



## PATHOLOGY MUSEUM A NEED OR JUST A PROTOCOL – A REVIEW

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### ABSTRACT

The history of pathology can be traced to the earliest application of the scientific method to the field of medicine, a development which occurred in the Middle East during the Islamic Golden Age and in Western Europe during the Italian Renaissance. Early systematic human dissections were carried out by the Ancient Greek physicians Herophilus of Chalcedon and Erasistratus of Chios in the early part of the third century BC. The first physician known to have made postmortem dissections was the Arabian physician Avenzoar (1091–1161). Rudolf Virchow (1821–1902) is generally recognized to be the father of microscopic pathology. Most early pathologists were also practicing physicians or surgeon. In order to understand the diseases more correctly and to convey the knowledge from one generation to another they finally decide to preserve the samples and specimen, and there come the need of museum. Silverstone states that 'museums are in many respects like other contemporary media. They entertain and inform; they tell stories and construct arguments; they aim to educate; they define, consciously or unconsciously; effectively, an agenda; they translate the otherwise unfamiliar and inaccessible information into the familiar and accessible. Even the small museums in addition to their educational value, play a part in recording the history of medicine, since the common diseases of today may well be the rarities of tomorrow. To fulfill such purpose it is essential that the original shape, color of the specimen should be retained. Relevant photographs, radiographs, presentation, labeling, cataloguing are of equal importance.

### INTRODUCTION

The medical museum which first appeared in the 16 and 17th centuries were museum of anatomy, such as that of Frederick Ruysch in Amsterdam. Toward the close of the 18th century, more general museums involving extension into natural history, physiology was developed by William Hunter and his brother John, for use primarily in their schools of anatomy in London. John Hunter's [1] collection was bought by the states in 1799 and housed in the Royal College of Surgeons in London while that of William Hunter, in accordance with his will, was moved to Glasgow in 1807. John's Hunter collection was of over 14000, specimen and was confined mainly to anatomy,

natural history, physiology and pathology. Whereas William Hunter's collection was much broader, including 35000, specimen [2].

### Preparation of the specimens

- Good museum specimens are obtained & preserved by care & planning at the time of autopsy.
- Careful treatment after removal.
- Careless removal of sections »» »» can easily ruin a specimen

Preparation of the specimens should be done in such a way that

- Cut surfaces should be smooth & even.



- By using a continuous stroke with the long –bladed , sharp knife.
  - Tissue should be taken either from back of the specimen intended for preservation or from the front with a scalpel [3].
  - Specimen should be put into a fixative almost immediately.
  - Containers with formal-saline should be always readily available to theatre staff.
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#### Methods of color maintenance

- Primary fixation » » » » 10% formal saline
- Then the specimen transferred to a special fixative afterwards.
- The technique most widely used » » » » modification of the method by **Kaiserling (1900)**

#### The original technique employed three solutions:

1. Fixing
2. Restoring color
3. A mounting fluid

#### Pulvertaft's modification (1936)

A method of restoring color to tissues by the addition of reducing agent to the mounting medium [4].

Reducing agent » » » » **sodium hydrosulphite**

#### Pulvertaft-Kaiserling method

##### Solutions

##### Kaiserling's fluid I-fixing fluid

- ✓ Formalin 400ml
- ✓ Potassium nitrate 30g
- ✓ Potassium acetate 60g
- ✓ Tap – water to 2000ml

NOTE: Specimens may be transferred to this fluid either after fixation in formal saline or directly fixed in it.

##### Kaiserling's fluid II- To restore color

- ✓ Ethyl alcohol 80%
- May be used to restore color in an emergency (color photography).
- Not necessary when using a sodium hydrosulphite mounting fluid.
- The time should be carefully controlled (30 mins-4 hrs).

NOTE: Continued immersion in alcohol has a permanent bleaching effect & the color so lost is not afterwards restored by the mounting fluid.

#### Pulvertaft – Kaiserling mounting fluid III

- ✓ Glycerin 300ml
- ✓ Sodium acetate 100g
- ✓ Formalin 5ml
- ✓ Tap – water to 1000ml
- 0.4% sodium hydrosulphite is added immediately before sealing the jar.
- If the solution is not crystal clear:
  - ✓ Should be filtered through a paper pulp filter.
  - ✓ 30ml of saturated sol. of camphor in alcohol should be added to 1 litre of the solution.

