

Section

1

Histopathology

1. Microscopes
2. Cell Injuries
3. Inflammation
4. Splenic Enlargement
5. Infarction
6. Amyloidosis
7. Neoplastic and Preneoplastic Lesions
8. Skin Tumors
9. Oral Malignancy
10. Other Important Tumors
11. Postmortem Examination
12. Histopathological Techniques

1. MICROSCOPES

Galileo first invented microscope at the end of first century. In 1676, Antonio von Leeuwenhoek first developed and used it. Robert Hook developed compound microscope. Now we have varieties of improved microscopes. These microscopes are helping us a lot in our diagnostic work.

DEFINITION

It is an optical instrument, which magnifies the image of an object. It is used to study structural details of the cell, microorganisms, etc.

TYPES OF MICROSCOPE

Light Microscope

- Simple
- Compound

Simple microscope gives magnifying image of an object by using a single convex lens. Usually, it magnifies 10 times of an object. It is used to study insect, structure of plants, etc.

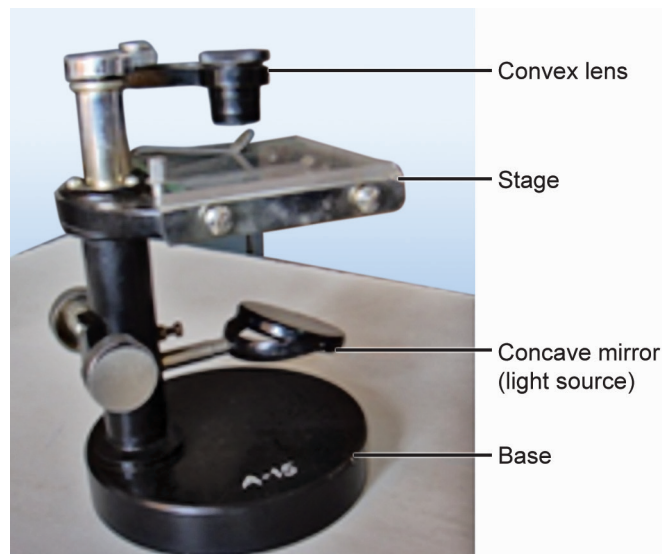


Fig. 1.1: Simple microscope

Compound microscopes are the most commonly used microscope in our laboratories. We get maximum magnification of an object by using two convex lenses in the same axis. We have varieties of compound microscopes like monocular, binocular, trinocular, etc.

Monocular microscope has only one eyepiece. It is cheaper and used where the work is less. It causes eyestrain, so it is not good for prolonged use. Binocular has two eyepieces. As we use both the eyes, magnification and resolution are good.

It is less straining to eyes and so it is used routinely in laboratories. However, it is expensive. Trinocular microscope has three eyepieces. It is used for photography and for teaching purpose. Teaching microscopes has multiple eyepieces so that many study the cell details at a time.



Fig. 1.2: Compound monocular microscope, trinocular microscope and teaching microscopes

Parts of Compound Microscope

Supporting System (Base, Tube and Stage)

Base is made up of heavy metal, which gives stability to microscope. Tube is a hollow tube measuring 160 mm in length. It supports objectives and eyepieces. It can be moved up or down by coarse and fine adjustment. Stage is a platform on which slide is placed. It has an apparatus for light to pass through and has a clamp to fix slide. Stage is movable, so that the slide can be moved in different directions.

Illuminating Systems (Light Source, Condenser, Filter)

Light source is mostly built in lamp with Halogen lamp of 6V and 10W to provide exact light. If tungsten light is used, it gives yellow color. Therefore, we must use blue or green filter. Daylight illumination by using concave lens is not good, as it is variable and it is not adequate for oil immersion.

Condenser is a movable lens below the stage with iris diaphragm. Its function is to focus desirable amount of light on an object and to avoid the glare. Thick section, heavy stained slide and oil immersion lens needs more light. Thin section, unstained slides need less light. For low power, condenser is lowered, for high power brought to the middle and for oil immersion raised completely. Iris diaphragm regulates the amount of light. More light will fall after opening the diaphragm. Filter alters the intensity of light to increase contrast and resolution. It protects the eye in fluorescent microscopy.

Magnifying System (Objectives and Eyepieces)

The convex lenses are arranged in same axis. Objective produces real image and the eyepiece produces virtual image. They magnify the image in multiple times.

Objectives are the lenses near the object. Four different types of objectives are used with different magnification.

- Scanner (4X) Used for rapid location of specimens, etc.
- Low power (10X) Used for rapid diagnosis.
- High power (40X) Used to observe finer details of a section.
- Oil immersion (100X) Used to get maximum magnification for blood and marrow cells, etc.

Eyepieces are the lenses near eye. It is available in different magnification like 5X, 10X or 15X. The distance between two eyepieces in binocular microscope can be adjustable (reading also can be noted). The distance between eyes should match the eyepiece and the two field should merge.

Total magnification = Magnification of objective $\times$ magnification of eyepiece.

Method of Focusing of an Object

Place the microscope nearly 15 cm from the edge of the table. Keep the slide on the stage. Adjust the desirable amount of light by condenser and iris diaphragm. Bring the scanner in position. Move the objective by coarse adjustment until we see the image. Finally get sharp image by adjusting fine adjustment. Bring the 10X to position. Look through eyepiece and adjust fine adjustment until maximum resolution. While using oil immersion use plain mirror and raise the condenser. Always sit straight while working with the microscope. The oil used for oil immersion lens should be of same optical property of glass. Therefore, it prevents bending of light and increase resolution.

Microscope Maintenance

- Clean the microscope lenses with lens paper before and after use.
- Hold the microscope in upright position by using both the hands.
- Keep the microscope in dust free atmosphere.
- Do not touch the glass parts with fingers and do not clean it with solvents.
- Keep the fine adjustment in the middle.
- If any dark shadow is seen, check the eyepiece.
- Keep the microscope with cover when it is not in use.
- After use clean the lenses including oil immersion with lens paper. Bring the 10X into position, lower the body completely, keep it in cabinet, and close the door.

Special Microscopes

Dark Field Microscope

In this microscope, the condenser has central black area, so the direct light will not fall on the object. The reflected light from outer edge is passed obliquely and the object is seen against dark background. Here we get maximum contrast for transparent objects. This microscope is used to see delicate organisms like Treponema, Leptospira, Vibrio, Microfilaria, etc. We can prepare this condenser ourselves.

Fluorescent Microscopy

Instead of visible light rays, we use UV rays. UV light is not visible. The cell and organisms are stained with fluorescent dyes, which convert invisible UV light to visible light. We can see glowing organism against dark background like “stars at night”. Proper filter must be used to filter all harmful rays. This microscope is used in immunogenetics and cytogenetics.

Electron Microscope

The resolution power of light microscope is limited. It is not possible to get magnification more than 1000 times with good resolution. It is possible if we use electron beams. In electron microscopy instead of visible light, electronic beams are used, magnified by electromagnets and focused on a fluorescent screen. The object should be ultra-thin and held in the path of electron beams. Some parts of the cells are electron dense and allow less number of electrons to pass through it and appear as a dark bodies compared to other. It is used to study cell structure, virus, etc. We can get a magnification up to 1 lakh times with good resolution. Electron rays are scattered in gas medium. Electron rays are passed in vacuum inside this microscope.

QUESTIONS

1. Who invented microscope?
2. What is microscope?
3. What are the types of microscope you know?
4. What are the parts of microscope?
5. Which light source is better?
6. When will you change position of the condenser?
7. How to calculate total magnification?
8. How to take care of your microscope?
9. In which conditions do you use dark field, fluorescent and electron microscopes?
10. What is the principle of electron microscope?

2. CELL INJURIES

Pathology is study of the structural and functional changes in cell, tissue, organs due to various injuries. All diseases are caused by injuries at the cell level. Injuries may be lack of oxygen, physical, chemical, biological, immunological and nutritious. Physical agents are high or low temperature, high or low pressure. Chemicals exposures are acid, base, hormones. Biological agents like bacteria, virus, fungus, parasites, etc. Nutritional problems are deficiency or excess. Immunological injuries are due to deficiency, hypersensitivity or autoimmunity.

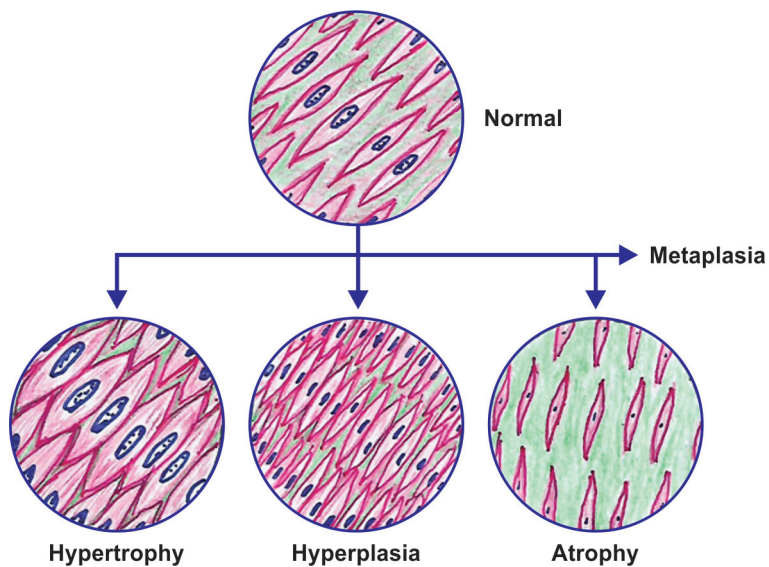
Depending of the length and severity of injury ultimate result of the injury are:

Adaptation (mild injury causes hypertrophy, atrophy, hyperplasia, metaplasia)

- Reversible injury (moderate injury causes cell swelling, intracellular accumulation of water, fat, cholesterol, protein, glycogen, pigments, etc.).
- Irreversible injury (severe injury causes necrosis and apoptosis)
- Pathological calcification (dystrophic or metastatic). Let us study each of them

ADAPTATION**Table 2.1:** Different types of adaptation with example

<i>Adaptations</i>	<i>Morphology</i>	<i>Example</i>
Atrophy	Decrease in the size of cells	Physiological—postmenopausal uterus, breast, etc. Pathological—muscle atrophy after poliomyelitis
Hypertrophy	Increase in the size of cells	Physiological—cells in pregnant uterus Pathological—ventricular hypertrophy in hypertension.
Hyperplasia	Increase in the number of cells	Physiological—cells in pregnant uterus Pathological—benign prostatic hyperplasia
Metaplasia	Change in morphology of cells	Pathological—columnar to squamous cells in chain smokers

**Fig. 2.1:** Different types of adaptation to stress

When the stress or injury is mild, body tries to adapt. Different types of adaptations are atrophy, hypertrophy, hyperplasia and metaplasia. It is a reversible change and may be physiological or pathological.

HYPERTROPHY

Hypertrophy is increasing in the size of cells and may be organs. Hypertrophy can be physiological or pathological. Physiological hypertrophy is seen in pregnant uterus, muscle of body builders, etc. (Fig. 2.2) pathological hypertrophy is seen in heart muscle in hypertension (Fig. 2.3).



Fig. 2.2: Physiological hypertrophied of uterus. Specimen is identified as uterus by its shape, cervix and endometrial cavity. Fallopian tube is seen at upper and lateral side. There is uniform increase in its size of this uterus (normal average $8.5 \times 5 \times 4$ cm) cut section is homogeneous gray white. Endometrial cavity is seen and no other secondary changes like necrosis or hemorrhage. Features are those of hypertrophy of uterus. On microscopic examination, each cells increase in size with normal central nucleus

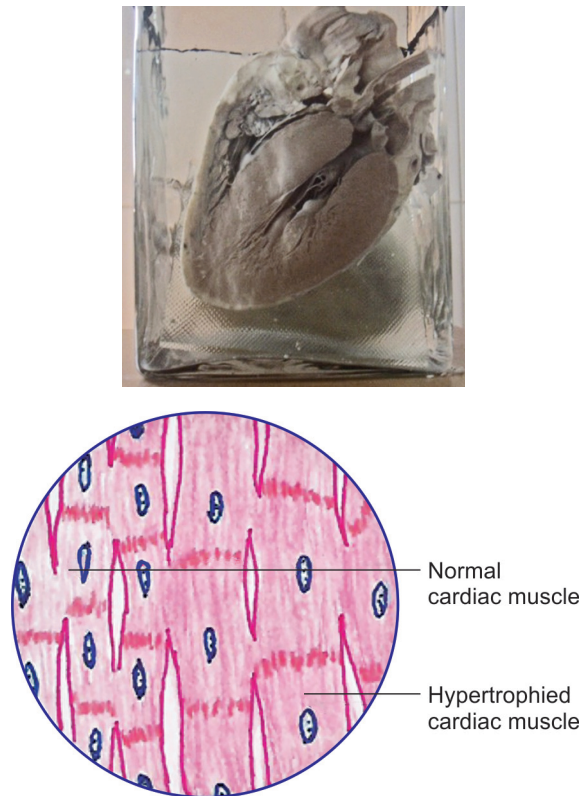


Fig. 2.3: Pathological hypertrophy of left ventricle—gross and microscopy. The specimen is identified as heart by its shape, size, color and cut section showing cardiac chambers. Cut section of heart shows left ventricle thickness has increased and the chamber size is decreased. Features are those of left ventricular hypertrophy. It needs microscopic confirmation. Microscopic section shows increase in the size of each cell compared to normal cardiac muscle. Nuclei are seen at the center and no abnormal accumulation inside the cells

ATROPHY

Atrophy is decrease in the size of a cell and may be organ. Physiological atrophy is seen in uterus (Fig. 2.4) or breast after menopause, etc.

Example: Pathological atrophy is skeletal muscle atrophy after poliomyelitis, etc.



Fig. 2.4: Physiological atrophy (atrophied uterus). The given specimen is identified as uterus by its anatomical appearance like cervix canal and endometrial cavity. Attachment of fallopian tube is seen. The uterine size is decreased uniformly (normal average $8.5 \times 5 \times 4$ cm). Cut section shows homogeneous gray white color. Cervical and endometrial cavity is seen. No other significant changes are seen. Features are those of atrophy of uterus

Hyperplasia

Hyperplasia is increase in the number of cells. This also can be physiological or pathological. Physiological hyperplasic is seen in pregnant uterus, lactating breast, etc. Pathological hyperplasia is seen in benign prostatic hyperplasia, endometrial hyperplasia, etc. (Figs 2.5 and 2.7).

