VIII TECHNIQUES (SPECIAL)

(a) Staining Methods

- In certain cases it is useful to **stain the macroscopic tissue** before mounting in order to show the contrast between certain structures and their surrounding elements.
- The purpose is to make **satisfactory and permanent gross preparations** which show to the naked eye the important micro-chemical reactions and the various pigmentations.
- When such stained slices are used for teaching purposes, they are presented in conjunction with the corresponding unstained slices.

1. Staining of Gross Tissues containing Fat and Lipoids.

- This method is applied in staining atheromatous lesions of arteries, differentiating fatty degeneration and infiltration of the liver, heart and kidneys, illustrating cases of cirrhosis of the liver, cancer of the breast with infiltration of the surrounding tissue and fat-necrosis of the pancreas.

- ***Procedure :***
  1. Cut a slice of the tissue **2.5 cm, in thickness**.
  2. Immerse in **10% formaldehyde solution** (2 days).
  3. Wash in running **water** (5 minutes).
  4. Transfer to a **saturated solution of scarlet red in alcohol**.
  5. Counter-stain in **haematoxylin** (1 minute).
  6. Wash in **running water and transfer to lithium carbonate**.
  7. Wash for 10 minutes in running water and **bleach in bichloride of mercury** till differentiation and brilliancy result.
  8. Mount in **10% glycerine to which 1% formaldehyde solution has been added**.

  The result: Fat: ***Bright red.***

2. Demonstration of Haemosiderosis in the Gross.

- This method is used in cases of anaemia, haemochromatosis, acute haemolytic poisoning, old extravasations, organizing haematomata and thrombi. It is commonly applied to the liver, spleen, lymph-nodes and bone-marrow.
- *Procedure :*
  1. Fix the specimen in **Kaiserling-solution No. 1.**
  2. Stain with a solution formed of equal parts of
     - **2% hydrochloric acid and**
     - **5% potassium ferrocyanide.**
  3. Wash in running water.
  4. Mount in 80% alcohol or 5% formol saline.
- The result: Haemosiderin granules: **Blue.**

3. Demonstration of Calcium in the Gross.

- This method is particularly useful in demonstrating calcium in the soft parts and its distribution in bone-tumours.
- *Procedure :*
  1. Section the specimen smoothly with a hand-saw.

2. Fix thoroughly in any fixing solution.
3. Wash in **running water** then in distilled water.
4. Place in **1% silver nitrate** solution in the dark.
5. Rinse the specimen in **distilled water**.
6. Place in **5% hypo-sulphite solution**.
7. Wash several times in running water.
8. Preserve in 50% alcohol or Kaiserling solution No. 3.

- The result: Calcium: **Black.**

4. Demonstration of Amyloid in the Gross

- *Procedure :*
- Amyloid is coloured **mahogany-brown by iodine-potassium iodide solution** in the fresh state. The colour changes to bluish-violet by 1% sulphuric acid.
    1. Fix the specimen in 10% formaldehyde solution.
    2. Cut into slices 2 cm. thick.
    3. Place in a fluid formed of
        - 2% sulphuric acid (1 part) and
        - 2 % Lugol's solution (1 part).
    4. Mount in liquid petrolatum.
- **The result: Amyloid: Violet**

5. Demonstration of Connective Tissue Hyaline and Fibrosis

- This is done by the use of a weak solution of **acid fuchsin and counter-staining with haematoxylin or by using the van-Gieson stain.**
- It is particularly of value to show
    - **Cirrhosis,**
    - **Scar tissue,**
    - **Healed tuberculosis,**
    - **Contracted kidney,**
    - **Fibromyomata, and**
    - **Other diseased conditions characterized by the presence of excessive fibrous tissue.**

- *Procedure :*
    1. Fix the specimen and cut into two halves.
    2. Put in **water** before staining each half separately.
    3. Stain with Wiegert's iron haematoxylin.
    4. Immerse in **water then in 1% acid alcohol then in water.**
    5. Counter-stain in the van Gieson mixture (concentrated watery solution of picric acid and acid fuchsin) until the rose-red colour of the fibrous tissue stands out sharply.
    6. Preserve in jelly.
        - The result: Fibrous tissue: **Rose-red.**

6. Demonstration of Nervous Tissue in the Gross

- This may be used to display lesions of the
    - Cerebrum,

- Cerebellum and
- Other nervous tissues.

