VII- GENERAL TECHNIQUES

- **Selection of Organs and Collection of Material**
- Specimens can be made more and more valuable by the modification of the methods of collection of materials and their preservation and mounting with characters as near as possible to the fresh condition.
- In the selection of a specimen to be displayed, one is guided by **general principles applied to all organs and special rules applied to certain types of tissue.**
- Tissues are usually obtained from human material (autopsies, surgical operations) and from experimental animal material.
- To afford good materials, a circular is issued to autopsy-operators, physicians and surgeons explaining how specimens are prepared and preserved.
- The aim is to ensure that the specimens will not be destroyed before they are handed over to be mounted.

- A list of instructions, issued to them, may contain the following:
  1. **Examine the gross specimen** and write a brief description of its important features, giving measurements, colour, shape and consistence.
  2. **Make clean, smooth sections** through the entire mass, using a sharp amputation-or sectioning-knife.
  3. Choose those portions illustrating **important characteristics** and cut slices not over 3 cm. thick, trim them neatly with scissors or knife, gently wipe free blood and fluids from surfaces with a towel and, without washing in water, place at once in the preservative.
  4. **Mucous membranes** and other surface specimens which need a special dissection should be gently stretched out and pinned on thin boards.
  5. Where time will not permit, or the specimen is too soft, containing cysts or abscesses, or the proper plane of sectioning is not apparent, or it is desired that the entire specimen be **preserved in situ and in its entirety**, place it in the preservative and send at once to the pathologist.
  6. Accompany each specimen with the **proper history** of its source and the clinical data, and express your wishes in respect to its preservation.
  7. In a small bottle **containing preservative, place thin bits of tissue** for microscopic examination. These should be carefully selected and not over 4 mm. thick.
  8. If possible, accompany material with the **patient's photograph, X-ray pictures and laboratory findings.**

- **The surgical theatres and autopsy rooms should have handy large and small jars** with preservative to be readily accessible for transferring the material to the preparation-room.
- Accompanying these jars should be a card with the name of the patient, age, sex, hospital number, date of admission, nature of operation or number of autopsy and the surgeon or doctor in charge.
- To obtain good material, necropsies should be performed as soon as possible after death.
- The student (future surgeon and physician) rather than the pathologist must know that there is a certain plan of performing the postmortem dissection.
- **The following general principles should be carefully followed out :**

1. In cases where the stomach and intestinal tract are intended to be preserved, 7% formol-

saline should be injected intraperitoneally or orally-introduced just after death.

2. In cases of brain, the carotid arteries may be injected with 4% formol-saline.
3. **Do not allow specimens to get in contact with water; they may, however, be washed with saline to prevent drying otherwise autolysis will set in.**
4. *Ample tissue should be taken from the specimen for microscopic study in order to have both the gross and histologic pictures of the specimen. Material for histological examination should be selected before mounting the specimen and should not injure its gross appearance.*
5. Do not open hollow viscera until fixation is complete. This is achieved by filling the hollow organ by diluted formalin by means of a syringe and needle and immersing it in the fixative for 24 hours.
6. Care should be taken that the tissue is - not crushed or injured before it is selected, the *specimen should be prepared in good shape by trimming and dissecting so that orientation is possible.*
7. With large solid organs, such as the liver, two parallel surfaces or one flat surface should be prepared by a single stroke of the knife which should be smooth and even; and, one long continuous cut made rather than numerous saw-like ones.