Metaplasia

Metaplasia is change of one type of cell to other types. It is seen in bronchial epithelium of chronic smokers. Normal pseudo-stratified ciliated columnar cells of the respiratory epithelium is replaced by stratified squamous epithelium as shown in Fig. 2.8.

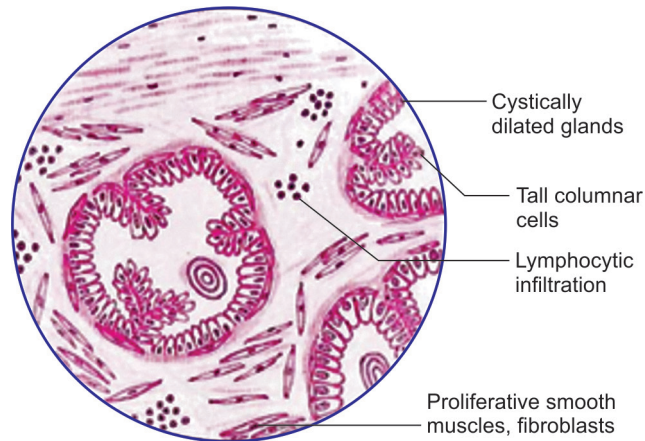


Fig. 2.5: Benign prostatic hyperplasia. Prostatic glands are cystically dilated and lined by tall columnar epithelium. Layers of flat cells are seen at the base of the glands. The epithelial cell forms the papillae. In between the glands, fibrous tissue and smooth muscle are seen infiltrated with lymphocytes. Corpora amylacea may be seen within the glands

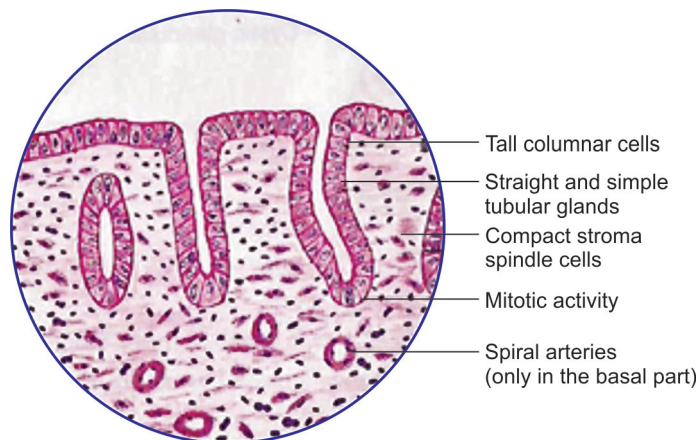


Fig. 2.6a: Proliferative phase of endometrium, proliferative phase shows straight tubular glands lined by columnar cells. Stroma is compact and made of spindle cells showing mitosis. Spiral arterioles seen at the base

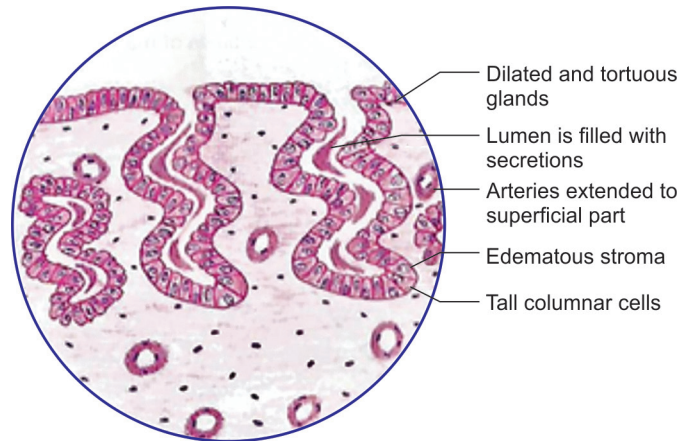


Fig. 2.6b: Secretory phase of endometrium in secretory phase there are dilated and tortuous glands lined by columnar epithelium. Their lumen is filled with mucoid secretions. Stroma is edematous and the arterioles are extended up to the superficial part

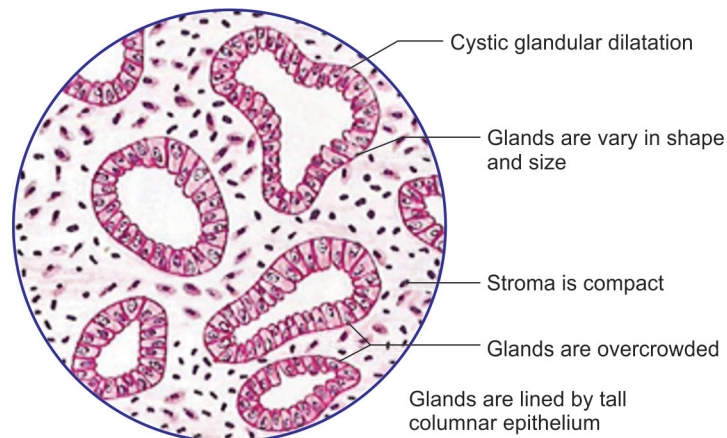


Fig. 2.7: Endometrial hyperplasia cystically dilated glands of different shape and size lined by tall columnar cells. The stroma is compared to normal endometrium like secretory phase

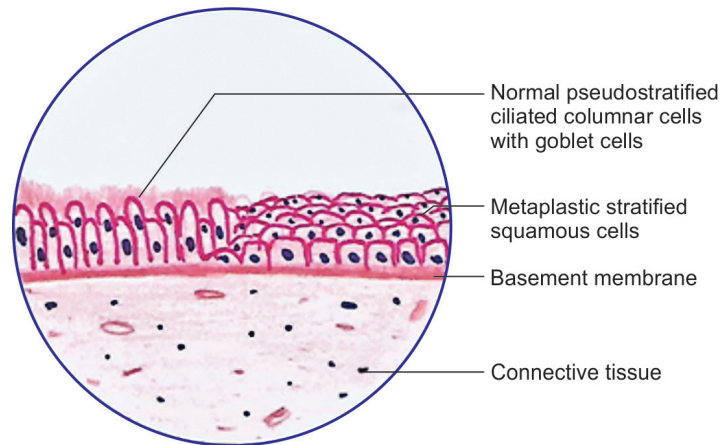


Fig. 2.8: Squamous metaplasia in the respiratory epithelium. Normal pseudostratified ciliated columnar epithelium with goblet cells are changed to stratified squamous epithelium in the right side

REVERSIBLE INJURY

When the injury is for short time or mild it causes reversible changes in the cells.

Example: Reversible injuries are cellular swelling, fatty liver, atherosclerosis, etc.

Cell Swelling

Cells are swollen due to defect in ATP leading to Na K pump. Movement of Na, Ca^{++} and water into cell cause cell swelling.

Fatty Liver and Atherosclerosis

Normally liver synthesizes fatty acids and apoprotein. Toxins like alcohol, CCl_4 may interfere in the liver apoprotein synthesis. In this case, fatty acids are unable to excrete from the liver and will start accumulating. This will lead to fatty liver. It is a reversible change (Figs 2.9 and 2.10). Atherosclerosis is a change due to chemical injury. There is accumulation of fibro fatty tissue below endothelium of blood vessels. Fat accumulates within macrophages. Study proved that it might be reversible with exercise (Figs 2.11 and 2.12).

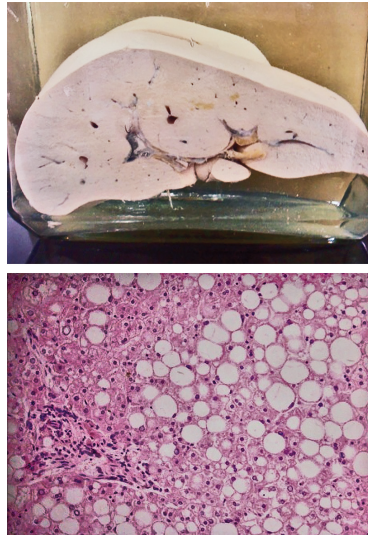


Fig. 2.9: Fatty liver—gross and microscopy. The organ is identified as liver by its appearance, size and wedge shape. The liver is enlarged and the surface is smooth, its weight is increased and it is soft to touch. Liver shape is maintained. Cut surface is yellowish and greasy. Features are those of fatty liver. Fat is demonstrated in frozen section with special stains like oil red O, Sudan IV. PAS staining is necessary to rule out glycogen

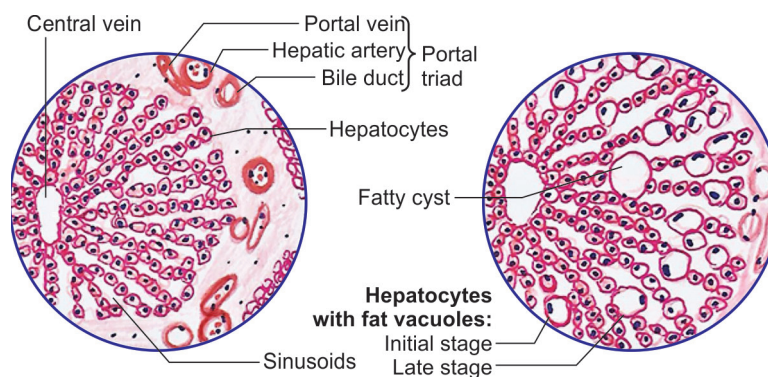


Fig. 2.10: Microscopy of normal liver and fatty liver. Section shows normal architecture in both. Note in the fatty liver hepatocytes are swollen and filled with triglyceride, which pushes the nucleus to periphery. At places, fat cell may rupture to form fatty cyst



Fig. 2.11: Gross specimen of atherosclerosis

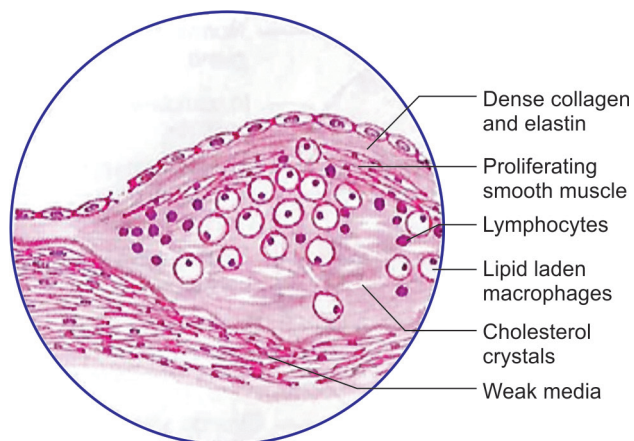


Fig. 2.12: Microscopic section of atherosclerosis. Specimen is identified as aortic wall by its color and vascular wall appearance its luminal surface shows patchy areas. Some small dot like and some yellowish streaks of atheroma are seen. Features are those of atherosclerosis—aorta. It needs microscopic confirmation. Microscopy shows subendothelial accumulation of fibro fatty tissue is seen. In addition, thinned out tunica media is seen. Intimal deposition is composed of lipid-laden macrophages, cholesterol crystals, proliferating smooth muscles, lymphocytes, collagen and elastic fibers

IRREVERSIBLE INJURY

If the injury is severe or prolonged cell may not recover and go for irreversible injury. This cell will die and go for necrosis or apoptosis. Enzymes of cell itself or infiltrated WBC degrade the injured cells. In necrosis, nucleus, organelles and membrane will breakdown. Cytoplasm becomes more eosinophilic. Nucleus disappears by fading, fragmentation or shrinking. Lysosomal enzymes released into blood or outside to cause inflammation. Types of necrosis are coagulative necrosis, caseation necrosis, liquefactive necrosis, fat necrosis, fibrinoid necrosis.

Classical example of irreversible injury is myocardial infarction (coagulative necrosis Fig. 5.1), brain injury in cerebrovascular accidents, etc. (liquefactive necrosis is described in other section).

Coagulative Necrosis

If there is denaturation of lysosomal enzymes, dead cell will not go for lysis. Cell architecture is preserved for few days. It is most commonly seen in ischemic injury of organs like heart. Myocardial infarction is discussed in the Chapter 5 Infarction.

Red infarction: It is a rare type of infarction occurs only 1–2% of total infarction. In red infarction is tissue involved will be red or brown in color and seen due to venous occlusion, in tissue which are not solid, tissue with double blood supply like lung, GIT, etc. there is infarction with accumulation of blood. It is not clear wedge shape and its margins are not well made out. It is most commonly seen in lung due to block in the pulmonary artery. Blood supply from bronchial artery is not sufficient.

Liquefactive Necrosis

The dead cells enzymatically digested to liquid. The enzymes liberated by WBC are responsible for it. It is seen in nervous tissue and infected tissues.

Caseation Necrosis

It is most commonly seen in tuberculosis. Microscopic changes are in between coagulative and liquefactive necrosis. The cell architecture is lost but fragmented cells are seen as pink granular structures in H& E section. Explained in the Chapter 3, Fig. 3.10.

PATHOLOGICAL CALCIFICATION

Calcification is accumulation of calcium salt. It may be physiological or pathological. Physiological calcification is seen in the normal bone formation. Pathological calcification is deposition calcium salts, with iron, magnesium, etc. in abnormal site. It is due to loss of mineral balance or some abnormalities in the tissues. Dystrophic and metastatic are the two types of pathological calcification.

1. Dystrophic calcification is seen in injured, dying or dead cells. It is seen in the intima of blood vessels, tuberculous lymph node, breast, brain, etc.
2. Metastatic calcification is abnormal calcification seen in normal tissues due to increase in blood calcium. Organs commonly involved are blood vessels, lung, kidney GIT, etc. It may be seen in vitamin D intoxication increased parathormone level, bone destruction, etc.

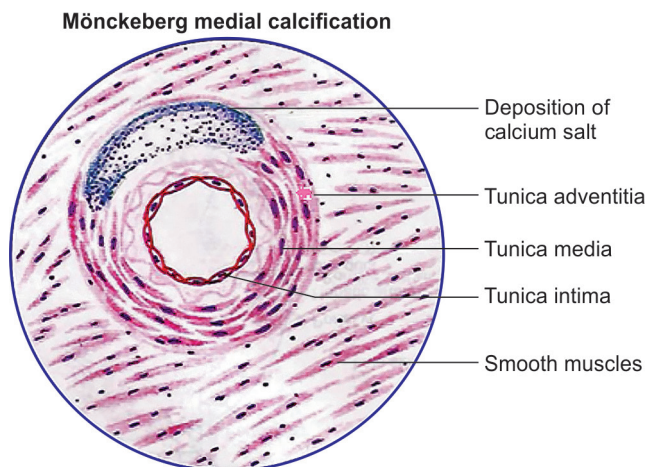


Fig. 2.13: Pathological calcification. Section studied shows a medium blood vessel. There is deposition of calcium salt in the tunica media. This is basophilic amorphous granular substance deposited both intra- and extra-cellular

3. INFLAMMATION

Inflammation is reaction of vascular tissue to injury. Injury may be by physical, chemical, biological, etc. as discussed already. It is a protective phenomenon, which eliminates pathogens, neutralize toxins followed by healing and repair, but sometimes it may be harmful also. There are mainly two types of inflammation acute and chronic. Actinomycosis often called subacute inflammation.