- Several staining procedures are available whenever the specimen is cut into relatively thin slices.
- Marchi's and Weigert's methods may be used for this purpose.

- *Procedure :*

1. Fix the gross tissue thoroughly in 10% formaldehyde solution.
2. Cut into thin slices.
3. Wash each tissue-slice in running water then in distilled water.
4. Immerse in a **fluid formed of**
   - **Phenol crystals… 32 g,**
   - **Copper sulphate crystals… 4 g,**
   - **Hydrochloric acid… 1 ml,**
   - **Distilled water to… 1000 ml.**
5. Immerse in **water.**
6. Transfer to **1% ferric chloride solution** in distilled water.
7. Wash in **running water.**
8. Place in **1% potassium ferrocyanide** in distilled water.
9. Wash in **running water.**
10. Moisten in **70% alcohol**.
11. 11. Mount.

The result: The grey matter stains **blue.**
Cerebral neoplasms will stand out sharply.

• (b) Colouring Methods For Gross Specimens

- Some methods are used to give permanent brilliant colour contrasts to certain types of specimens.
- Carbon monoxide (in the form of illuminating gas), when made to run through the solution containing the specimen unites with the haemoglobin.
- The method gives a brilliant colour provided the gross tissue contains muscle or blood-colouring matter.
- When a satisfactory colour is attained, the specimen is transferred to the mounting fluid.

• (c) Macro - Microscopic Methods

- The simultaneous study of gross and microscopic preparations helps the observation of tissue-changes.
- It enables one to apply one's knowledge much better than when the lesions in gross and microscopic pathology are presented separately.
- The preparation of **stained sections of whole organs** allows the study of the structure of the diseased areas and their relationship to the normal parts.
- Such preparations are valuable for scientific investigations, practical diagnostic problems, instruction and display.
- They offer a convenient means to study the problems concerned with **the processes of spread of diseases and tumours in single organ regions** by direct inspection.
- They can be used as transparencies by transmitted light in the projection-apparatus or

under the microscope.

- Various researches upon the appendix, prostate, lungs, eyes, ovaries and tumours can be **made with these large sections**.
- Furthermore, they offer a new means of storage of specimens and of dissemination of information.
- ***Procedure :***

1. **Cut slices 1 cm. thick and keep in 10% formaldehyde solution (2 days).**
2. **Wash in running water (several hours).**
3. **Embed in gelatin.**
4. **Freeze the block gradually and attach to a microtome having a large freezing chamber and a platform.**
5. **Cut sections (20 to 30 microns), float them in water and mount on glass.**
6. **Arrange the mounted sections in the exact position in which they were related before they were cut.**
7. **Pour a thin alcohol-ether-celloidin solution over the section.**
8. **When dry, dip in water.**
9. **Carry out the desired staining in covered glasses.**
10. **Dehydrate in alcohol, clear in carbol-xylol and mount in glycerine-gelatin.**

- This method is used for microscopic purposes only.

- Another method is used for obtaining representative sections of the entire organ for naked-eye examination only.
- *Procedure :*

1. **Remove the organ with its covering.**
2. **Distend it, either via the vessels in solid organs (liver) or through the bronchi and natural passages (lung) or through the orifices of hollow organs (bladder).**
3. **Place the organ in the fixing fluid and cover with a wet cloth.**
4. **Cut slices, each is 2 cm thick.**
5. **Wash in running water.**
6. **Place each slice separately in the following solution :**

1	**Gelatin**	**100 ml**
2	**Propylene phenoxetol**	**10 ml**
3	**Capryl alcohol**	**5 ml**
4	**Water to**	**1000 ml**

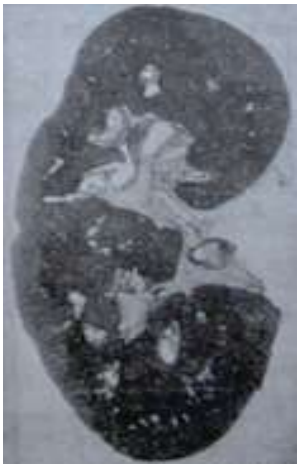
7. **Place the specimen in an incubator, 37^0C (few days).**