- **In dealing with some particular organs, the following guiding principles may be followed out :**
- **Lungs:**
- Choose the anterior or posterior half of one or both lungs, including if necessary the corresponding half of the bronchi and trachea.
- Tissues should be **instantly fixed after cessation of circulation** to prevent autolysis; when this is not practically possible, **they should be kept in a refrigerator.**
- The problems to be overcome in preserving a specimen are **postmortem decomposition** and **drying of tissues** as well as the **proper time for penetration**.
- **Heart:**
- Demonstrate the entire organ preferably opened.
- **Liver:**
- Only a slice of liver not over 5 cm. in thickness, taken through the long axis, is sufficient.
- In some cases about **one half of the organ may be selected**.
- Slices and thick sections are generally to be preferred to the whole organ.
- If there is a focal lesion, the specimen should be taken to include both the lesion and the adjoining normal tissue.
- **Spleen:**
- One half of the spleen or a slab 2 to 4 cm in thickness may suffice.
- The specimen should be taken through the long axis.
- **Kidneys:**
- One entire kidney or one half of one kidney or both kidneys with the corresponding ureters and the bladder may be chosen.
- **Pancreas:**
- The entire pancreas as such or cut into two longitudinal halves.
- **Intestine:**
- A segment of the intestine not over 30 cm. in length including the adjacent mesentery.

- **Thyroid:**
- Either the entire thyroid gland, preferably still attached to the trachea, or that half of the thyroid which contains the lesion.

- **Genital tract:**
- The entire genital tract and adjacent structures such as
- The testis, epididymis and vas; or the prostate, urethra, seminal vesicles and bladder in the male;
- The uterus with the cervix and vagina or the uterus with the tubes and ovaries or the uterus, bladder and rectum in the female.
- **Brain:** The entire brain or a half of it cut coronally or sagitally.

(b) Fixation

- Certain reagents are used to act upon the tissue-elements in order to preserve the tissue in the condition **which approximates that of the living state**.
- Most fixatives are mixtures of different reagents and the choice of the proper fixing reagent is a matter of experience and depends on the type of tissue and the nature of the pathological lesion present.
- A common fixative used for pathological materials is **diluted formalin** **because it permits large varieties of staining methods**.
- It is cheap and can be used in combination with many fixing mixtures after correcting its natural acidity by **shaking the diluted formaldehyde solution with calcium or magnesium carbonate.**
- Formaldehyde solution, however, when highly concentrated, tends to over-harden the outer tissue-layer while the inner parts are still underfixed and soft.
- ***Therefore, it should be used in moderate concentrations and at a low temperature to penetrate and to cause only little shrinkage.***
- Certain disadvantages may be encountered when formalin is used.
- It brings about **conversion of haemoglobin to acid haematin** and thus the characteristic colour of fresh tissue is lost, or a **dark brownish black deposit may be formed**..
- When there is a need for histological preparations, **it does not preserve glycogen**, and the **colour reaction in amyloid may be diminished**.
- In order to fix any tissue-specimen, **place it in the fixative without distortion, bending or twisting,** and do not cover it with paper-towels.
- If the tissue floats, **cover it with a fine layer of absorbent cotton;** then, cover the container tightly.
- With opened hollow-viscera, pin the tissue on a board. With closed hollow-viscera, fill with the fixative and tie off at both ends before immersion in the fixing fluid.
- **Un-opened cystic cavities should be injected** with the fixative before immersion.

- Certain solid organs such as the brain and limbs may be **fixed by vascular injection** before immersion in the fixative.
- The brain should be put in a separate container and hung by a certain device.
- The spleen and kidney should not be cut before fixation unless there is fear of non-penetration by the fixative as in case of splenomegaly, or a huge tumour of the kidney. The gall bladder should not be put with other organs in one vessel.
- Specimens containing pigments, or stained by bile, must be fixed and stored apart from others as they will stain them.

- **Over-fixation is as undesirable as under-fixation**.
- The length of time for which tissues should be left in fixing fluids is a matter of experience and depends on the rapidity of penetration of the fixative, and on the nature

and thickness of the tissue.

- In all cases use a container deep enough to permit suspension of the specimen. A relatively-**large quantity of fluid should be used**, and the specimen has to be laid out and held in the natural position to show the correct anatomical relationships. Care should be taken not to cut a new surface after fixation except when indicated.