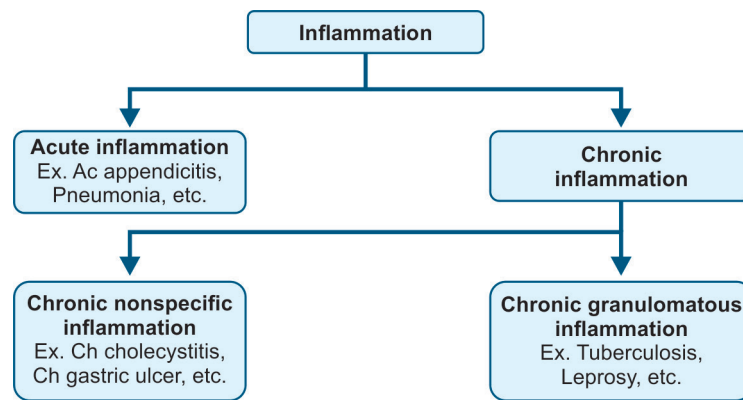


Fig 3.1: Classification of inflammation with examples



Fig. 3.2: Lobar pneumonia. The specimen is identified as lung by its shape, color and appearance. Its pleural surface is seen. Cut section shows part of the lung is red, gray or rusty brown in color. It looks like liver. Consistency of the diseased area is solid and like liver because of accumulation of RBC, WBC and plasma. Features are those of lobar pneumonia

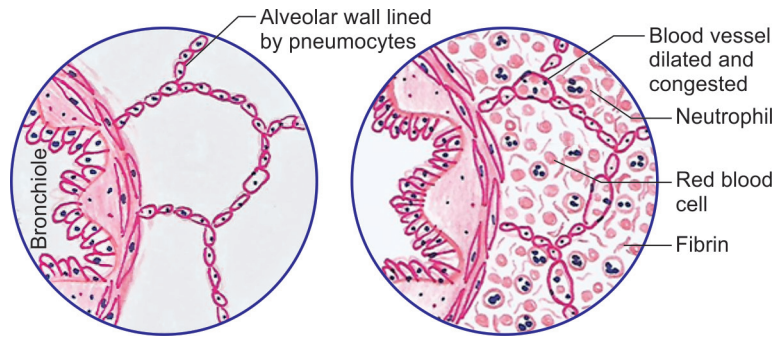


Fig. 3.3: Microscopy of normal lung and pneumonia. Alveolar lumens are filled with neutrophils, RBCs and fibrinous exudates. Alveolar blood vessels are dilated and congested. Features are those of lobar pneumonia

The specimen is identified as appendix by its worm like appearance with its mesoappendix. The normal glistening serosal surface appendix is changed into dull granular because covered with fibrinous exudates. Dilated and congested blood vessels may be seen on the surface. Cut section may show obstructions due to worm, stone, fecalith, etc. Lumen may be filled with pus. Features are those of acute appendicitis.



Fig. 3.4a: Acute appendicitis—gross

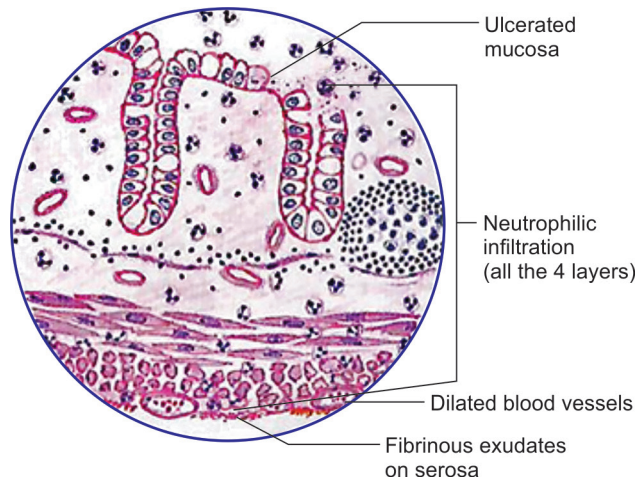


Fig. 3.4b: Acute appendicitis—microscopy

Microscopy shows ulceration of mucosa, transmural infiltration of neutrophils, dilated and congested subserosal blood vessels and fibrinous exudation on serosal are seen. Features are those of acute appendicitis.

The covering of the brain is dull granular, shows many dilated blood vessels. Features are those of acute meningitis.



Fig. 3.5: Acute inflammation of the meninges

ACUTE INFLAMMATION

Acute inflammation is a sudden response to injury. It starts in minutes. Here blood vessels dilate increases its permeability, blood flow increases, neutrophilic and protein rich fluid will come to the site. There are five cardinal signs. They are:

1. Pain (due to release of chemical prostaglandins)
2. Heat (due to increased blood flow)
3. Redness (due to increased blood flow)
4. Swelling (due to fluid come out of blood vessels)
5. Loss of function.

They all resolve/disappear in few days.

Examples are acute inflammations are appendicitis, pneumonia, tonsillitis, meningitis, etc.

Actinomycosis

Actinomycosis is a subacute infection caused by gram-positive anaerobic bacteria Actinomyces.

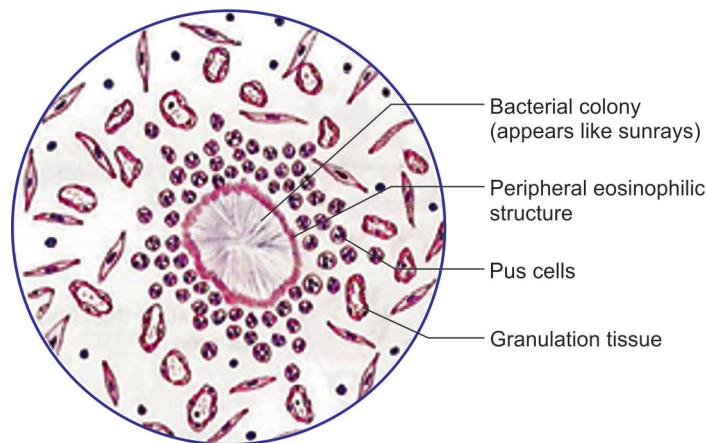


Fig. 3.6: Actinomycosis microscopy shows bacterial colony of actinomycosis arranged like sunrays. Club shaped eosinophilic structure is seen at the periphery. The colony is surrounded by neutrophils. The entire lesion is covered by granulations tissue and fibrosis

Rhinosporidiosis

Rhinosporidiosis is a chronic infection of upper respiratory tract caused by a fungus *Rhinosporidium seeberi*.

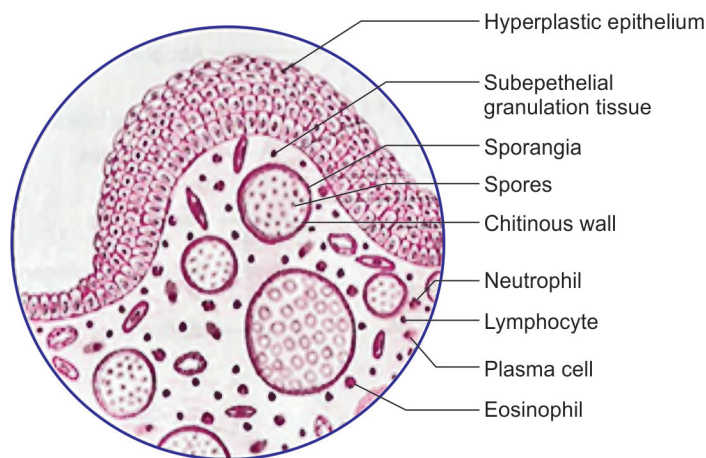


Fig. 3.7: Rhinosporidiosis—microscopy shows hyperplastic epithelium of nasal mucosa. Subepithelial granulation tissue shows multiple sporangia of *Rhinosporidium seeberi* at different stage of maturation. These sporangia are having multiple spores inside and covered by chitinous wall. These spores are surrounded by neutrophils, eosinophils, plasma cells and lymphocytes

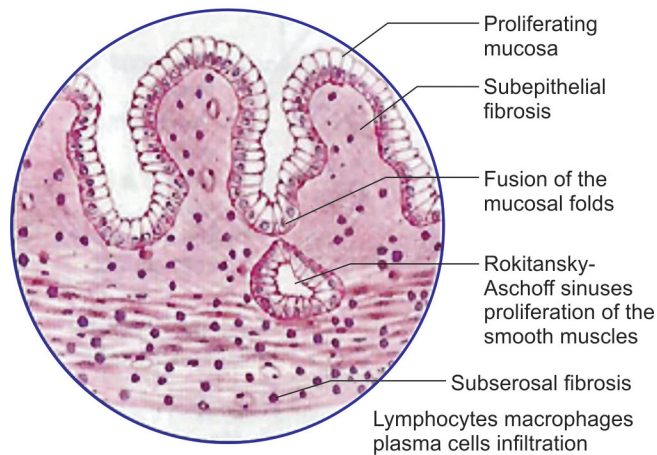
CHRONIC NON-GRANULOMATOUS INFLAMMATION

The specimen is identified as gallbladder by its pear shaped cystic appearance. Gallbladder wall is thick and fibrotic with ulceration of mucosa; Size of gallbladder is increased/normal/shrunken. One or more stones are usually seen in the lumen. Their size varies from pin head to apple size, cholesterol stone is yellowish to brown in color, pigment stones are dark in color composed of bilirubin and calcium salt.

Microscopy of the gallbladder is showing diffuse infiltration of lymphocytes in all the layers. Mucosal proliferation and submucosal fibrosis is seen. Mucosal folds are fused to form Rokitansky-Aschoff sinuses. Excessive proliferation of smooth muscle is seen. Subserosal fibrosis is seen.



(a)



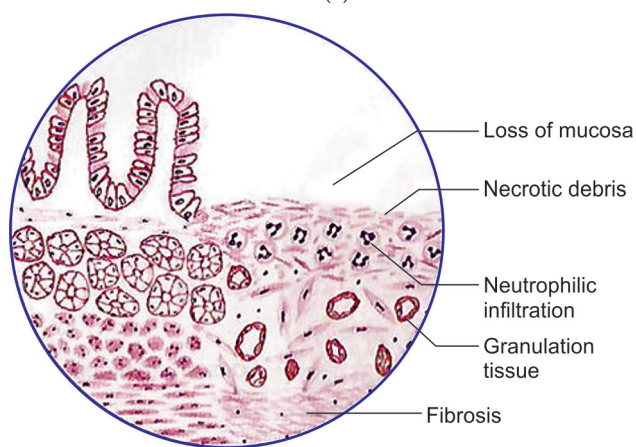
(b)

Fig. 3.8a and b: Chronic cholecystitis—gross and microscopy

Specimen is identified as stomach by its shape and appearance. Luminal surface shows appearance of normal stomach in between there is in ulcer in the lesser curvature. It is regular, round or oval ulcer around 2 cm in diameter. It has perpendicular border and smooth base. Surrounding radial folds may be seen. Features are those of chronic ulcer of the stomach.



(a)



(b)

Fig. 3.9a and b: Chronic gastric ulcer—gross and microscopy

Microscopy shows loss of mucosa. The superficial layer of ulcer is made of dead necrotic tissue and neutrophilic infiltration. Below this, a layer of granulations tissue is seen. The basal layer is fibrous tissue replacing muscularis externa.

Chronic inflammation is reaction after acute inflammation it will be there for weeks, months or years. Here infiltration of macrophages, lymphocytes and plasma cells are seen. It may follow acute inflammation if failure to heal or it will start even

without acute inflammation or it may be autoimmune in nature. Example: Peptic ulcer, tuberculosis, atherosclerosis, active hepatitis, hashimoto thyroiditis, etc. Chronic inflammation leads to many complications including heart disease, diabetes, asthma, emphysema, cancer, etc. Defect in the process of healing causes disease like cirrhosis. There are two types of chronic inflammation, nonspecific and granulomatous.

In non-specific inflammation, there is proliferation of connective tissue fibroblasts, new blood vessel formation. In granulomatous tissue, aggregation of epithelioid cells (modified macrophages) lymphocytes, fibroblasts, e.g. tuberculosis, leprosy, fungal infection, foreign body, syphilis, etc. Chronic infection is seen when the agent is strong (tuberculosis) prolonged exposure (atherosclerosis) abnormality in the immune reaction (hypersensitivity, autoimmunity). Example of nonspecific chronic inflammation are chronic cholecystitis, chronic peptic ulcer, etc.

Diagnosis: Diagnosis is depends on microscopic examination. Elevation or ESR and C-reactive proteins are not specific tests, which may increase in inflammation and other conditions.

Table. 3.1: Differences between acute and chronic inflammation

	<i>Acute inflammation</i>	<i>Chronic inflammation</i>
Onset	Sudden	Slow
Duration	Hours to days	Weeks, months or years
WBC infiltration	Neutrophils	Macrophages, lymphocytes, plasma cells
Tissue reaction	Vasodilatation, increase permeability	Vasoproliferation, fibrosis
Types	Only one type	Two types: Non granulomatous, granulomatous
Cardinal signs	Prominent	Not prominent
Resolution	Mostly without complications	Mostly with complications

CHRONIC GRANULOMATOUS INFLAMMATION

Example 1: Tuberculosis

Tuberculosis is an infectious disease caused by mycobacterium tuberculosis, *M. bovis*, etc. It is more in Asia and Africa. Disease spread from one person to other by cough, sneeze, etc. Inhalation of even 5–10 bacilli is enough to cause disease. *M. bovis* infect by ingestion of bacilli. Primary tuberculosis. The bacilli are inhaled by alveolar macrophages. Macrophage is unable to digest the bacilli because of its tough cell wall. Bacteria may multiply inside and spread to different site. After a gap of 3 weeks, macrophages present antigen of mycobacterium to CD4 (TH1) lymphocyte. CD4 cells secrete IFN- γ . This IFN- γ recruits more macrophages and convert them to epithelioid cells. These epithelioid cells will secrete nitric oxide, tumor necrosis factor γ , free radical, etc. All these factors together can destroy Mycobacteria. This is called delayed type of hypersensitivity. It may heal and calcify but bacteria may be live inside. This is called Ghon's focus. If it involves the regional lymph nodes also, it is called primary or Ghon's complex. If the infection localized to the site it is called latent infection.