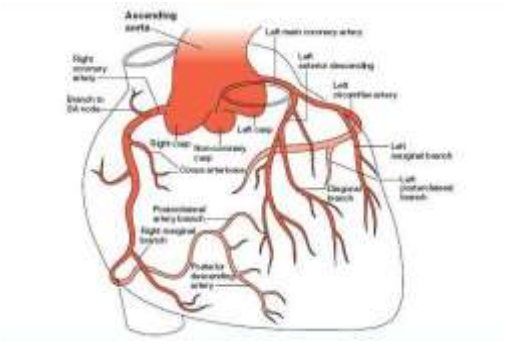
8. **Transfer to the following solution:**

1	**Gelatin**	**250 ml**
2	**phenoxetol**	**20 ml**
3	**Water to**	**1000 ml**

9. **The gelatin is cast into a block in perspex mould and, using the same gelatin solution, pours into the mould to cover the bottom.**
10. **Place the specimen in the mould, cover with the rest of the gelatin and allow setting.**
11. **When the block is thoroughly cooled, remove it from the mould.**
12. **Immerse the gelatin block in 10% formaldehyde solution for few days.**
13. **Fix the block to the microtome, cool and cut.**
14. **Put the cut sections into a weak formaldehyde solution.**
15. **Keep the sections under water till they are mounted in perspex-jars.**

- A further modification **is the dry mounting of sections on paper** as well as in fluid in thin cases of perspex.
- The principle is to fix the organs in a weak formaldehyde solution, then to embed in gelatin, cut the sections and mount them on a particular type of paper which prevents the curling.
- These sections are put together in a catalogue for inspection and study.



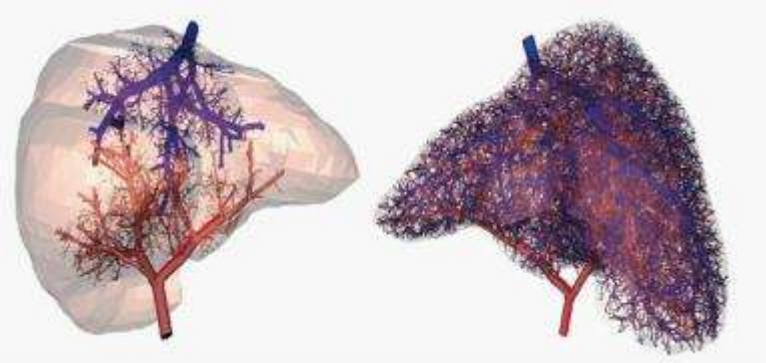


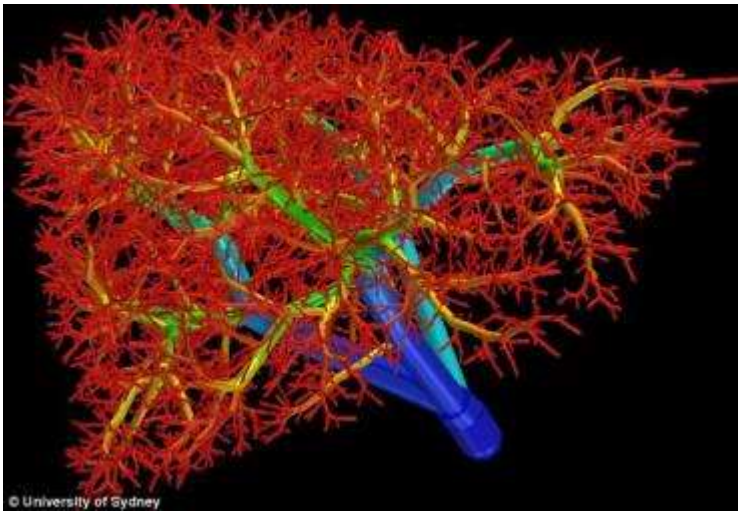


- The most recent method is that in which the author could obtain large serial sections of entire organs stained and mounted on thin large glass slides to be studied macroscopically and microscopically at the same time.