- Specimens undergoing fixation **must not touch each other**. They must lie on lint or be suspended by linen-thread.
- The fluid should be changed if it becomes dirty or if the specimen is not fixing properly. Cleanliness is essential in dealing with any fixing or preserving process which should be carried out away from bright light. The specimens are then labelled and kept for some time in opaque jars or tanks made of glazed porcelain.

(c) Preservation

- In preserving surgical or post-mortem material for diagnostic and educational purposes, **<u>the two requirements to be kept in view are :</u>**
  1. The permanent preservation of **the external appearance** of the object.
  2. The bringing out of **all morbid anatomical peculiarities in a conspicuous way**.

- Attempts should be made to **prevent discolouration of the specimen** or-and to **restore the colour** by some secondary procedure.
- The question of preserving natural colour in gross pathological material has led various investigators to publish a vast number of formulae for preservatives.





2. Keep the specimen in this solution until no blood can be expressed. The time required for fixation is variable, depending upon the type, size and density of the specimen. The duration may be one day for a small piece of stomach, or 3 days, for a slice of spleen or liver.
3. After changing the position of the specimen wash it thoroughly in cold running water for 5 hours until formalin is removed.
4. Place the specimen in solution No. 2, which is 90% alcohol, for 10 minutes to 3 hours. The alcohol converts the acid haematin into alkaline haematin which is redder in colour and permanent. This may restore the original colour.
5. Wash the specimen in cold running water for a few hours until the alcohol is removed.
6. Put the specimen in the final mounting fluid which is solution No. 3 with the following formula:

1	Potassium acetate	130 ml
2	Glycerin	260 ml
3	Dist. water to	1000 ml
4	Thymol crystals	0.25 g



- Whilst many modifications have been made of **the original Kaiserling's method**, many investigators have tried to find a substitute.
- **One of these is Khalil's method.**
- The procedure of which is carried out as follows:
- The material, when obtained fresh from the surgical theatre or as soon as possible from the autopsy room, is received in a container which is filled with a solution made up of :

• Potassium nitrate	19 g
• Sodium sulphate	11 g
• Sodium bicarbonate	10 g
• Sodium chloride	09 g
• Potassium sulphate	01 g
• Chloral hydrate	25 ml
• Formalin	25 ml
• Water	1000 ml

- The specimen can be kept in this "Receiving" solution for any time provided it is trimmed and mounted in the position in which it will be finally exhibited.
- Meanwhile, the specimen can be handy for gross pathologic examination as well as taking pieces for histologic examination.
- When it is decided to preserve the specimen for demonstration purposes, it has to be finally preserved and mounted in a freshly-prepared "Receiving" solution in which the water used is saturated by arsenious acid and to which traces of camphor are added.