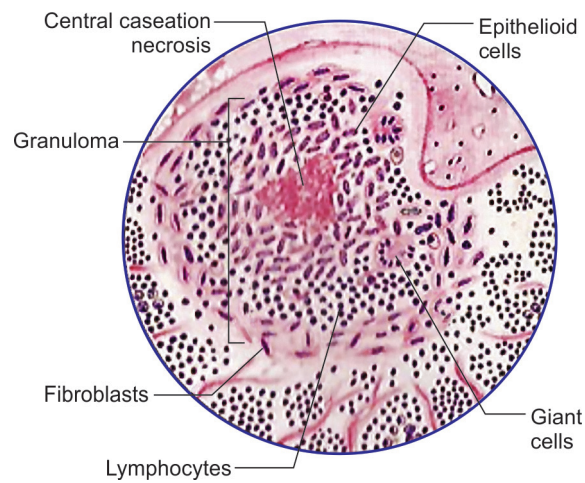
Secondary tuberculosis is infection with external bacilli once again or reactivation of the primary tuberculosis due to reduced immunity. Lesion will be more severe.

Mycobacteria attacks lungs, lymphatic system, GIT, bone, etc. macroscopic and microscopic lesion are similar in all the areas. That is what we see as caseating granuloma. About 5% of the infected person will die of it and 95% patient will suffer from chronic disease. It is clinically presented with chronic cough, evening rise of temperature, loss of wt and appetite. It is diagnosed by sputum macroscopic examination, culture, chest X-ray, etc. Morphological features are explained with the diagrams.

Specimen is identified as lymph node by its shape, size and color. On gross examination the lymph node is enlarged, matted (multiple lymph nodes joined together), firm in consistency. Cut section shows, multiple cheesy white spots of different sizes replace normal parenchyma. It is due to granuloma of



(a)



(b)

Fig. 3.10a and b: Tuberculosis lymph node—gross and microscopy

different sizes with central caseous necrosis. Features are those of tuberculosis lymphadenitis.

Microscopy shows lymph node with granuloma. The granuloma is composed of aggregated epithelioid cells around central caseation necrosis. These epithelioid cells are large oval cells with more eosinophilic cytoplasm. Langhans type of giant cells and lymphocytes are seen. Fibroblasts are seen at the periphery in chronic cases.



Fig. 3.11: Tuberculosis lung. Specimen is identified as lung by its shape, appearance and its pleural surface. Cut section shows irregular cavity in the upper lobe of lung, fibrous wall is seen at the periphery. Wall may be smooth or nodular. Thickening of the pleura is common. Features are those of tuberculosis lung. It needs microscopic confirmation

Example 2: Leprosy

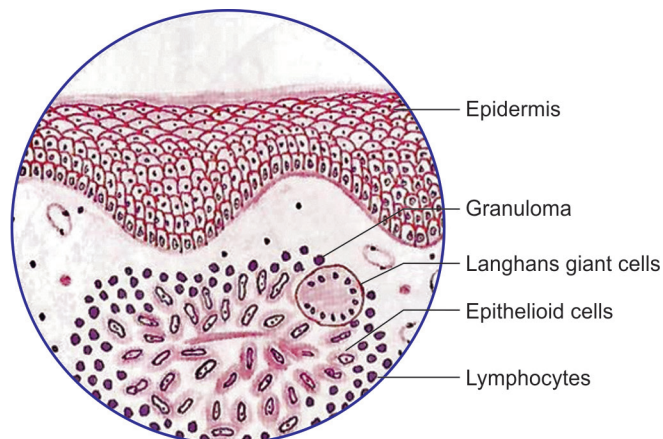


Fig. 3.12: Tuberculoid leprosy of skin. Microscopy of the skin shows epidermis and dermis. Dermis show non-caseating granuloma around nerve fiber. Granuloma composed of aggregation of epithelioid cells, Langhans giant cells and peripheral lymphocytes

Granulation Tissue

It is entirely different from granulation tissue. It is a tissue seen once the inflammation is controlled. There will be new blood vessels and fibroblast proliferation to replace the dead tissue by scar tissue. The stage of new blood vessels and fibroblast proliferation looks like granular. That is why it is called granulation tissue.

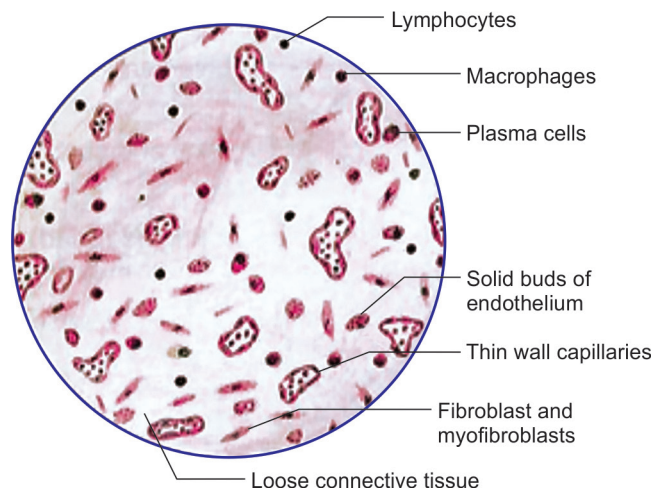


Fig. 3.13: Granulation tissue. Microscopy shows rich vascular tissue with newly formed blood vessels lined by endothelium. Scattered fibroblasts, macrophages and lymphocytes are seen within the loose connective tissue

4. SPLENIC ENLARGEMENT

Normal spleen is soft purple organ in the lymphatic system. It is situated in the left upper quadrant of abdomen. It removes the old and damaged RBC, provides T and B-lymphocytes to fight against infection. It stores the blood cells, platelets, WBCs. Its size varies approximately clinched fist size, i.e. 1 × 3 × 5 inch in length breadth and thickness. It is weighing 7 ounce situated between 9th and 11th intercostal space. During accidents, etc. it is likely to rupture and cause severe internal bleeding and shock. It has two or three notches on its superior border (between diaphragmatic and gastric surface). It is nearly

oval or fist shaped organ. Enlargement of the spleen is called splenomegaly.

CAUSES OF SPLENIC ENLARGEMENT (INCASH)

- Infection bacterial (typhoid, tuberculosis), viral (infectious mononucleosis) parasitic (malaria, kala-azar)
- Neoplastic—lymphoma, leukemia, CML, multiple myeloma.
- Congestive—CCF, cirrhosis, portal or splenic vein thrombosis.

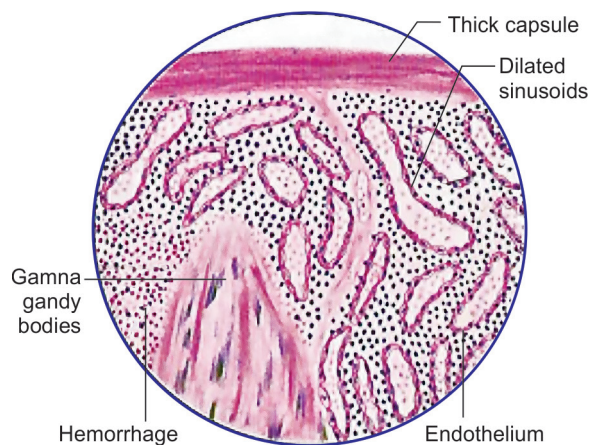
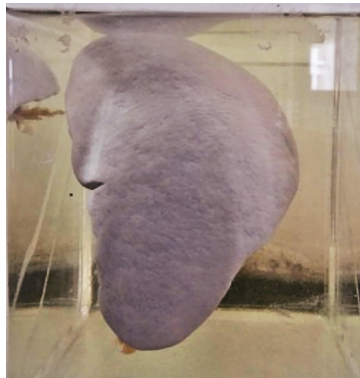


Fig. 4.1: Splenic enlargement in chronic venous congestion (CVC) gross and microscopy

- Autoimmune—SLE, rheumatoid arthritis
- Storage disorders—(Niemann-Pick, Gaucher's disease, etc.)
- Hemolytic anemia—sickle cell anemia, spherocytosis, G6PD deficiency, etc.

The specimen is identified as spleen by its color, appearance and splenic notches. The spleen is uniformly enlarged. Normal spleen is about 150 g. It is enlarged, increased in weight and firm in consistency. In chronic case spleen will be tense, cyanotic with thick capsule. Initially the organ is bluish red in color. In chronic case because of fibrosis, it is tan in color.

Microscopy of the spleen shows thick capsule and dilated sinusoids. The sinusoids have thick wall lined by prominent endothelium. Foci of hemorrhage are seen. Gamna gandy body is thick collagen fibrous tissue with deposition of iron and calcium.

5. INFARCTION

Infarction is death of cells due to lack of blood supply. It may be due to obstruction of the vascular lumen by thrombus, vasoconstriction or external pressure. Arteries or veins may be involved. Organs like heart, brain, lungs, etc. are affected. Cells will die without oxygen, nutrition. Development of infarction depends on tissue and its blood supply. There are two types of infarcts, red infarct and white infarct.

White infarct is seen in solid organs like heart, having single blood supply, mainly deficient in arterial blood supply. It is the most common type of infarction. It is usually wedge shaped infarction; the vascular occlusion is at its tip. Initially margins are red hemorrhagic due to inflammation but pale and demarcated in time. Microscopic examination shows coagulative necrosis, in time it goes for inflammation and scar formation. Myocardial infarction is discussed in the beginning is infarction of the brain. Infarction of the spleen, foot, and intestine is explained with diagrams. In myocardial infarction there will be coagulative necrosis during initial 12 hours. After 12 hours, there will be neutrophilic infiltration and edema. Up to 3 days, neutrophilic enzymes dissolve myocardium. In 3–5 days, neutrophils are replaced by macrophages. After

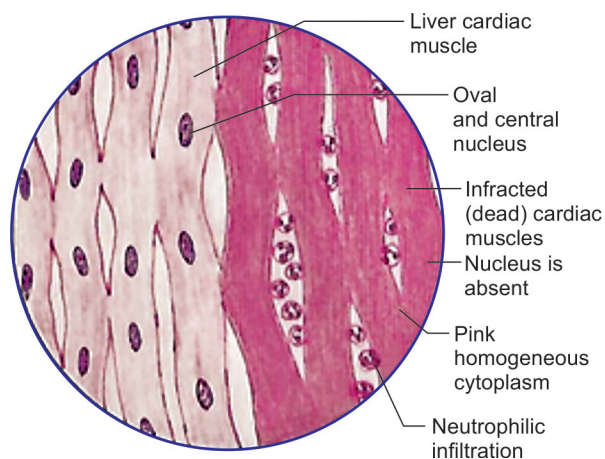
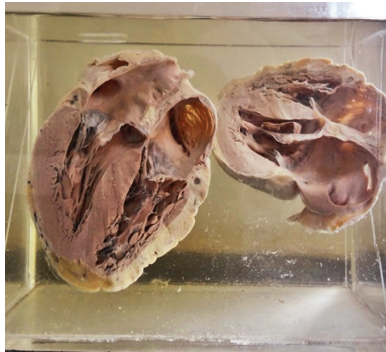


Fig. 5.1: Myocardial infarction—gross and microscopy

7 days development of granulation tissue starts and replacement of dead tissue by fibrous scar.

The specimen is identified as heart by its shape, size, color and cut section showing cardiac chambers. Cut section of heart shows white collagenous scar in the left ventricular wall. Infarction may be transmural (entire thickness of heart muscle is involved). Subendocardial (infarction of muscle below endocardium) features are those of myocardial infarction. It needs microscopic confirmation.

Microscopy shows coagulative necrosis and normal cardiac muscles. Dead and necrosed cardiac muscles are pink without nucleus. Plenty of neutrophils are infiltrated between the dead muscles. Features are in consistent with myocardial infarction.



Fig. 5.2: Infarction of spleen. The given specimen is identified as spleen by its oval shape, dark-red color and splenic notches. Cut section shows wedge shaped paler area of infarct is seen within normal dark brown tissue. Thrombus in the branch of celiac artery causes wedge shaped infarction of spleen. It is pale in color so it is called white infarct. The site of vascular occlusion form the apex and periphery or the serosal surface forms the base of wedge. Initially rim of hyperemia may be seen at the border but with time, it will be well demarcated. Microscopy will show coagulative necrosis of the splenic tissue similar to myocardial infarction



Fig. 5.3 Gangrene of toe. Specimen is identified as foot with its heel and toes. Specimen will be dry and shrunk. Part of the specimen show color change to pale, purple, blue or black. This color change is due to gangrene of the tissue. Color change is due to hemoglobin liberated by RBC. Discharge and swelling may be seen. Features are those of gangrene toe



Fig. 5.4: Gangrene of intestine. The specimen is identified as intestine by its appearance. The part of intestine becomes dark, and edematous. Normal surface glistening is lost. The unaffected part of intestine is gray white in color with glistening surface. Features are those of gangrene intestine. Microscopy will show liquefactive necrosis.

6. AMYLOIDOSIS

Amyloidosis is a misnomer it is not related to amylase or carbohydrate. Amyloidosis is accumulation of amyloid an abnormal fibrous protein. During protein synthesis these proteins not folded properly due to quantity or quantity defects. Enzymes degrade normally misfolded proteins but in amyloidosis it is not. This misfolded and undegraded proteins became unstable and form fibrils. They bind with different cell constituents, deposit in between the cells, and disturb the cells and organs. Clinically it does not cause any specific symptoms or inflammation. Only it cause organ enlargement and pressure effect on the adjacent cells. Commonly it involves kidney, spleen, liver, thyroid, heart blood vessels, etc. Amyloid will disturb their functions and cause organ failure.