(d) Vascular Injection

- It is often necessary to inject the vessels of an organ or a tissue, either for preservation or in **order to distend them before they are hardened** or cut up.
- The method has recently been employed for diagnosis and the study of normal and diseased conditions.
- The practice of vascular injection with coloured masses has had wide application in the **study of the circulatory system of various organs**.
- It serves well for gross preparations and those **intended for clearing and study under low magnification.**
- Various types of injection masses are used for the study of the **vascular architecture** of the body-organs.
- They are either
    - **Fluid-masses injected at room temperature, or**
    - **Warm injection-masses allowed to set in the vessels, or**
    - **Masses opaque to x-ray, or**
    - **Specific stains applied only to living tissue or**
    - **Neoprine and plastic-injection materials.**





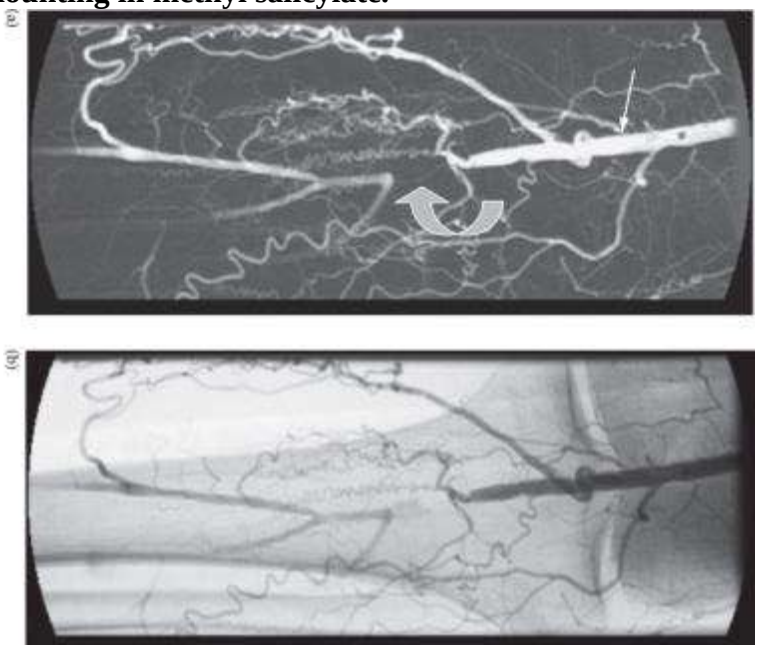
- **The literature contains several techniques** for the use of the injection-masses.
- In most of these methods **carmine-gelatin is used** as an injection-mass to demonstrate the vascular tree.
- The principle is to prepare **neutral colloidal carmine and add to it the gelatin** or to

dissolve carmine in water by means of heat and ammonia water, then mix with the gelatin solution.

- Sometimes, **before removing an organ from the body**, it is advisable to inject its vessels by a fixing fluid for the purpose of better preservation in a more natural state.
- In such cases, it is recommended to use either weak formaldehyde solution or the following fluid :

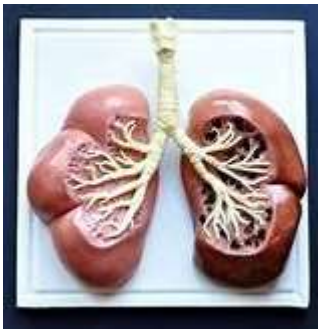
1	**Crystallized Carbolic acid**	**(1)**
2	**Methylated spirit**	**(80)**
3	**Pure glycerine**	**(80)**

- In certain cases in order to demonstrate the injected vessels, the specimen must be made transparent by replacing the tissue fluids by other **fluids of a high refractive index; and, oil of winter green may be used for such preparations**.
- The principal steps are
    1. **Fixation in formaldehyde solution**,
    2. **Bleaching in hydrogen peroxide**,
    3. **Washing in water**,
    4. **Dehydration slowly in alcohol** and
    5. **Transference to benzene and benzyl-benzoate**
    6. **Before mounting in methyl salicylate.**