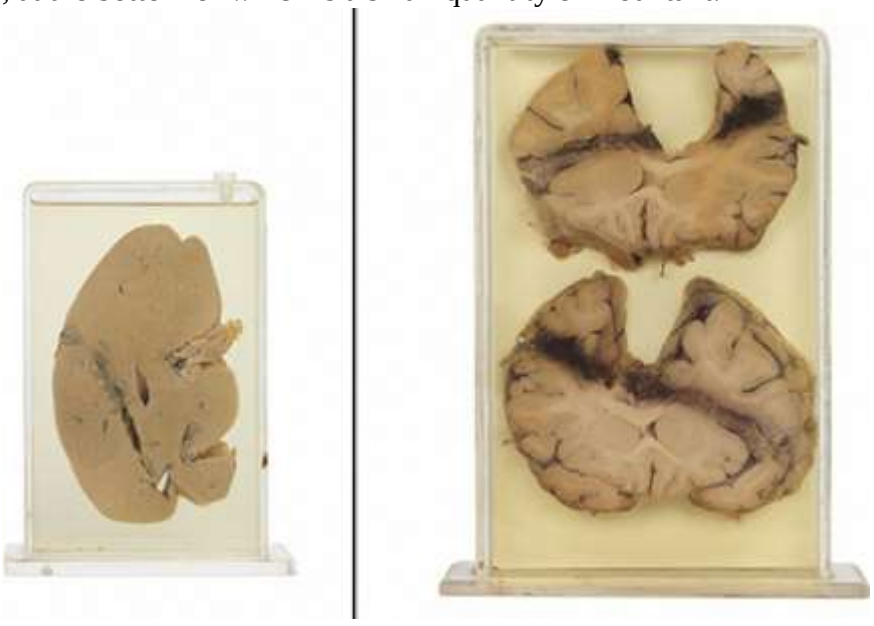
(d) Mounting

- ***Mounting in Fluid.***
- After fixation and preservation, of a specimen, it has to be mounted in oval, circular or rectangular glass-jars.
- The glass must be clear and of a good quality, and the top edge has to be grounded and the cover made from thick glass (Fig. 18).
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- Another suitable type of mounting containers is that made up of plastic, being unbreakable, resistible to the action of the preserving fluids, clear, water-proof, easily cleaned, easy to construct, light in weight, durable, pleasing in appearance and allows the specimen to be viewed from all angles without distortion (Fig. 19).

- In order to make the perspex containers non-expensive, it is advisable not to buy but to construct them.
- The principle in making them is simple and the technique is easily carried out by sawing the plastic to the required size and then the edges are joined and cemented together.

- ***2. Mounting in a Minimum of Fluid.***
- **Some investigators introduced the method of mounting with a minimum of fluid.**
- The principal steps are to fix the tissue thoroughly and then to place the preparation in a suitable jar, at the bottom of which is a small quantity of mountant.



- The method almost universally-used for the preservation of gross tissue specimens is that of **storing the material in a container which is filled with a preserving fluid.**
- This is called the **wet method of preservation**.
- It is a relatively simple process which may be adapted to all types of material.
- By this method, permanency is assured so long as the fluid lasts and it is possible to retain in the specimen a certain amount of naturalness as regards size, shape, colour and consistence.
- Moreover, the specimens usually possess validity for further dissection and microscopic examination should these become indicated at a future time.
- The process familiarly known as **"Kaiserling's"** with some modification is the most widely-used, particularly for ordinary pathological preparations.
- However, the use of **chloral hydrate** and **arsenious acid** made distinct advances in the preservation and permanency of natural colours.

- **The following procedure should be known by every medical person.**
- **The steps are as follows:**

- **The tissue is fixed in solution No. 1**
  Made up according to the following formula:

1	**Potassium acetate**	**30 g**
2	**Potassium nitrate**	**15 g**
3	**Formaldehyde 40% (i.e. full concentration)**	**200 ml**
4	**Dist. Water to**	**1000 ml**

- ***3. Mounting in Watch Glasses.***
- This method can be used to preserve certain pathological specimens and membranes, mesentery, slices of liver, kidney, the appendix, adrenals, ovary, as well as pieces or gross sections of neoplasms.
- It can beautifully demonstrate parasites, insects and calculi.
- Watch glasses are rather expensive and need a great experience.
- Therefore, cover-glasses made up of plastic material are used in the preparation of permanent museum mountings.
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- ***4. Mounting in a Semi-Solid.***

- **The semi-solid or gelatin method of mounting specimens** has been much used as the containers are rarely damaged and there is no trouble from fluid-loss.
- Moreover, the colour preservation is very satisfactory.
- One has to prepare gelatin to which formalin is added just before use.
- With face uppermost, the specimen is coated with gelatin, and when the gelatin is set, it is placed face downwards in the jar.
- After setting the jar is filled with prepared gelatin.
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- ***5. Dry Mounting.***

- The literature contains numerous methods of making dry preparations either by **mummification or infiltration**.
- The true mummification method consists essentially of sterilizing the specimen with an **antiseptic fixing-fluid** and then removing the water content of the material.
- This method which can be traced back to Ancient Egyptian times is only of historical interest.
- In it, they used
    - **Alcohol,**
    - **Turpentine or**
    - **Strong antiseptic fixing fluid or an**
    - **Air-drying method.**
- The method is valueless as regards pathological specimens and worthless for histological examination.
- The infiltration method, in which the water content is replaced by a substance which facilitates preservation in the dry state, is carried out with
    - **Linseed oil,**
    - **Glycerine,**
    - **Modified gelatin,**
    - **Calcium chloride,**
    - **Paraffin or wax.**
- Dry mounting is limited to those circumstances under which size, colour and consistence may be somewhat sacrificed in favour of the preservation of the specimen's morphology.