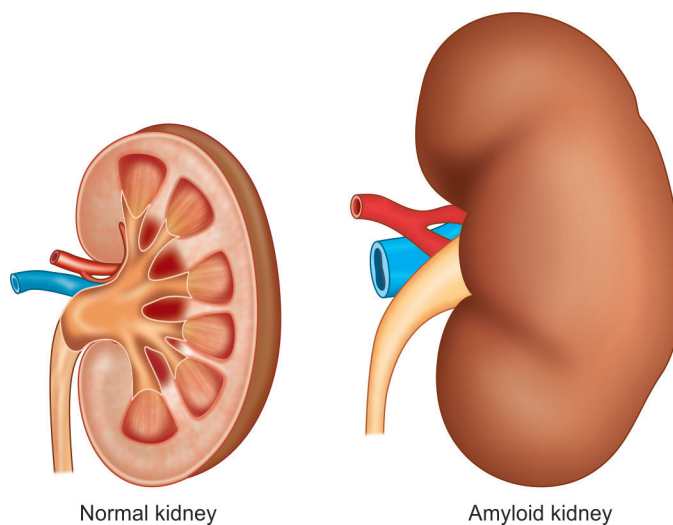


Fig. 6.1: Cut section of normal kidney and amyloid kidney. The specimen is identified as kidney by its bean shape and presence of ureter. Specimen is enlarged; its external surface is smooth. Cut section is pallor with waxy appearance. There is loss of normal corticomedullary junction appearance of kidney. Features are those of amyloid kidney

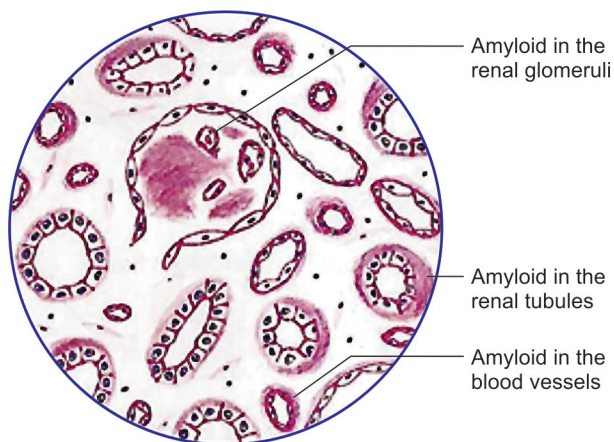


Fig. 6.2: Amyloidosis of kidney. Section of the kidney shows amyloid deposition in the glomerulus, tubular basement membrane and wall of the blood vessels. It is pink in H and E stain. It appears reddish pink in Congo red stain. It appears yellow green when observed under the polarizing microscope

Amyloid contains more than 20 different types of proteins. Chemically it can be AA, AL, A β , transthyretin, β 2-microglobulin types. Clinically, it is classified into localized, systemic, endocrine, age related.

In localized amyloidosis, it is deposited to one organ like skin, tongue, etc. There are plasma cell depositions around amyloid suggest that amyloid is deposited from these cells. In endocrinal type, it is deposited in the pancreas to cause diabetes or in thyroid (medullary Ca). Amyloid may be seen universally after age 80 mainly in the heart or brain.

Systemic amyloid is seen in the patient suffering from plasma cell proliferation, chronic inflammation like tuberculosis osteomyelitis, malignant neoplasms like renal cell carcinoma, Hodgkin's lymphoma. It is common in certain families due to genetic mutation.

Initially, it is difficult to recognize. In advanced case affected organs looks like glassy or wax like appearance. It turns brownish on applying iodine and H₂SO₄. Microscopically in H and E section, it takes eosin color. With congo red special stain, it appears red-pink color in light microscope and yellowish green under polarizing light.

Kidney is the most common organ affected. Gross appearance of the kidney is enlarged. (Normal kidney measuring 11 × 6 × 3.5 cm approximately) Features of Amyloid kidney are: Kidney is enlarged (may be smaller in the end stage); shape of the kidney is maintained, surface is smooth. Cut section shows loss of normal corticomedullary appearance. Kidney is lost its normal dark red color and it looks pallor with waxy in appearance.

7. NEOPLASTIC AND PRENEOPLASTIC LESIONS

NEOPLASTIC LESIONS

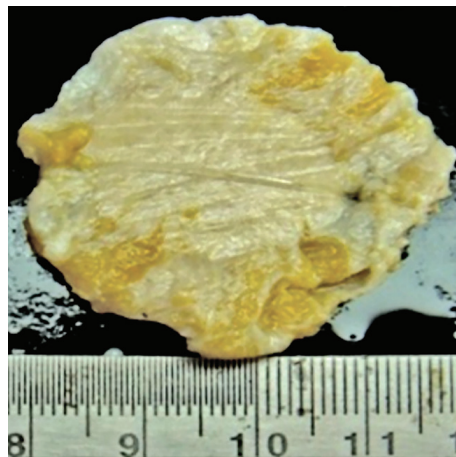
Neoplasia is abnormal growth due to genetic mutation. Growth persists even after removal of the cause. Depending on the behavior, it is classified into benign and malignant tumors. Differences between them are as follows.

Table. 7. 1: Differences between benign and malignant tumors

<i>Characters</i>	<i>Benign</i>	<i>Malignant</i>
Growth	Slow	Fast
Nature	Mostly innocent	Mostly dangerous
Spread	Remain local	Invade and metastasis to distant site
Capsule	Complete capsule	No capsule
Tumor	Well demarcated	Irregular
Cut section may be	Uniform	Necrosis and hemorrhage
Cells	Well differentiated	Poorly differentiated
Microscopy	Monomorphic	Pleomorphic
Nuclear color	Normochromic	Hyperchromic

EX OF BENIGN LESIONS

The specimen is identified as breast tissue by its appearance. It is a spherical/multi lobed mass of size 5 cm in diameter. There is a well-circumscribed nodule within the fibro-fatty tissue. Its borders are made out with well-developed capsule. Cut section is homogenous gray white and no secondary changes. It is rubbery in consistency. Features are those of fibroadenoma of

**Fig. 7.1a:** Fibroadenoma breast—gross

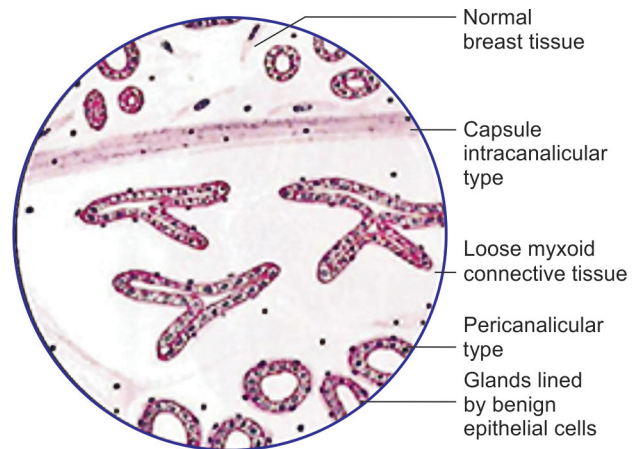


Fig. 7.1b: Fibroadenoma breast—microscopy

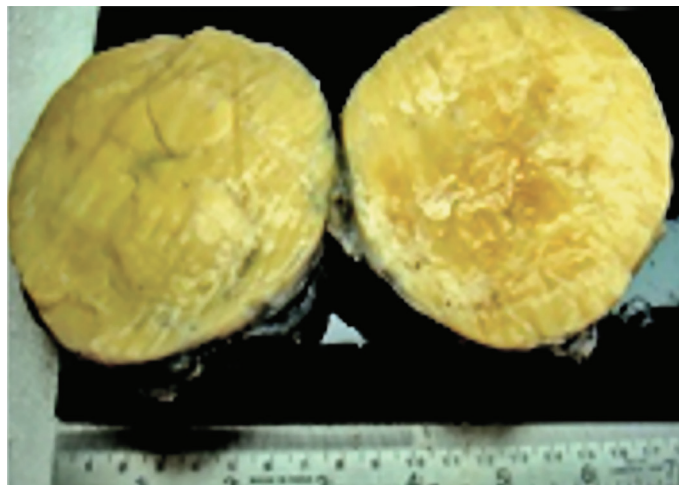


Fig. 7.2: Benign tumor (lipoma). Well-demarcated, capsulated and cut section is uniform

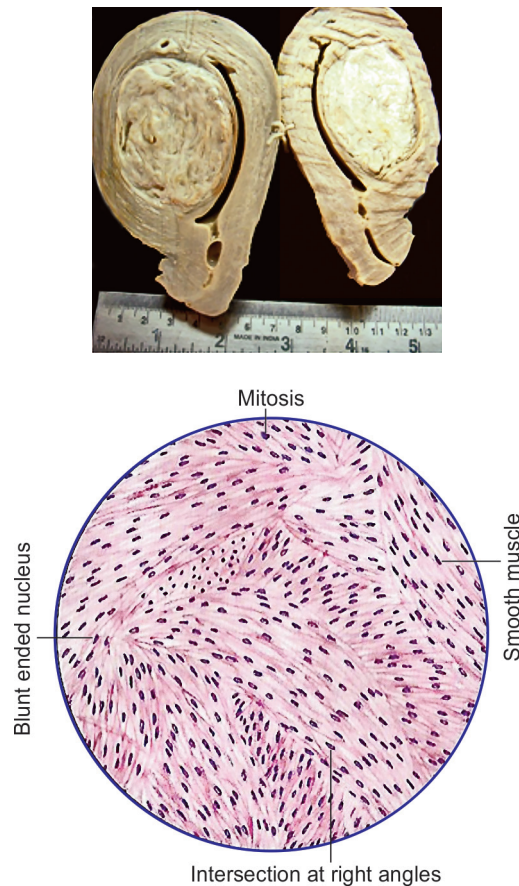


Fig. 7.3: Leiomyoma uterus—gross and microscopy. The specimen is identified as uterus and cervix by its appearance, cervical canal and endometrial cavity. Cut section shows one submucosal, globular mass of few cm in size, surrounded by pseudocapsule. Smooth surface, firm in consistency. The mass is protruding into endometrial cavity. Degeneration (hyaline) and necrosis is seen at the center due to lack of blood supply. Various secondary changes may be seen like hyalinization, cyst formation due to liquefaction, calcification, etc. Features are those of fibroadenoma uterus. Microscopy shows dense cellular tissue made up of spindle shaped smooth muscles. These cells are running in different directions and intersect at right angles. The cells are having elongated blunt ended nucleus with eosinophilic cytoplasm. Occasional mitosis may be seen but less than 3/10 high power field

breast. Microscopically the lactiferous ducts are compressed to slit like spaces by fibrous tissue in intracanalicular type. These ducts are appears normal round to oval in pericanalicular type. One or more layers of epithelial cells line these ducts. Abundant connective tissue is seen in between. Entire lesion is covered by intact capsule. Normal breast tissue is seen at the top.

EX OF MALIGNANT TUMORS

The specimen is identified as breast by its appearance with nipple and areola. Nipple is inverted and the skin shows

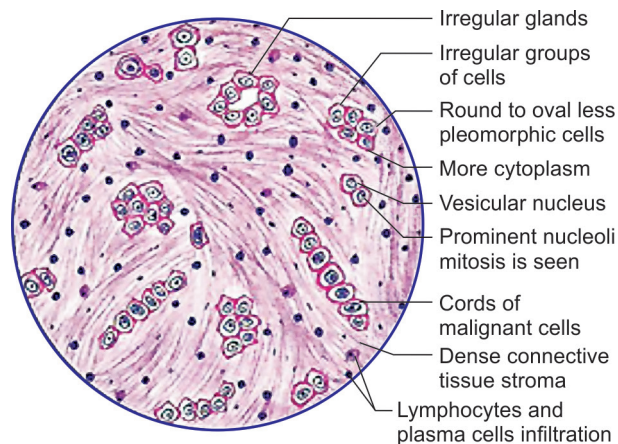


Fig. 7.4: Carcinoma of breast

multiple dimples. Cut section shows a growth measuring around 5 cm in diameter. The growth is dense white in color in yellowish fatty tissue, borders of the growth are not well made out and no capsule is seen. Lump is hard in consistency. Features are those of carcinoma breast. It needs confirmation by microscopy.

Microscopy shows dense connective tissue stroma infiltrated with malignant cells. These malignant cells form cords, clusters and glands. The cells show mild pleomorphism. They have round to oval vesicular nuclei and prominent nucleoli. Lymphocytic and plasma cell infiltration may be seen in the stroma. Features of infiltrative ductal Ca breast.

OTHER MALIGNANT TUMORS



Fig. 7.5 Malignant tumors (bronchogenic carcinoma, cervical cancer, ovarian malignancy, colon, Ca penis) showing growth is irregular, no capsule and cut section may show areas of necrosis, hemorrhage

Preneoplastic Lesions

Preneoplastic lesions are the lesions that are at an increased the risk of developing cancers. Examples are mucosal leukoplakia, actinic keratosis, lentigo maligna, Bowen disease. Bowenoid papulosis, polyps in the colon, dysplasia of cervix, oral erythroplakia, lichen planus, etc. they are all reversible conditions. If the cause is removed it will become normal, if not they may go for cancer.

Leukoplakia: It is a white plaque seen most commonly in the mouth of a person addicted to tobacco, alcohol. This plaque cannot be scrapped. Microscopy shows epithelial dysplasia.

Actinic keratosis: It is sandpaper like rough lesions on the sun exposed areas of skin. Microscopy shows hyperkeratosis and parakeratosis. It is mostly due to UV rays.

Lentigo maligna: It is a dark macule of irregular border seen on the sun-exposed areas of skin. Microscopically groups of atypical melanocytes are seen.

Bowen's disease: It is irregular scaly lesion on the skin. It is due to UV rays or papilloma virus. Microscopically epidermal cells are pleomorphic and hyperchromatic but not crossed the basement membrane.

8. SKIN TUMORS

Skin is the largest organ of our body exposed to radiation, etc. Neoplastic lesions in skin are a common problem. It may be benign and malignant. Benign lesions like actinic keratosis, Seborrheic keratosis are common problem. Malignant tumors are relatively less common but prognosis is not good so they need early diagnosis and treatment. Malignant tumors are caused by exposure to radiation, chemical carcinogens, poor immunity, etc.

BENIGN TUMOR—SQUAMOUS PAPILLOMA

Squamous papilloma is a benign proliferation of squamous epithelium seen on mucus membrane or skin. Many times clinically, it may mimic malignant tumor so histopathological diagnosis is necessary. It is mainly due to HPV infection.