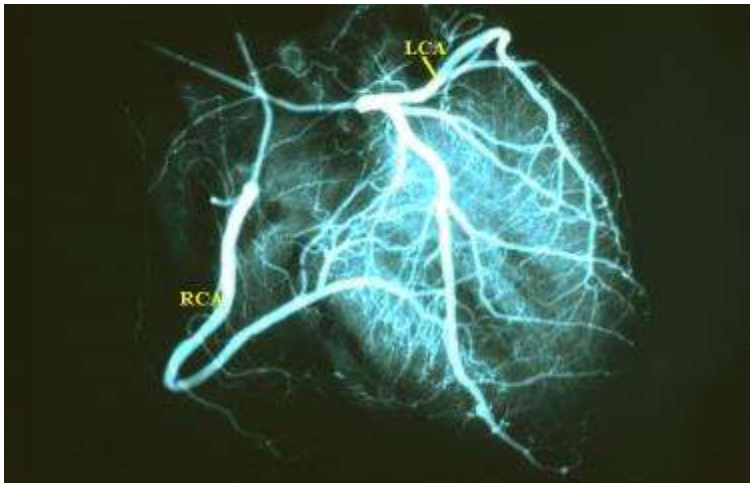


- **Various methods have been used to demonstrate the vascular tree of various organs** (lung, kidney, brain, stomach and liver) by injections with neoprine or plastic injection-masses.
- Most of these methods are easy in the hands of an expert but difficult to the beginner.
- They give highly satisfactory corrosin-specimens in **more than one colour** if desired, allow **roentgenologic** and **histologic** study to be made on the same organ and are now practiced by several authors and research workers.
- **The renal circulation** can be studied histologically and roentgenologically by means of neoprine injection in the renal artery and ureter.

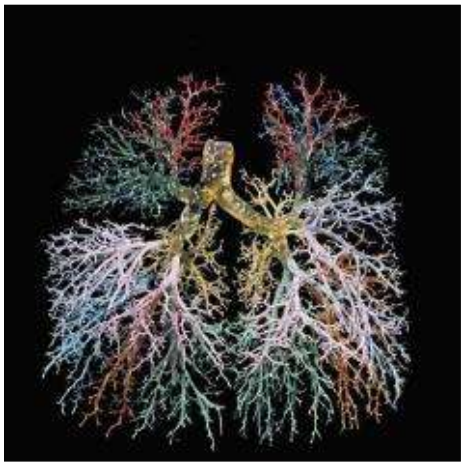
- The collateral circulation of the lungs can be studied by making Broncho - pulmonary plastic casts.
- One can utilize the technique of plastic-injection to study the vasculature of the various organs in the body in relation to their particular diseases and by combined pathologic (gross and microscopic) and roentgenologic examinations.

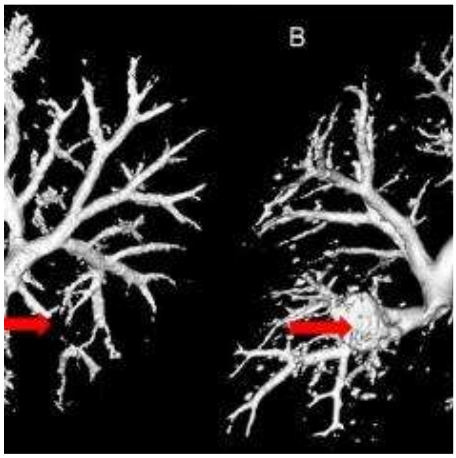




- In general the principal steps in any technique must accomplish the following:
    1. Remove the organ with its artery, vein, natural passages and some of the surrounding peri-organ adipose tissue.
    2. Cannulate the artery and wash the specimen and its vasculature by a tap flowing slowly.
    3. Place the specimen in a covered dish in an ice-box (several hours).
    4. Place at room temperature (few hours).
    5. Connect the cannula, already tied into the artery, by a clamped rubber tube to the bottle containing the "neoprine" regulated by a mercury-manometer.
    6. Release the clamp and allow the neoprine to be injected at a constant pressure.
    7. Ligate the artery and vein just beyond the cannula before releasing the pressure.
    8. Immerse the entire injected organ in hydrochloric acid and put in an incubator, for one day, in order to obtain the corrosion specimen.
    9. Wash in warm water.
    10. When all tissue-debris is removed, the specimen is ready for study by putting in water and inspecting by a binocular dissecting microscope.
    11. If it is desired to keep the specimen for illustrative purposes, the dried cast is sprayed with shellac and suspended by a glass or plastic-hook.
    12. If X-ray pictures were needed, one has to use a 10% solution of vinylite in acetone containing sufficient lamp-black (to render the mixture black) and 10 gm of bismuth oxychloride per liter. After a brief interval during which the injection-mass is allowed to harden in the smaller vessels, the first roentgenogram is taken to demonstrate the pattern of the vascular (or renal or bronchial or cerebral) tree.
    13. If histological study was needed, blocks might be cut and placed in acetone then in two changes of xylol or chloroform and then in paraffin.