**ISRAEL & YOUNG (1978)** used pure liquid paraffin as the final mountant after color restoration with alcohol.

NOTE: This procedure reduces chances of discoloration of the mounting fluid by pigments in the specimen.

#### Wentworth methods (1938,1939,1942,1957)

- In a series of papers Wentworth described modifications of Pulvertaft method .
- Which dispensed with the alcohol step for color restoration.
- He used only sodium hydrosulphite & omitted glycerol from the final mountant.

##### Fixation of the specimens

- Additional rules should be followed in fixing of museum specimens:
  - ❖ Specimens should be always injected with fixative if possible, to ensure adequate fixation (e.g Brain).
  - ❖ Specimens containing much blood must not be washed in water at any time, either before or after fixation.
  - ❖ Fresh specimen should lie on a thick layer of cotton wool covered by lint.
  - ❖ The specimen with it's attached structures, must be fixed.
  - ❖ Cystic cavities, if unopened, are inflated or if opened are packed with cotton wool soaked in fixative so as to maintain their natural shape.
  - ❖ Bile stained or bile containing specimens must be fixed & stoared separately.

#### Storage of the specimens

**storage of the specimens should be done in following manner:**

- ✓ The method must permit easy & certain identification of each specimen.
- ✓ A reference book should be maintained to record necessary details about the specimens.
- ✓ Specimens should be stored in separate containers to avoid damage caused by contact with other specimen.
- ✓ The containers should be labeled adequately on the outside & label should be tied to specimen with linen thread.



### Mounting of the specimens

- When specimens are brought from the storage some amount of attention will be required towards them before mounting:
  - ✓ Slight irregularities developed on the surface of the specimens may need to re-cut.
  - ✓ If specimens of membranes, skin, intestine have been pinned on the cork board outer edges will require trimming.
  - ✓ After the removal of cotton wool the natural cavities should be filled with arsenious acid gelatin.
  - ✓ Color perspex arrows or rods & black horse hair used to identify delicate anatomical structures.
  - ✓ Friable specimens may be covered with a thin layer of arsenious acid gelatin & used locally to hold fragments such as blood clot in position.
  - ✓ Bile specimens should be soaked in saturated sol. of CaCl for 24 hrs.

### Procedure for mounting

- The specimen should be laid on a flat, water proofed preferably formica covered bench.
  - Position should as far as possible, be anatomically correct .
  - Specimen is then measured, allowing a 1cm clearance at the top & sides & 2cm at the bottom.
  - The extra clearance at the bottom is to enable a label to be fitted without obscuring part of the specimen.
  - Depth is measured & approx. 5mm added for the entire plate.
  - A suitable box is then taken from stock, ordered or made.

### Museum jars & boxes

- Perspex boxes are used almost universally today.
- They available commercially or made in the laboratory.
- The laboratory made boxes have advantage that such boxes may be designed to fit each specimen exactly.
- Perspex boxes made by cutting four sides, a top & a bottom from Perspex sheeting cementing them together with a Perspex cement.
- An alternative better method is to bend a strip of Perspex to form top & sides of the box.

### Centre plates

- Commercial boxes may be obtained already fitted with centre plates.
  - Colored opaque Perspex centre plates may be used to enhance the color of the specimen or to enable specimens to be attached to both the sides.
  - the great majority of specimens may be simply stitched to a centre plate, which just fits in to the box with about 5mm clearance at the top.
- Attaching the specimens to the centre plate
- Specimen is arranged in desired position & with a scribe crosses are made on the centre plate.

- The no. of stitches depend upon weight & consistency of the tissue.
- Stitches should not be placed through the pathological lesions.
- After marking on the centre plate 1-2 mm diameter holes are drilled at those points.
  - In case of nylon thread : 2 holes 1mm in diameter
  - In case of linen thread with glass bead: one hole is drilled at each point.
  - The bead should be slightly larger than the hole to act as a retainer for the tie.
  - Centre plate is thoroughly washed & dried on the fluff less cloth.
  - The treads should be emerged from the specimen about 1 cm apart so as to form a V of tissue on which to tie.
  - A reef knot is tied.
  - Gives a tight tie which will not sleep.
  - An alternative method used for attaching is to cement spikes of Perspex made up from Perspex rod.
  - After placing it in the suitable position press the specimen on to the spikes.