6. Mounting in Solid Perspex.

- This is embedding of biological or pathological materials in a transparent solid plastic

- ***1- Preparation of the Embedding Syrup.***
  - **(a)** The methyl methacrylate monomer is washed with 5% sodium hydroxide. The alkali is then removed by washing with water.
  - **(b)** The monomer is dehydrated with flaked calcium chloride, it is then filtered and the catalyst (Benzol Peroxide 0.1%) is added followed by the plasticiser (Dibutyl Phthalate 15%).
  - **(c)** The monomer is partially polymerized over a boiling water-bath.

- ***2- Preparation of the Specimen.***
  - The specimen should be dehydrated after fixation, by taking it through ascending grades of alcohol. It is then placed in chloroform and finally into a thin syrup for penetration.
- ***3- Embedding.***
- **A-**
  - A layer of syrup is poured into a special mould and polymerized at 40°C for few days, to form a supporting layer.
  - Another layer of syrup is poured into the mould and the specimen is positioned in it.
  - Further layers may be needed to cover the specimen adequately, and to allow for evaporation and incomplete polymerization.
- **B-**
- When polymerization is complete, the block can be shaken out of the mould, cut and trimmed to size and shape and finally polished.

• (e) Supporting

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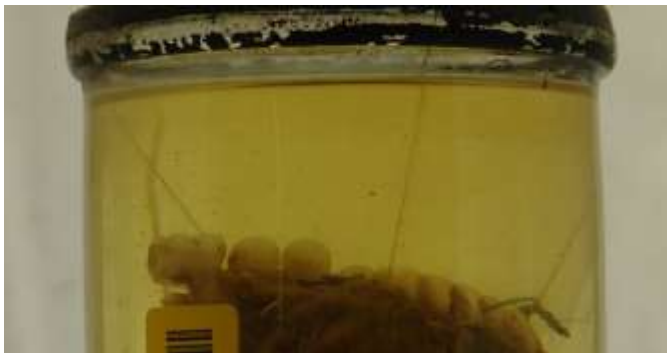
- When a gross tissue specimen is chosen, fixed and preserved it has to be mounted and supported inside a container.
- Gross-pathological specimens are held in the correct position inside their containers by means **of supporting frames**.
- In case of plastic jars the specimen may closely fit the jar that no need exists for a support