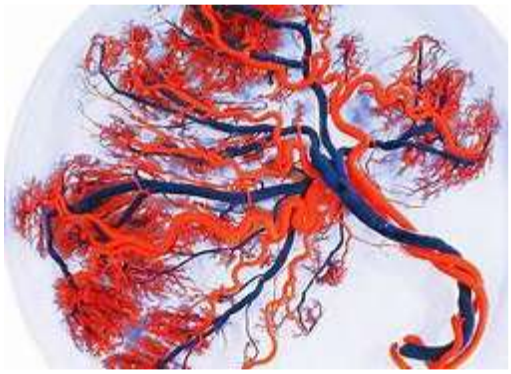


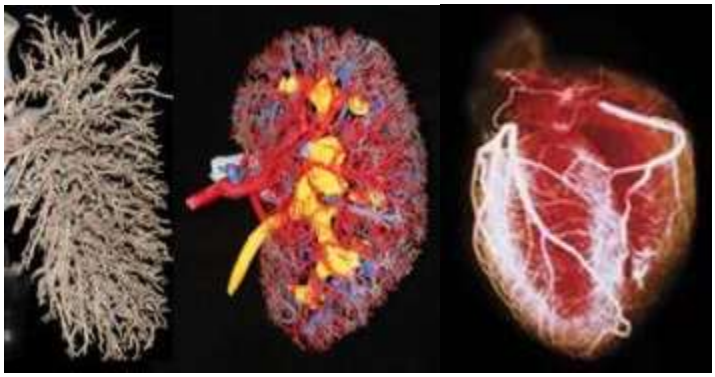
- To study the **collateral circulation of the lungs** and to obtain broncho-pulmonary plastic casts, the following steps are recommended.
- **The thoracic aorta** with its branches is injected in situ with black plastic.
- The lungs, aorta and associated mediastinal structures are then removed en masse and the lungs inflated in a large vacuum-jar.
- While inflated, the bronchial tree and pulmonary arteries and veins are injected with white, red and green plastic respectively.
- Several blocks are removed and fixed in formaldehyde solution for subsequent histologic study.
- After digestion of the lungs in acid, casts are obtained which will demonstrate the collateral vessels and their relationship to other structures.