### Fixing the centre plate

- The centre plate with the attached specimen is put on to the box.
- Marks are made with the grease pencil, if stops are required to hold the plate in position.
- In case of using a deeper box 2 rectangles of 3mm perspex measuring 5mm square are cemented to the wall of the box .

### Filling & sealing

- 0.4 % sodium hydrosulphite has been added to museum fluid & is run in to within 1 cm of the top.
- Trapped air bubbles are released with a broad-bladed spatula.
- For introducing remaining mounting fluid a hole of 3mm is drilled in to corner of the lid.
- Top of the box is wiped dry & perspex/ ethylene dichloride is applied with a Pasteur pipette.
- After 30 sec the surplus cement is carefully removed.
- After 30 sec a lead weight is applied & left for atleast 1 hr (2-3 hrs preferably).
- A short length of perspex rod is tapped into the hole & specimen should be left for 24-48 hrs.
- After removal of the perspex plug the box is filled with the museum fluid.
- After removal of the last air bubble perspex plug is replaced & after drying it is sealed with the perspex cement.
- An alternative method includes tapping the hole & plugging it with a nylon screw.

### Mounting in glass jar

- The specimens are mounted in jars in the same manner as on the centre plate.
- Except the holes are drilled with an engraver's tool.



- By using camphor dissolved in turpentine as a lubricant.
- While drilling holes the glass sheet must be on absolutely flat surface.
- Sealing of the glass jar is done with the help of asphaltum-rubber compound.
- Flame with a Bunsen burner until picein runs completely cover till ground edge.
- Placing the specimen in the jar & jar should be filled with mounting fluid to within 1cm of the top.
- Hold the lid with a pair of forceps & after heating the lid it should be placed firmly on the top of the jar with the help of cloth.
- After setting removal of excess Picein is done & edges are painted with a black enamel / asphaltum varnish.

#### Gelatin embedding

- Applicable for the delicate/intricate structures which are difficult to stitch.
- Material used- arsenious acid gelatin.
- Method –after embedding the tissue in the arsenious acid gelatin specimen should be mounted by routine method .
- A trough is formed by applying sellotape around the edge of centre plate to a depth of 0.1-1cm & filled with gelatin.
- Specimen may be mounted in a solid block of gelatin by fixing the specimen in position with a thin layer on one surface of the jar.
- The jar is filled to within 1cm of the top with gelatin after setting of layer.
  - Disadvantages of using gelatin:
    - ✓ Tendency to become yellow with age
    - ✓ Undergoes liquefaction with resultant air bubbles formation.
- Embedding in a solid plastic blocks
  - Ideal method for presenting a museum specimen.
  - But unfortunately there is as yet no method available to protect the color of the soft tissue specimen.

#### Macerated specimens of bone

- Clean cut even surface of the bony specimens are best achieved by cutting it on a circular saw/ band saw .
- It can cut the slices as thin as 3mm.
- Specimens are easiest to cut if they have been frozen hard.

#### MACERATION

- ✓ Used to demonstrate bony lesions, such are produced as:  
Osteogenic sarcomas, osteomas, chronic osteomyelitis & TB.
- ✓ Technique employed is depend upon the degree & type of the lesion.
- ✓ For the preservation of the finest spicules of bone-  
Peutrefaction method.
- ✓ As a routine method, the specimen should be boiled in tap-water / very dilute (N/100) sodium hydroxide.

- ✓ At the intervals during boiling the softened tissue should be removed from the specimen.
- ✓ A gross method for the compact bone is autoclaving in N/10 sodium hydroxide for 5 min.
- ✓ But this will also remove the fine bony spicules along with the soft tissue.

#### Degreasing & bleaching

- ✓ Fat is removed by immersion of the bone in chloroform for 3-4 hrs.
- ✓ Specimens are then dried in an incubator & bleached in hydrogen peroxide.