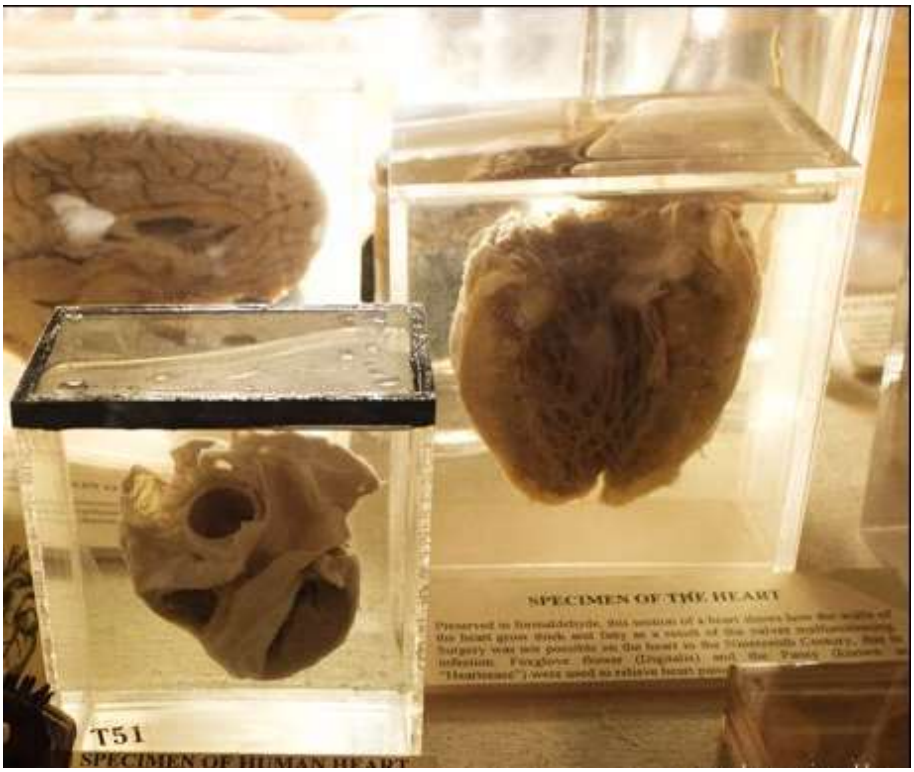
- Very often however, the specimen has to be fixed on a sheet of perspex which is cut to fit into the container.
- For glass-jars, the type of selected supports depends upon the specimen peculiarities.

- The frames are made up of glass-rodding or tubing and are done individually for each jar **with the help of Bunsen flame** and the specimen is suspended on the frame by a thread or ligature.
- Several workers prefer celluloid films or cellophane for suspending specimens in glass-jars.
- Another means is to mount the specimen on a center-plate and, two holes are drilled and the specimen stitched on with nylon thread.
- The centre-plates are held in position by perspex strips cemented in place.

(f) Sealing



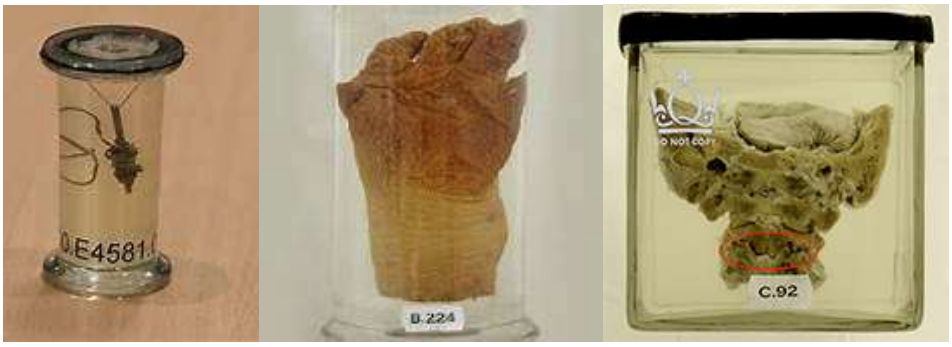
- The method for **cementing museum-jars** depends upon their construction.
- In case of glass-jars, the main ingredient of most cementing substances **is asphalt**.
- In case of plastic jars, one has to use **perspex dissolved in chloroform**.



- The ideal sealing of museum-jars is attained at by using a substance which is **easy to apply or remove and, which does not dissolve in the mounting fluids.**
- A very efficient method is to use "Sicotine".
- It is applied to the top of the jar and, the cover is placed carefully on the jar, compressed by weights and left to harden in few hours.

(g) Labelling

- Various methods are used during the process of fixation and preservation, others after the specimen has been **carried through the mounting fluids**.
- Labels differ according to their site **(in or outside the jar)** and, whether they carry numbers or descriptions as well.
- In any case, the ideal label must be of such a nature that it is **not destroyed by the fluids**.
- The label is of the same colour for surgical disorders, of another colour for medical disorders and so forth.