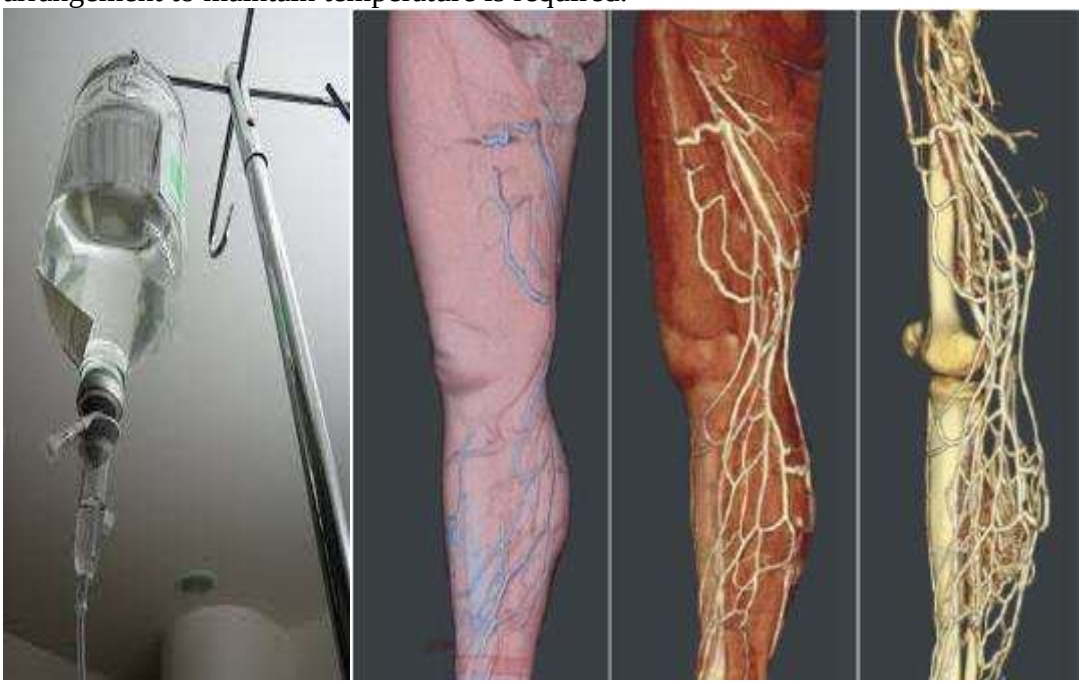








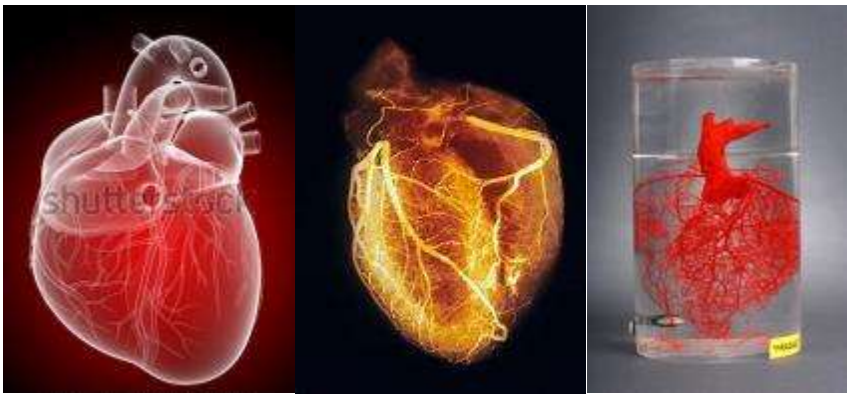
- For the technique of injection, there should be available, cannulas of different sizes (made up of glass), glass-tubing, brass-syringes, a constant pressure apparatus and brass-tubing with stop-cocks.
- The tubes should be filled with the injection fluid before attaching to the cannula in order that no air may get into the vessels.
- The glass cannulae should be provided with a slight bulbous enlargement on the end so that they can be tied in.
- To avoid tears in the veins when introducing the cannula, the latter should have the opening on the side of the bulb and distal to the constricted area around which the tie is made.
- With large human organs, some types of gravity-perfusion apparatus with a heating arrangement to maintain temperature is required.

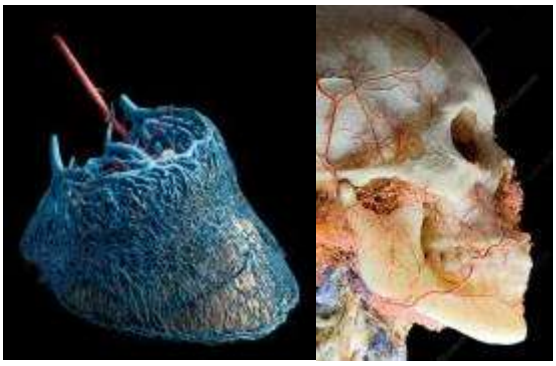


- A manometer is attached to the injection system with a T-tube and the pressure regulated.
- To ensure a good injection preparation, the system should be a closed circuit so that no air gains entrance during injection. The blood vessels may also be washed out with either cold or warm water or saline solution according to the injection mass used.

(e) Wax Modelling

- Wax-models of anatomical organs 'and wax casts of skin lesions, deformities and pathological specimens can be made very realistic if prepared under the direction of a medical man.
- They are invaluable for **teaching purposes** and as **permanent records of morphologic**, surgical and autopsy (human or animal) materials.
- Wax-models showing surgical technique are the most recent methods of medical exhibits.
- The principle is to make a clay-model from which a plaster-mould is taken to make the wax-positive.
- Sketches and snap-shots made during the operation are used as supplementary guides (Fig. 28c).
- When such models are made with the elimination of the un-essentials and accentuation of the essential surgical principles, their teaching value is highly augmented.
- Fresh pathological material can be reproduced in wax for the purpose of keeping the fresh colour of the original specimen, its minute details and the appearance of plasticity.
- The technique in each case has to vary with
    - The **nature of the organ** (hollow, solid) and
    - **The aim of the study** (pathologic-physiology, gross or microscopic-pathology, roentgenology) and
    - The **type of injection mass** used (plastic, neoprine, vinylite in acetone, red or green pigments and with or without bismuth oxychloride).