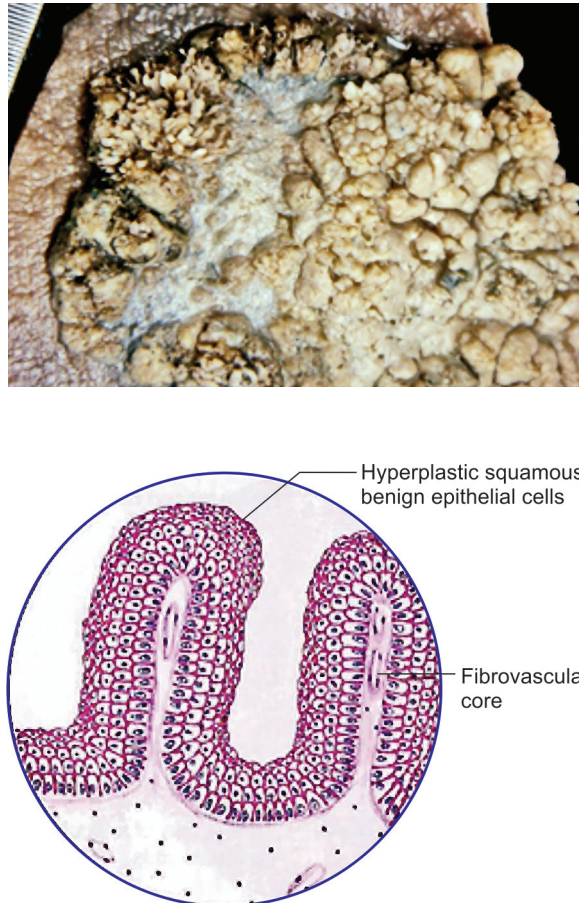


Fig. 8.1: Squamous papilloma—gross and microscopy

Specimen is identified as skin by its appearance. There are multiple finger like/sessile/pedunculated lesions. No ulceration or other secondary changes are seen. Features are those of papilloma skin.

Microscopy shows finger like projection with central fibrovascular core covered by hyperplastic squamous epithelium. These epithelial cells are benign. They are uniform in shape and size, having small central nucleus.

MALIGNANT TUMORS—SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma is the most common type of malignancy in the skin and other tissues. More common in India mainly oral and cervical cancers. The causes are exposure to radiation and chemicals in skin and oral cancers and papilloma virus in cervical cancers. Prognosis depends on the stage, grade of tumor. So early diagnosis, better prognosis.

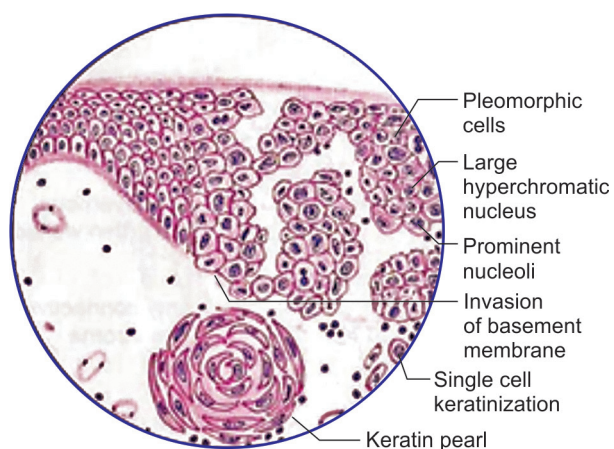


Fig. 8.2: Squamous cell carcinoma—gross and microscopy

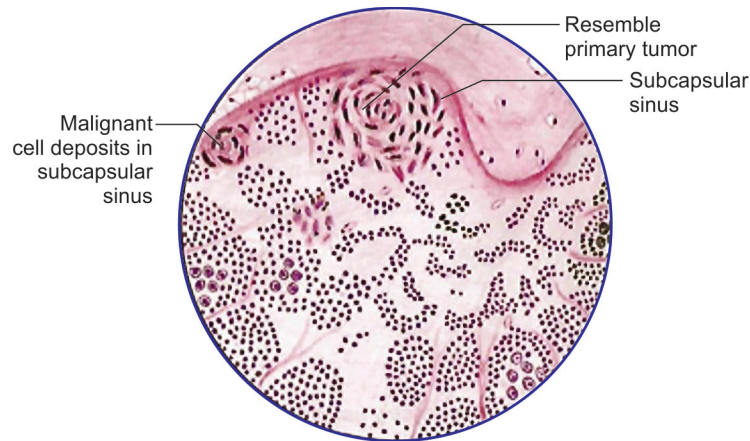


Fig. 8.3: Metastatic deposits in the lymph node. Microscopy of the lymph node showing deposition of metastatic tumor cells in the subcapsular sinus. This metastatic tumor resembles the primary malignant cells

Specimen is identified as skin by its appearance. There is an irregular cauliflower like growth measuring about 5 cm in diameter on the skin with irregular borders. Areas of ulceration are seen. Features are those of squamous cell carcinoma skin.

Microscopy shows sheets of malignant cells invading the basement membrane. These cells are hyperchromatic with pleomorphic nuclei. At places, these cells are arranged concentrically to form keratin pearls. Good number of mitosis is seen. Lymphocytes and plasma cells are infiltrated in the stroma.

BASAL CELL CARCINOMA

Basal cell carcinoma is another type of skin tumor. It is malignant tumor of basal epithelial cells, a slow growing, highly invasive tumor rarely metastasize. Risk factor is exposure to radiation and chemicals in fair colored people. Gross specimen is a pearl colored nodule or cystic lesion. Prominent blood vessels may be seen over the nodule.

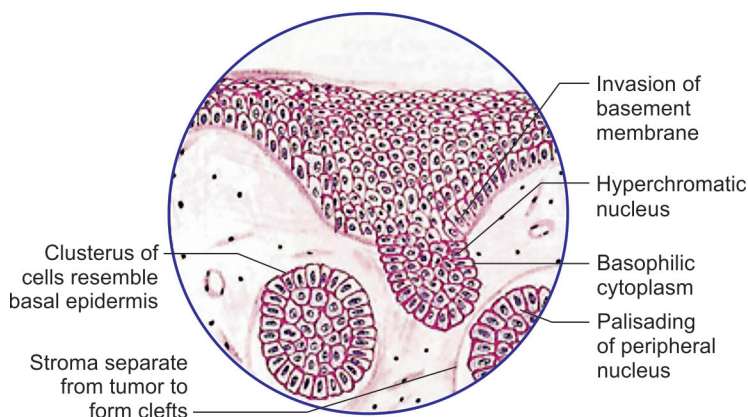


Fig. 8.4: Basal cell carcinoma. Microscopy shows clusters of closely packed round to oval cells invading the basement membrane. These cells resemble the basal cells but having hyperchromatic nuclei. The peripheral cells are columnar and arranged radially (peripheral palisading). Clefts may be seen around the clusters of cells. Mitosis is seen at places. Lymphocytic infiltration is seen at the periphery

MALIGNANT MELANOMA

Malignant melanoma is a malignant neoplasm of melanocytes. It may occur in the skin or other sites. It has very bad prognosis and cause death in young age. The risk factor is UV rays. Initially it grows horizontally, will not metastasize has good prognosis. In time it grow vertically down, will metastasize and prognosis is worst. Even though it is asymptomatic, it can be diagnosed easily by its external appearance.

Specimen is identified as skin and subcutaneous tissue by its appearance. There is an irregular growth on the skin. There is irregular black to brown pigmentation is seen on the growth. It is an asymmetric lesion, border and color are irregular. Its diameter is more than 6 mm. Features are those of malignant melanoma. Microscopy of the skin is showing epidermis and dermis. Clusters and isolated melanocytes invading basement membrane are seen. These melanocytes are pleomorphic with hyperchromatic nucleus. Junctional activity is seen at the dermoepidermal junction. Mitosis is seen at places. Pigment laden macrophages and chronic inflammatory cells are seen.

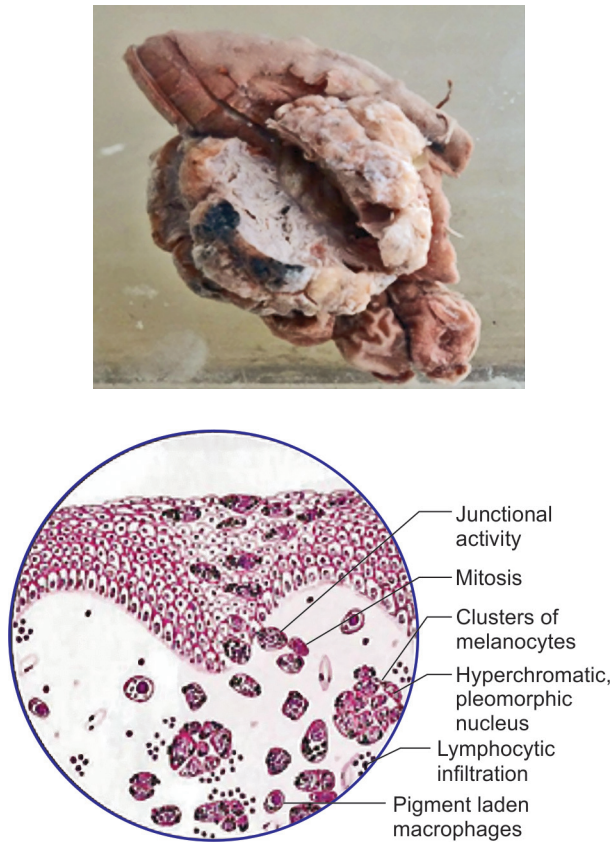


Fig. 8.5: Malignant melanoma—gross and microscopy

9. ORAL MALIGNANCY

INTRODUCTION

It is the third common cancer after cervical and breast cancer in India. It accounts for around 30% of all cancers and 90% of oral cancers are squamous cell carcinoma. It is common in both men and women. In India >50,000 deaths per year are due to oral cancer. All over the world, <150,000 deaths are due to oral cancer. It means for every one hour 6 Indians are dying of oral cancer, comparatively only one American dies of it.

DEFINITION

Oral cancer means malignant neoplasm of lip, tongue, floor of the mouth, inside cheek, gingiva and palate. The frequency at different site is as shown in the Fig. 9.1.

CAUSATIVE FACTORS

The causes are tobacco (chewing, smoking), alcohol, HPV, betel nut, poor oral hygiene. Indian habit of using powdered tobacco with slaked lime and betel nut is one of the main causes apart from smoking. Combined use to tobacco and alcohol will multiply the risk of oral cancer.

Both incidence and mortality is increasing day by day. Oral cancer is increase with age. As tobacco is easily available and lack of awareness may be the reason. Treatment and prognosis is not good even today. Smoking and alcohols are the preventable cause of oral cancer. So the prevention of oral cancer by public awareness programs and reduce the free availability is need of the day.

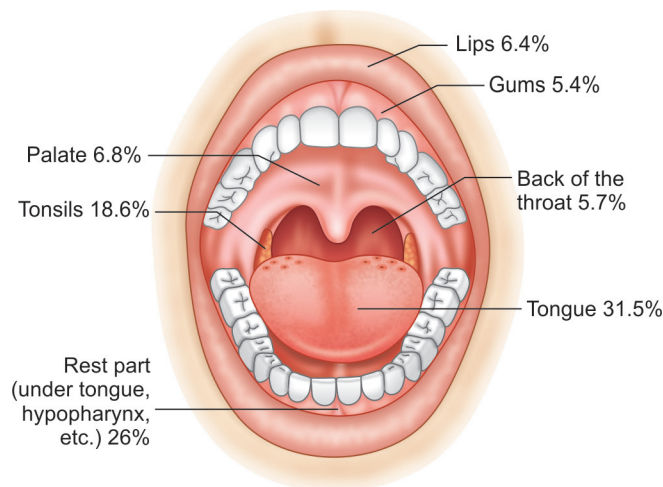


Fig. 9.1: Incidence and risk factors of oral cancers

Tobacco

Tobacco is used in the form of cigarette and other forms chewing tobacco, etc. It causes neoplastic as well as non-neoplastic diseases. Non-neoplastic diseases like COPD, atherosclerosis, TAO, etc. Tobacco contains >4000 chemicals out of which >70 are carcinogenic chemicals. For example, aldehyde, benzene, polycyclic aromatic hydrocarbons, etc. It contains both initiators and promoters of cancer. These carcinogens or their metabolite reacts with DNA. There will be change in the DNA (mutation). If there is mutation in the oncogenes, tumor suppressor gene, apoptotic gene, etc. there is a chance for repair. If the cell divides before repair, mutation may be fixed. Multiple mutations like this accumulate and finally lead to uncontrolled cell proliferation.

Alcohol

Alcohol is also contain multiple carcinogenic compounds like arsenic, aflatoxins, benzene, cadmium, ethanol, etc. It causes cancer of mouth, esophagus, colon, liver, breast, etc. It is metabolically converted to toxic material like acetaldehyde, which are mutagenic. Ethanol is also a direct toxic substance to cell. Associated risk factors smoking, poor nutrition, poor oral hygiene aggravates causes of cancer. Alcohol causes other adverse effect on health like alcoholic hepatitis, fatty liver, cirrhosis, pancreatitis, etc.

OTHER CAUSES

Like betel nut chewing, poor oral hygiene, nutritional deficiency and exposure to sunlight are also to be kept in mind and avoided. Genetic factor have minor role so regular checkup with doctors is necessary.

PREVENTION OF ORAL CANCER

Mostly oral cancer is a preventable by:

- Avoid tobacco in any form
- Avoid alcohol
- Avoid betel nut

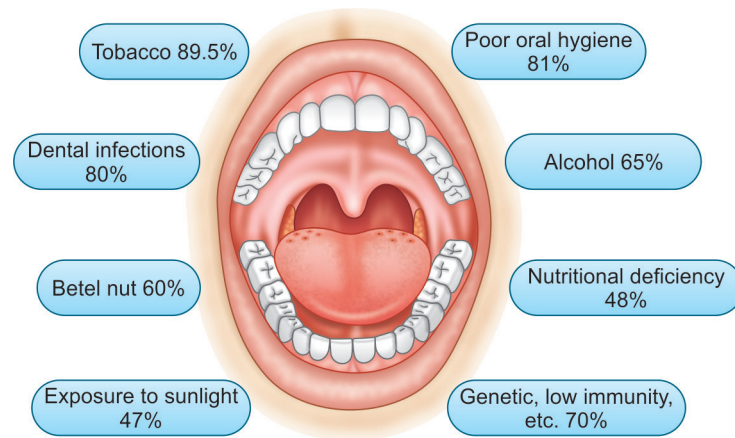


Fig. 9.2: Oral cancer patient gives the history of exposure to the above risk factors

- Maintain good oral hygiene
- Take fresh fruits, vegetable and balanced diet
- Regular medical checkup
- Microscopic examination if any white patch, etc.
- Educating the community.

