium ferrocyanide and 2% hydrochloric acid in distilled water until blue coloration is well developed (usually 15 to 20 minutes).

Wash in running water and mount in Kaiserling I solution. The blue colour tends to fade, but can be redeveloped with 20% hydrogen peroxide, after which the

specimen is washed thoroughly with KI solution, and the pot refilled and sealed [10].

Fat

There are many stains available for the demonstration of fat in tissue, but the one which gives the most striking results is Sudan IV or Scarlet R. The fixed tissue is thoroughly washed in running water, placed in a saturated solution of the stain for one hour, differentiated in 70% alcohol, and mounted in KI solution. This technique is often used to demonstrate atheromatous plaques in blood vessels [10].

Calcium

Small areas of calcification on the surface of gross specimen can be demonstrated with alizarin red S, but due to the weak contrast produced, it is more usual to clear the bulk of the tissue so that only the red-stained calcified areas show up. The method recommended is that of Dawson's Technique [27], which was designed primarily to demonstrate developing bone. This method depends on clearing the surrounding tissues with potassium hydroxide and staining the bone with alizarin red S [27].

Alizarin Red S Method for Gross Staining of Calcium

- Fix specimen in 95% alcohol for two or three days.
- Place in 1% KOH until calcified areas are clearly visible through the cleared soft tissue
- Transfer specimens to a solution of alizarin red S (1mg in 100cm³ of 1% KOH) and leave until the calcified areas are stained.
- If all the stain is taken up, transfer to fresh solution.
- Transfer tissue to 20% glycerine in 1% KOH.

- Place specimens in increasing concentrations of glycerine and mount the specimens in pure glycerine [27].

Injection Techniques

It is often necessary to demonstrate both the normal or abnormal vasculature in an organ. This can be done by:

- Injection of a radio-opaque substance and subsequent demonstration by X-ray.
- Injection of colored gelatin and demonstration by clearing of surrounding tissue
- A combination of the above methods
- The injection of coloured latex rubber and subsequent dissection.
- The injection of polyester resin which, after polymerization, allows the digestion of the surrounding tissue, leaving a 'corrosion cast' [28].

Organisation of Specimens

Although initially only a few specimens may be received and ultimately mounted, it is essential that they are easily located and are accompanied by all the relevant information that will be helpful for visitors, students and researchers [4].

Lighting

A poorly lighted museum is useless, and in any museum the best lighted exhibits are the ones most frequently examined and visited. Fluorescent lighting is the best giving better results [3].

Arrangement

Specimen can either be classified by an anatomical system, i.e all liver conditions together, all heart conditions together, etc, or by disease system, i.e. all tuberculous specimens, all parasites together, etc. most medical teachings are carried out by anatomical systems and this usually prove

the most practical arrangement. However, if a disease process needs to be studied, a simple numbering system enables these specimens to be easily selected from the general collection [3].

Numbering

Having decided on anatomical division to the collection, a simple prefix letter is given to each, e.g **C** for Cardiovascular, **R** for Respiratory, **S** for Skeletal, **B** for Breast.

This letter would be followed by a number to indicate the main anatomical breakdown/division of each system e.g C1 for Heart, C2 for Valves, C3 for Arteries, C4 for Veins.

This is then followed by a point and then a number indicating the disease, e.g. C1.1 for congenital conditions of heart, C1.2 for inflammatory conditions of heart, C1.3 for Parasites. These numbers are then followed by the appropriate specimens' e.g C1.11, C1.12, C1.13 would all be examples of congenital heart conditions. In this way, later additions or amendments do not interfere with the general arrangement [22].

Cataloguing

It is essential that a plan of the museum should be visible to the visitor on entering; each section should be clearly labelled. The simple loose-leaf catalogue is sufficient to hold all the necessary information relating to each specimen and as the collection enlarges, it is helpful if there is a separate catalogue for each anatomical system. If possible, all the information relating to each specimen should be contained on one catalogue sheet. This sheet will have the diagnosis of the condition (with its museum number) at the top, and these are followed by a brief description of the specimen and a brief case history. Different color bands can also be used for each condition. e.g., red for malignancy, yellow for inflammatory

processes and green for normal condition [22].

Labelling

In some of the older medical museums, can still be seen some of the attractive glass jars with their hand painted labels, and in some modern museums are seen the Perspex jars with machine engraved labels. The former are easily chipped off and can become confusing or illegible, and the latter are expensive to produce and are not easily replaced. A compromise which, whilst not as attractive as the first, but cheap and easily renewed, is to use a conventional 'Dymo' tape label. The range of colors available allows for a differential labelling system; thus specimens with a red label are examples of rarer conditions or conditions which the undergraduate need not to concentrate on, yellow labelled specimens are good examples of common conditions which the undergraduate should concentrate on and the green labelled specimens are less satisfactory examples of common conditions, or less common conditions [22].

Methods of Presentation

The ideal pathological museum should present a visitor or student with the full picture of human disease; it should show, by photographs, the appearance of the patient if possible, the etiological and environmental factors that lead to the disease. Some attempt should also be made to impress upon visitors or students the statistical significance of each disorder, and the changes which are taking place in the incidence of each. X-ray and laboratory findings should be illustrated. Somewhere also there should be room for a few sentences from the writings of those who were inspired to make original and fundamental observations. Color codes can also be used for etiology, epidemiology, pathology, clinical treatment, prognosis and prevention [3].

Discussion

Samples to be mounted in museum jars must be handled with proper care right from the reception. Samples must be properly fixed with the right and adequate fixatives as recommended by Kaiserling [9]. Factors that can cause deterioration of tissue samples must be duly avoided.

Restoration of samples colours can best be achieved with the use of Kaiserling II (95% alcohol) solution [14]. The use of 40% glycerine solution is the best for preserving specimen. Glycerine has a refractive index similar to that of the Perspex jars used in presenting samples. Samples are to be measured before they can be finally mounted into Perspex boxes. This is to avoid cramming of big samples into a small mounting jar or using an over large mounting jars for small samples [22]. The presence of normal or abnormal constituents of tissue samples can be demonstrated by applying special stains. For example, Amyloid can be demonstrated by staining the sample with Iodine or Congo red; Haemosiderin and free iron with Perl's Prussian Blue; Fat with Sudan IV or Scarlet R; Calcium with Alizarin Red S.

Museum rooms must be properly lighted, and mounted tissue samples must be well labelled, arranged and organized.

Conclusion

Medical museum as an institution has been seen to be the only place where rare pathological conditions or specimens of historical interest can be stored and exhibited for self-examinations, or as a research tool for students and visitors.

Studies have revealed that the use of Kaiserling solution (KI, KII and KIII) is still one of the best solutions and methods that can be used in preparing tissue samples for final museum mounts in histopathology.

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Not Applicable

CONFLICT OF INTEREST

No conflict of interest

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