- *Procedure :*

1. Wash quickly the fresh specimen **in saline.**
2. Immerse in a diluted solution of **sodium hydroxide**.
3. Rinse again **in saline**.
4. Dry carefully, put on **board and surround by gauze**.
5. Depressions are packed smoothly with oiled cotton.
6. **Oil the specimen** with olive oil.
7. Pour **plaster of Paris** over the surface.
8. Allow the **plaster-cast to dry out**.
9. Immerse in **cold water**.
10. Dry and **pour the wax** to which was added the suitable colour.
11. Orifices are made by probes introduced into the model.
12. **Calculi should be well dried out** and affixed to their original position on the moulage by means of balsam.
13. Variations on the wax composition may be used to reproduce bone, mucus, serous fluid, muscle, fat and so forth.
14. When the wax-reproduction is completed, it is mounted on a suitable board with the diagnostic-label, reference number and a descriptive card. It it then put inside a dust-proof glass-case**.**

(f) Photographs and Roentgenographs

- Photography is able to **demonstrate and permanently record changes in the size and configuration of organs.**
- It can be used **to register colour changes** which are often the characteristic demonstrable sign of certain diseases.
- Teaching must be supplemented and **amplified by**
  - **Coloured photographs,**
  - **Photomicrographs and**
  - **Other similar illustrative materials on the history and various aspects of the sick person and the diseased organs.**
- Photographs can be demonstrated together with
  - **Patients,**
  - **Gross specimens,**
  - **Histologic sections,**
  - **Micro-organisms and**
  - **Lesions of the skin and mucous membranes**
  - **roentgenograms**,
  - **illustrations** and
  - **Photostatic enlargements of charts and labels.**

- The application of colour photography to medicine helps **the demonstration of many pathological changes.**
- Any medical person should have a fair knowledge of the subject of clinical photography in general and photographing wet specimens in particular.
- The specimen has to be **submerged in water and anchored there so that no part floats or moves during the exposure.**

- Sometimes, when the surface of water is reflective and prevents the proper placement of lights, or there is oozing of blood and bile, photography by **means of polarized light is recommended.**
- This affords complete control of reflections on the surface of the specimen and obviates the submersion.
- To get good results by photomicrography, use the perfectly stained well illustrating sections.
- **A good field must be chosen** with no artefacts, scratches or foreign bodies.
- The selected fields should be circled before photographing the section.
- The glass-slide is rotated by means of the movable stage until the section presents a well-balanced appearance with the most important features in the centre of the field.
- In photographing a series of slides it is advisable to do at one time all the fields requiring the same magnification.
- It is interesting to know **that it is easier to take good high-power pictures than those of low magnification.**

- **Photographs and photomicrographs** may be printed and mounted in **lantern slides** so that they can be used in display-cases or by means of the projector.
- They allow more tissue lesions to be demonstrated than could possibly be preserved in jars.
- They can be filed according to disease under systems or may be mounted for display on panels.
- Such material can be available for display in the museum and lecture theatres as well as for projection at clinico-pathologic conferences and for staff-members in their reports and research work.

- The provision of **x-ray pictures** together with the gross and microscopic material facilitates the study of many diseases.
- In fact, an **X-ray pathology** dependent upon the varying penetrability of tissue to X-ray has become the basis for X-ray diagnosis, and many modern autopsy rooms are provided with an X-ray apparatus.
- The medical teaching needs the study of **X-ray films side-to-side with the gross tissues** from which the films have been prepared.
- By this means the X-ray can increase the interest in the specimens and augment their teaching value as the characters and features of certain specimens (osseous) are well demonstrated through the use of X-ray.
- The **viewing-box** which is used for exhibiting transparencies of photomicrographs can be used to display the X-ray plates.
- The reproduction of X-ray films on small films permits a maximum number of radiographs to be exhibited in a minimum amount of space.
- The use of the **combined-film-cabinet and viewing-box** is one of the best means of displaying X-ray films.
- Each frame carries a set of films illustrating one disease and the progress of a disease can be seen at a glance.
- The same viewing box can be used for transparencies of clinical pictures, enlarged photomicrographs, and colour lanternslides particularly of blood pictures.