10. OTHER IMPORTANT TUMORS

PLEOMORPHIC ADENOMA

Pleomorphic adenoma is a benign neoplasm seen in any salivary gland. It is more common in parotid gland. Tumor arises from the epithelial and myoepithelial cells. It is also called mixed tumor as mixture of both epithelial and mesenchymal tissue is seen. Clinically it present as a painless firm swelling of different size. Skin over the swelling is normal. As the lesion is painless, patient do not take any treatment. Surgical removal is the treatment of choice. However, recurrence is quite common. 5–10% of them go for malignancy.

Specimen of salivary gland is identified by its triangular shape and external appearance. External surface is lobulated and irregular bosselated mass. It is a circumscribed mass; Size varies from few millimeters to several centimeters. Cut section

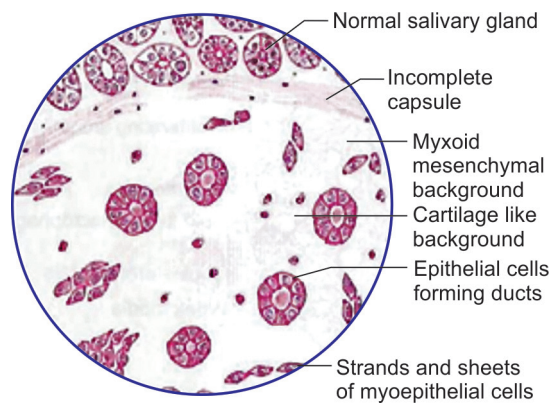
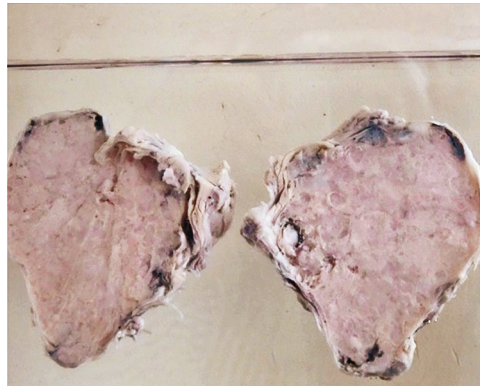


Fig. 10.1: Pleomorphic adenoma—gross and microscopy

is of varying in color. Irregular gray white in color and bluish areas are seen in between. Rarely cystic changes are seen. It is firm in consistency. Features are those of pleomorphic adenoma.

Microscopy shows normal salivary gland separated by pathologic lesion by incomplete capsule. Within capsule there are epithelial cells forming duct like structures within myxoid stroma and cartilaginous background. Strands of myoepithelial cells are seen at places.

NORMAL EXOCRINE GLANDS

Glands are organ that produces some important substance for our body. It may be endocrine or exocrine. Endocrine glands are not having ducts and secrets directly to blood. Exocrine glands are having ducts secrets their contents on surface by means of ducts. Examples of exocrine glands are sweat gland, sebaceous gland, salivary gland, lacrimal glands, etc. Depending on their secretions, exocrine glands are classified into serous, mucous and mixed glands.

Mucus glands are seen in the respiratory tract, sublingual salivary glands, etc. Its secretions are thick and mucus. Mostly helps in lubrication. Serous glands will secrets watery substance rich in enzymes and helps digestion. Parotid gland is made of mainly serous glands. Microscopic differences between them are shown in the diagram.

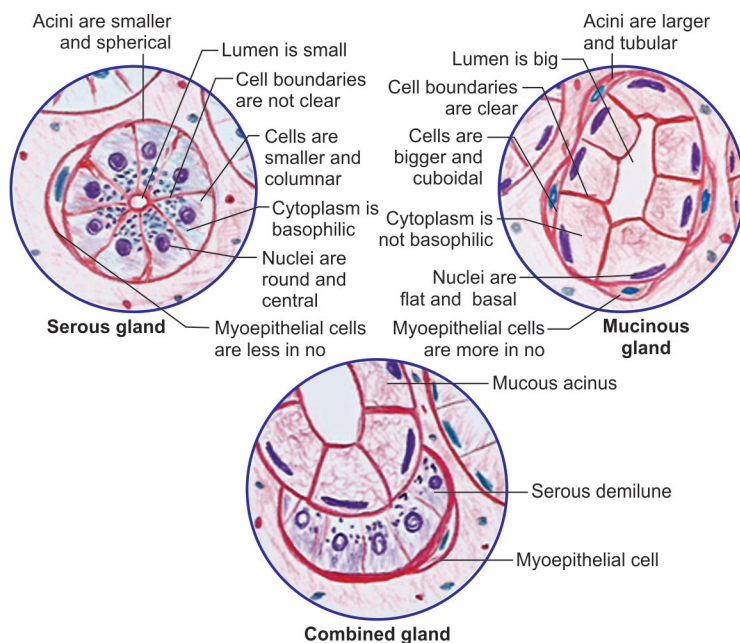


Fig 10.2: Differences between serous and mucous glands

AMELOBLASTOMA

Ameloblastoma is a neoplasm from the ameloblast (outermost part of teeth). Most of the time it is benign, rarely malignant. Clinically present with pain and swelling. Most common site is posterior part of mandible. Exact cause is not known. It may be due to genetic cause or repeated injury, viral infection, etc. Radiologically it has soap bubble appearance. There are four types; multicystic, unicystic, metastasizing and atypical. Around 85% are multicystic type. This multicystic has 6 subtypes (follicular, plexiform, acanthomatous, basal, granular and desmoplastic), in follicular type is made of island of cells with central mass of cells and peripheral cuboidal or columnar cells. Their nuclei are hyperchromatic and at the apex (reverse polarity). Cytoplasm has vacuoles. Central cells are polygonal and may have cystic degeneration. Plexiform type has anastomosing islands of cell with two layers of cells. Desmoplastic type has thick band of connective tissue and follicles are compressed. Acanthomatous have squamous cell metaplasia within the follicles. In granular type, the central

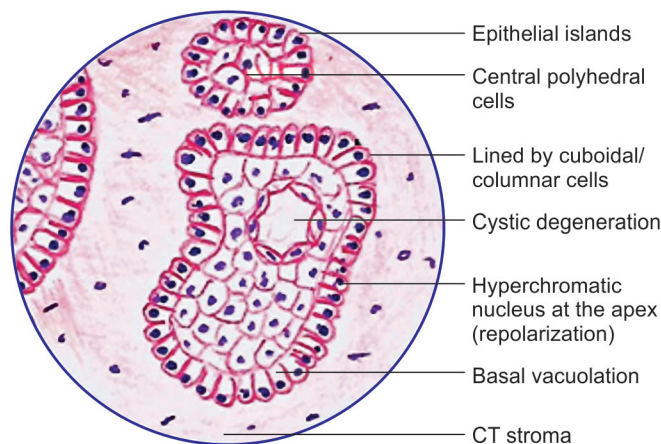


Fig. 10.3: Ameloblastoma follicular type. Microscopy shows islands of epithelial cells lined by cuboidal/columnar cells. These cells have hyperchromatic nucleus at the apex and vacuoles at the base. Cystic degeneration may be seen at the center and the cysts are lined by flat cells. In between follicles, there is connective tissue stroma

polyhedral cells contain cytoplasmic granules. In basal cell type, the cells look like basal cell of skin. Most of the time there will be combination of different types.

BONE TUMORS

Microscopy shows numerous osteoclastic type of giant cells, which contain >100 nuclei. Stromal cells are seen in between

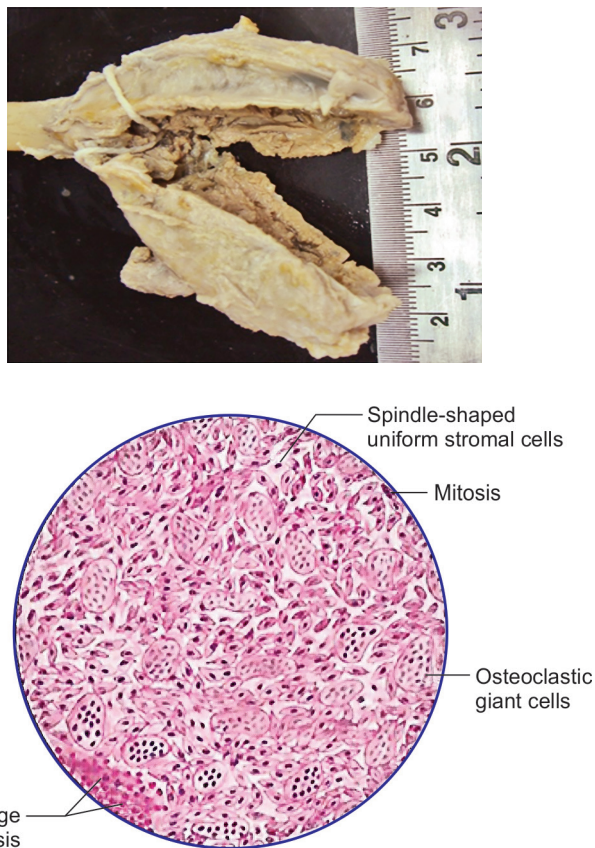


Fig. 10.4: Osteoclastoma—gross and microscopy. Specimen is identified as bone. There is a large, single well circumscribed lesion seen at the epiphyseal end of long bone. Cut section is brown in color. Massive destruction of bone is seen. Features are those of osteoclastoma. It needs microscopic confirmation

giant cells. These stromal cells are spindle shaped cells with large nucleus and scanty cytoplasm and indistinct boundaries. Mitosis may be seen at places. Areas of hemorrhage and necrosis are seen.

Bone tumors are less common. Many of the tumors occur at the younger age group. Risk factors also not know. Common tumors are osteoclastoma, osteosarcoma, Ewing's sarcoma, myeloma, etc.

Osteoclastoma (Giant Cell Tumor)

It is a common benign tumor of osteoblasts. It arises from the epiphysis of the long bone with complaints of pain or fracture. X-ray shows soap bubble appearance. Curettage and bone grafting treat it.

Osteogenic Sarcoma

It is the most common malignant tumor of the bone. It is a malignant tumor of the osteoblastic cells. Exact cause is not known but some genetic predisposition, mutation of the p53 (Li-Fraumeni syndrome), Paget disease, etc. is known risk factors. It occurs in two age groups one at young children of



Fig. 10.5: Specimen of osteosarcoma

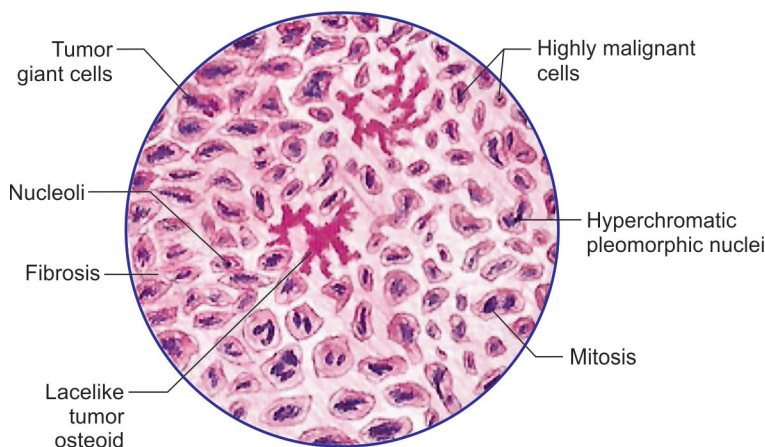


Fig. 10.6: Osteogenic sarcoma microscopy shows sheets of highly malignant cells. These cells are having large hyperchromatic pleomorphic nuclei with prominent nucleoli. Mitosis and tumor giant cells are seen. Lacelike tumor osteoid is seen in between

10–12 years complaining of pain and swelling. Another age group is old age due to Paget disease. It is seen mostly around joints or any other parts. Most common site is metaphysis of the long bone.

The specimen is identified as bone. It is the lower end of femur. Grossly there is a large growth arising from the metaphysis of long bones, its borders are irregular. Cut section is irregular gray white with areas of hemorrhage and necrosis and it may be hard to touch. Features are those of osteosarcoma. It needs microscopic confirmation.

11. POSTMORTEM EXAMINATION

DEFINITION

Autopsy or postmortem examination is the examination of the body after death.

Legal requirements

Medicolegal autopsy is done on request of police or magistrate with inquest. The report is handed over to the police only. It is done in daylight immediately.

Types of Autopsy

1. Medicolegal autopsy
2. Anatomical autopsy
3. Clinical autopsy
4. Imaging/digital autopsies done by MRI, CT scan.

Aims and Objectives of Autopsy

- Medicolegal autopsy
 - Identify the person
 - Time since death
 - Know the cause of death
- Clinical autopsies
 - Confirm clinical diagnosis
 - Get the organs for transplantation
- Anatomical autopsies
 - Display, the organs
 - Know the structure of the body.

Autopsy Room

The autopsy room must be well-ventilated with good light, water supply and drainage. The postmortem table is of size 7' × 3' with water drainage. One more dissection table is necessary for examination of viscera.

Instruments

The following instruments must be available. Scalpel, sharp dissecting knife, brain knife, plain and toothed forceps, scissors, retractors, probe, chisel, hammer, bone cutting forceps, councilman's blade saw, needle, sutures, gloves, mask, autopsy aprons and antiseptic lotions, etc. Formalin jars, swabs, slides, syringes are also necessary in some cases.

Procedure

Before starting autopsy note the history, clinical findings and investigations. Wear the clean glove, mask and apron.

External Examination

Examine the cloth for any stain or tears, etc. Note down height, weight, sex, nutritional status of the body. Examine all the

external orifices. Look for edema, lymphadenopathy, sclera for yellowish discoloration, nail deformity and external injury, etc.

Incision

The most common incision used is "I" shaped vertical incision extend from symphysis menti to symphysis pubis which is curving around the umbilicus. One more incision used is "Y" shaped. It starts from lateral end of clavicle to suprasternal notch and straight incision from suprasternal notch to symphysis pubis as usual.

Neck Dissection

Dissect the skin flaps until the lower border of mandible and other neck structures exposed. Now dissect the tongue from mandible by knife passing along the inner surface of mandible.

Chest Dissection

Dissect the skin flaps on the chest. Open the sternoclavicular joint and dissect it. Cut from second and the entire down ribs 1 cm lateral to costochondral junction on either side. Now cut the first rib with rib shears taking care not to injure the lung. Lift the sternum and cut all the soft tissue attach to it.

Peritoneal Opening

Make incision deep until reach peritoneum. Lift the peritoneum up with toothed forceps and make nick in it. Introduce 2 fingers through the nick and cut abdominal wall in between the fingers from xiphisternum to symphysis pubis. Note any gas escaping. Note fluid presence in the peritoneum. If fluid present, note its nature and volume.

Dissection of Diaphragm

Pull the abdominal and thoracic viscera and cut the diaphragm on both side along the costal margin including attachment to vertebral column.