#### Mounting

- ❖ Macerated bones are mounted dry, either on the centre plate or with Perspex supports designed for individual specimens.
- ❖ Nylon thread should be used for the tying on the centre plate & A drop of Perspex cement is applied to the knot to give added support.

#### Calculi

##### ❖ Older method:

- Calculi are often presented by -
  - a) Dry mounting in boxes with reversible glass lids.
  - b) Mounting in gelatin to which formalin has been added.

#### Disadvantages

In the former the specimens become very dirty & in the latter the gelatin slowly dissolved.

#### Recent method

To utilize both the halves of calculi by polishing & mounting one half & labeling other half with Indian ink ,the latter being kept for the students to handle & study more closely.

#### Drawbacks of the routine formalin fixed techniques

- ✓ Irritating odor.
- ✓ Bleached colorless parts.
- ✓ Do not give naturalistic idea.
- ✓ Difficulty in maintaining the sections.
- ✓ The luminal architecture, dimensions, branching patterns are almost impossible to imagine in a dissection.

#### Plastination

- ✓ A technique of preparation of dry, colored, non toxic, durable, odorless, natural looking specimens.
  - Types :
    - ✓ Whole Organ Plastination
    - ✓ Sheet Plastination
    - ✓ Luminal Cast Plastination

#### Material used

1. Jars for storage & procesing
2. Acetone
3. Silicone rubber
4. Resin, catalyst, accelerator
5. Color paints
6. Dissection instruments
7. Syringes, needles.



### Whole Organ Plastination

- Helps in better understanding of total structure & relationships.
- Method:
  - ✓ Washing of the specimen with tap water.
  - ✓ Immersion in preservative:
    - a. 95% alcohol-280ml
    - b. Formalin- 1320ml
    - c. Glycerin- 80ml
    - d. Phenol- 120ml
    - e. Distilled water- 800ml
  - Time required for complete fixation is 2-3 days
  - Washing of formalin in running tap water
  - Transferring of specimen to acetone jar
  - Then to a jar of resin & left in it for about 1-2 weeks
  - Finally the specimen is immersed in a mixture of resin (general purpose resin), catalyst (5%) & accelerator(0.1%)
  - After few hours specimen becomes non sticky
  - Specimen is mounted on a suitable base & properly oriented.

### Sheet Plastination

- Useful method for the preparation of thin transparent or thick opaque body sections.
- The sheets are totally portable, the whole body may be cut into slices & stored dry.
- The inner relationships are best appreciable.

### Method

- ✓ After deep freezing for 24hrs thin sections have been made with a band saw or frozen parts are sectioned at 1cm slices.
- ✓ Section has to be cast in the form of sheet.
- ✓ The two glass sheets 4mm thick are separated by a rubber tubing of 6-8mm diameter clips, hold the glass sheets together & make a leak proof chamber.
- ✓ The processed section is immersed in the chamber(resin+catalyst+accelerator).
- ✓ After 24 hrs clips are removed & glass sheets are separated from the resin.
- ✓ Sheet edges are trimmed, polished & specimen is labeled.

### Luminal Cast Plastination

- Useful to study the dimensions & architecture of different cavities of organs & to study the tubular structures (arteries, veins, ductal branches & their variation).

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- Principle:
  - ✓ Filling up of the lumen with material & dissolving the surrounding tissue.
  - Used for tracheo- bronchial cast of lungs, cerebral ventricles, bony labyrinth, vascular pattern of kidney, liver, lung, spleen, coronary vessels, etc.
- Why plastination is preferable & better museum technique???
- Provides very unique specimen at a very low cost equivalent to the plastination specimens of international quality.
- Has simple protocol.
- Best suited for :
  - ✓ Teaching purposes
  - ✓ Designing newer surgical procedures.
  - ✓ Practice skills
  - ✓ Research purposes.

### CONCLUSION

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/dental 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. 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. Thus, all teaching hospitals and colleges should have museums which serve many functions: permanent exhibition of common specimen for undergraduate and postgraduate teaching purposes, illustrating specimens of rarity, permanent source of histologic material and for gross and microscopic photography.