- The number of the specimen is preceded by a letter **(indicating the section of the museum to which the specimen belongs)** which is painted in green or blue.
- These numbers give the clue to both the clinical and museum **(pathological)** notes upon the case, as well as the biopsy or autopsy case.
- When perspex-jars are used, the labels can be made by cutting rectangles of coloured perspex, the numbers being stencilled on and, the rectangle is sealed in position with perspex cement.



(h) Cataloguing, Filing and Classifying

- For the purpose of recording the clinical and radiological data, rather than the pathological features, there must be descriptive cards and or catalogues for the specimens
- The labels which are attached to the specimen-jars may not carry any other description except the number of the jars as these are often used for examination purposes.
- Along the shelf and attached to its lower edge, a thin board is made to hold the cards on which are typed a resume of what is contained in the original catalogue.




- Only one side of the card is used and more than one card may be employed for each specimen.
- On each card is a number corresponding to the number of the jar.
- A very practical method is to use **descriptive catalogues**.
- The pathological nature (morbid anatomy and morbid histology) of the specimen together with a summary of the clinical history and investigations are written in the form of a report.
- Every fifty reports, under the same numbers as those of the jars, are bound together in a **single book-catalogue.**
- Another method is a combination of the usual **short descriptive cards** and the full-length catalogue.
- The **museum-number is placed at the top of the card** which carries only the pathological description.
- For further details, the student can refer to the original history of the case with full data enclosed in catalogues placed at his disposal on the museum-shelves or cupboards.
- Each specimen should be given a routine number and, complete records of the museum material are preserved in suitable binders.
- A card-catalogue should be kept of all museum specimens.
- These cards are **cross-indexed under "organ" and "diagnosis" headings**.
- The card for each specimen states its location, nature, surgical and biopsy (or autopsy) number, the name of the treating physician or operating surgeon and the pathologist in charge of the case.
- An "at-a-glance-filing-system" is essential for modern pathological museums.
- It consists of steel-panels holding 100 card-carriers.





- The card contains a clinical summary of the case and a pathological description of the specimen.
- The museum material after it is labelled and catalogued it has to be classified so as to be arranged in a systematic manner.
- This ordered-sequence increases its educational value and facilitates the finding and study of any specimen.
- Although it is not always necessary to adopt the *"International method of Classification*", but every medical person, rather than pathologists and museum-curators must know it.
- It is called the **"Decimal System".**
- A descriptive number is used in which the anatomical organ is represented by numbers before, and the pathological condition by numbers following the decimal point.
- The number of the individual specimen is indicated by a small index figure to the right of and above the descriptive number.
- The numbers of the specimens are used as a code which is filed by the usual method of cross indices so that specimens may be located anatomically and pathologically.



- Some museums employ **alphabetic letters** to indicate the organ or tissue affected.
- To the right of the letter, the numbers indicate the main morbid anatomical condition.

- Another method is to use capital letters denoting the systems:
    - **C for circulatory or cardio-vascular system;**
    - **R: respiratory,**
    - **D: digestive,**
    - **U: urinary,**
    - **M: male genitalia,**
    - **F: female genitalia,**
    - **E: endocrines,**
    - **N: nervous,**
    - **L: lymph-adenoid,**
    - **0: osseous and articular and**
    - **T: tegument-skin and muscular tissue.**





- The number of the individual specimen follows the letter.
- Small letters (or numbers) indicate sub-divisions or duplicates.
- If the museum is divided into small bays which are numbered, these numbers may be used to identify the jars contained in each bay.
- Some systems of classification of specimens may be easy to follow, but are not built on scientific basis and cannot act as the link of understanding in the world.
- Any scientific classification must allow an indefinite future expansion in the museum, as well as an easy interchange of specimens with the other museums.





- ***The pathological classification is rather long and often modified according to the progress of our knowledge on diseases and their causes.***
- ***It is usually adopted in conjunction with the anatomical classification.***
- ***The various organs and tissues are arranged anatomically, and the diseases of each organ pathologically.***