Removal of Viscera

Hook the tongue with finger and pull it out. Cut the soft palate including tonsils from hard palate and pull the tongue down.

The trachea and esophagus are separated from vertebra. Hold the tongue and larynx and pull it down to separate thoracic and abdominal viscera. It may need gentle stroke from knife. Transfer the viscera on the dissection table.

Examination of Viscera

See the tongue and tonsil for any change in color, ulcer or any growth. Open the esophagus along the entire length and see for any ulcer growth, stricture and for varices, etc. cut the larynx and trachea and see for similar changes. Open the entire aortic wall and note for any atherosclerotic patch, ulcer or any aneurysm, etc. note the thyroid for any enlargement.

Dissection of Lung

Separate both the lungs from main mass and see its surface for emphysema, puckering and thickening, etc. palpate and see for any consolidation. Cut the lung by large brain knife to see any cavity, mass fibrosis, collapse, etc. examine the bronchial tree. Examine the blood vessels for any thrombi or emboli.

Dissection of Heart and Pericardium

First observe parietal peritoneum, open it, and observe fluid in the pericardial sac. Separate the heart and weigh it. See the patency of coronary vessels. By cutting it, heart is dissected in the direction of blood flow.

Open the right atrium and auricle. Examine for septal defects. See the size of right atrioventricular valve. Normally it admits three fingers in adults. Now cut into right ventricle through this orifice. Examine chordae tendineae, ventricular wall and interventricular septa. Now go to the pulmonary trunk by passing finger into it. Cut the ventricle to open. See the pulmonary valve and vessel wall.

Next open the left atrium by cutting the pulmonary veins. Examine the left atrium. Note the size of mitral valve by passing 2 fingers. Now open the left ventricle by cutting through the mitral valve along the ventricular left border. Observe mitral valve, ventricular thickness. Open the aorta by dissecting along the inferior cusps of mitral valve. Examine the valve and lumen.

Dissection of Abdominal Viscera

Open the abdomen by incising at its anterior wall in between 2 curvatures. Note any ulcer, growth stricture, etc. continue the same incision to open the duodenum. Examine opening of common biliary duct, see for any ulcer. Open the gallbladder to see wall of the gallbladder and any stones. See the external surface of pancreas. Cut the pancreas into slices to see any pathology. Separate the spleen by cutting at its hilum and examine its surface, weight and cut section. Separate the kidney from other viscera by cutting near its hilum. Note the external surface. Cut the kidney into two halves by cutting along convex border until hilum. Strip the capsule. Note down any pathological findings. Adrenals are sectioned sagittally to see any pathology.

Separate the liver and note its external surface for nodularity, etc. make thin slices sagittally and note any growth, abscess change in color, etc.

Brain Dissection

Make a coronal incision starting from one side of mastoid process to other side. Strip the skin flaps by blunt dissection up to external occipital protuberance on posterior side and orbit on anterior side.

See for any skull injury after dissection temporal muscle. Make a mark from occipital protuberance to supraorbital margin. Saw the outer table of skull. Cut the inner table of skull by hammer and chisel. Take care not to injure brain and meninges. Remove the skull cap.

Incise the sagittal sinus and see for any thrombus. Cut the dura mater 1 cm lateral to midline on both sides anteroposteriorly. Now make one more cut right-angle to first one. So dura will come as four flaps and the brain is exposed. Gently pull sagittal sinus and falx cut its attachment to crista galli. Observe the lateral surface of cerebrum. Gently pull the frontal lobes back with fingers of left hand till optic chiasm is exposed and cut the optic nerves. Now cut the attachment of tentorium to petrous part of temporal bone on both side. Now cut all the cranial nerve attachment. Cut the spinal cord and vertebral arteries. Cell out

the cerebellum and pull that entire brain out. Examine the base of skull for any fracture after removing the dura matter.

Brain examination first inspect entire external surface of brain and meninges. Gently cut the corpus callosum with brain knife. See arachnoid villi and ventricles. Now cut the brain into two halves by dividing cerebellum and brainstem. Cut one-half into thin sagittal sections and other into thin coronal slices. Examine and take section for histopathology from any suspected areas. Impression is taken in case of suspected malaria. Dissection of eyeball, spinal cord and nasal sinuses are done in suspected cases.

Dissection of Urogenital System

It is the examination of urinary bladder, urethra and genital tract. Separate the bladder from symphysis by blunt dissection. Rectum along with uterus with its adnexa in female and prostate in male are separated from lateral pelvic wall and pelvic floor. Open the rectum along its long-axis and examine. Open the urinary bladder and see for any stones growths, etc. cut the prostate anteroposteriorly into thin slices and examine. Examine the prostate by cutting along its long-axis. In female, open the uterus along its long-axis for any pathology. Examine the tube and ovary for any pathology. Any suspected lesion, take biopsy and send to histopathology reporting.

Conclusion

The cause of death based on medicolegal autopsy.

Report

After finishing the examination, write the report in duplicate including, name, identification, date, time and place of examination, external and internal findings, viscera collected and cause of death. If exact cause is not made out, it can be written, as cause is unknown. It must be signed by the registered medical practitioner with his qualification and handover to the one who requested. In case of using any tissue for treatment, teaching, display, etc. proper consent must be taken from the near relatives.

12. HISTOPATHOLOGICAL TECHNIQUES

The whole purpose of histological techniques is to enable the microscopic examination of tissue for the purpose of diagnosis. This is done by cutting tissue into thin sections followed by staining. The principle ways of doing this are embedding into paraffin wax.

Frozen section (in emergencies, for brain specimen and fat tissue, etc.) electron microscopy (expensive and not available in all centers).

RECEIVING OF SPECIMENS

The sources of specimens are operation theater or from the autopsy for medicolegal purpose. Before accepting specimens, the specimen label and laboratory request should be crosschecked for name, age, sex, OP/IP number. Nature of specimen, clinical findings and clinical diagnosis are necessary. It must be kept in fixative solution 10–20 times the volume of specimen. All the details are entered into laboratory register with date and time of receipt of specimen. Once specimen is accepted, a laboratory number is given to it.

GROSSING

Grossing or sampling of specimen is taking the bits from representative area. Pathologist must do grossing. Describe the entire gross specimen, their number, color, shape, size, weight and volume. Cut the specimen into slices and describe the cut section. Take small bits from the representative area. The bits are taken from the edge to include normal tissue. The grossing bits must be $<3 \times 3$ cm in length and breadth. Thickness must be <4 mm any number of bits can be taken. If the tissue is tiny, put a drop of eosin on it and wrap it in tissue paper.

Labeling

Identify the tissue by its laboratory number. Write the laboratory number on a small paper with lead pencil to identify the blocks. Keep the tissue bits and number chit in capsule. Close the capsule and put in a fixative.



Fig. 12.1: Capsule

QUESTIONS

1. What is the purpose of grossing and tissue processing?
2. What are the precautions to be taken in specimen receiving?
3. What is grossing? And how will you do it?

TISSUE FIXATION

Once the tissue is away from blood supply, it will undergo autolysis or decomposition by the enzymes from cell or from bacteria. To avoid this tissue must be fixed. Fixation is a process by which the cellular constituents are maintained in original physical and chemical state to prevent autolysis or decomposition. It will maintain the tissue or cell morphology even after away from the blood supply. By keeping in fixatives Soft tissue like brain and breast, etc. will become firm in consistency, so it can be grossed easily. Various fixatives are available as solutions or vapors.

Ideal Fixatives

- Should not interfere in staining
- It should keep the tissue morphology as original as possible
- It should be rapid in action
- It should be isotonic, cheaper and stable

- It should not be too toxic
- No fixatives have all these properties. Formaldehyde is the better fixative even though it is toxic.

Mechanism of Action

All fixatives make enzymes insoluble and inactivate by cross linking.

Classification of Fixatives

- | | |
|--------------------|---|
| • Aldehydes | • Formaldehyde, glutaraldehyde |
| • Alcohols | • Ethyl alcohol and methyl alcohol |
| • Oxidizing agents | • KMnO_4 |
| • Physical | • Heat, microwave (so used for EM)
(microwaves at 50°C fixes tissue rapidly) |
| • Others | • Mercury, picric acids, etc. |

ALDEHYDES

Even though aldehydes are toxic, they are better in their action. They reversibly cross link amino acids. Various preparations are available with better action. Aldehyde fixation can be reversed by bringing the specimen to water for 24 hours.

Formalin

Most commonly used fixative. It is formaldehyde gas in water. 40% of formaldehyde gas by weight dissolved in water to form 100% formalin. It is mixed with methanol to avoid tissue hardening.

It is used as 10% formal saline

- Formalin 100 ml
- Distilled water 900 ml
- Sodium chloride 8.5 g

For 4 mm thick tissue it takes 8 hours to fix at room temperature. If agitated, it will fix in 4 hours. At 45°C it fixes in 2 hours but with some loss of quality.

Advantages of Formalin

- Cheaper
- Rapid in action with good penetration
- Does not interfere in staining
- Tissue will not be brittle therefore no need to prior washing
- Table for long-time
- It can be used for frozen section and nervous tissue also.

Limitations of Formaldehyde

- It is an irritating fluid causing allergic reaction
- If blood is present, it turns tissue black by formalin pigment
- As it denatures proteins, it is not good for electron microscopy
- It is not good for photography due to loss of original color
- It is inert to neutral lipids (mercury is good) and phospholipids diffuse in it.

Glutaraldehyde

Glutaraldehyde is available as 25% solution. For fixation, it is used in 4% concentration. It is less irritating and less allergic. It does not cause much shrinkage of tissue. Once the tissue is fixed we can keep it for months before processing. It is best for electron microscopy and nerve biopsy. But it is expensive, and it takes 12 to 24 hour to fix 4 mm thick tissue at room temperature. It is not good for PAS staining. Paraformaldehyde also can be used rarely.

ALCOHOLS

Methyl and ethyl alcohols are used in PCR, *in situ* hybridization, immunofluorescence and in enzyme studies. Alcohol acts by protein precipitation. It is used in Carnoy's fluid.

ADVANTAGES

- It is good for small fragments and for glycogen studies.
- Alcohol also causes dehydration and is rapid in action.

Limitations of Alcohol

It shrinks, hardens the tissue and lyse RBC. It is expensive than formaldehyde.

METALLIC FIXATIVE

Mercury is the most commonly used metallic fixative in Zenker's, Heidenhain's fixatives. It stains nucleus, chromosomes and connective tissues in better way. It is preservative of choice for photography. But it causes shrinkage and hardening of the tissue. After treating with Zenker's, potassium dichromate deposits should be removed by washing tissue in running water.

Chromium is the other metallic fixative which acts by forming complex protein which is better for chromaffin tissue like adrenal medulla, mitochondria, Golgi, etc. It bleaches melanin pigment. Tissue fixed in it must be washed to avoid formation of black precipitate.

Picric acid is used in Bouin's fluid. It is good for glycogen but not good for kidney. It is used for small tissues as it stains the tissue yellow. If left for long-time, tissue will become hard.

Acetone is used as fixative in enzyme study. Osmium tetroxide is also a fixative rarely used. Lead can be used, but it takes 24 hour to fix.

Volume of Fixatives

Volume of the fixatives must be 10–20 times that of the specimen.

Duration of Fixation

Routinely tissue takes 24 hours to fix in formalin solution. If we keep more time, tissue will shrink and become hard. Washing in running tap water will reverse it. Brain takes 2–3 weeks to fix in formalin.

Factors Affecting the Fixation

Mucus and blood both reduces the penetration and increases the time for fixation. Specimen is washed in water to remove blood and mucus before putting in fixatives.

Temperature

Fixation is rapid at higher temperature. We can use up to 60°C for rapid fixation. Bring the formalin saline into boiling point and put the specimen for 1 minute. Immediately cool and process the specimen. This technique is less commonly used as we have

cryostat. Some laboratories use 40°C for 5 hours. Routinely we use room temperature for fixation to avoid any alteration in cell morphology. For electron microscopy and histochemistry at 0–4°C is preferred in glutaraldehyde.

Tissue Thickness

Penetration of any fixative depends on tissue thickness, time given and fixative used. The tissue should be thinner. Larger tissues are sliced and kept in the fixatives.

pH of Fixatives

Normal pH will be 6–8. Otherwise, it alters the structure. For special tissues like stomach, we use pH 5.5 and adrenal glands at 6.5 by adding acetic acid.

Fixation of Some Important Tissue

Kidney specimen—make the specimen into 3 parts.

- a. 1st in—buffer formalin for paraffin section
- b. 2nd in—glutaraldehyde for EM
- c. 3rd in—liquid nitrogen for immunofluorescence.

Muscle specimen: It should be in a stretched condition, for that tie the specimen to glass slide. Put 1st in buffered formalin for routine staining. 2nd specimen in glutaraldehyde for EM and the 3rd specimen for enzyme histochemistry in cryostat.

- Liver, lung is fixed in buffered formalin
- Testis is fixed in buffered formalin solution and Bouin's solution.
- Needle biopsy specimens—immediately keep in formalin and 1 hour is enough for fixation.
- Hard tissues—wash overnight in water and put it in phenol to become soft.

QUESTIONS

1. What is a fixative? How will you classify them?
2. What is an ideal fixative?
3. What are the advantages and disadvantages of formalin?
4. Where glutaraldehyde and alcohols are used as fixatives?
5. What are metallic fixatives and where they are used?
6. What are the factors influencing tissue fixations?

TISSUE PROCESSING

Our aim is to cut tissue into thin sections. For this it should be embedded in a solid media which can support the tissue from all the sides. Embedding medias are mostly water insoluble and fixatives are water-soluble. We should bring the tissue from water-soluble media to water insoluble embedding media. Processing consists of 3 steps, i.e. dehydration, clearing and impregnation.

In the first step water and fixative between the cells are replaced with fluid, which mixes with both water and clearing agents. Second step is replacing dehydration fluid with clearing agent (fluid mixes with dehydration fluid and embedding media). Third step is replacing clearing agent with embedding media. Paraffin wax is the common embedding media.

DEHYDRATION

It is removal of water and fixatives in and around the cells. It is replaced with dehydration fluid like alcohol. Isopropyl alcohol is commonly used because ethyl alcohol is costly and methyl alcohol is too toxic. Acetone is volatile and flammable.

To minimize tissue destruction we use ascending grades of alcohol starting with 50% for brain and 70% for other tissue. The timing is given below. The volume of dehydration fluid must be 10 times of that of tissue, and it should be changed twice in a week.

Note: Freedom from water can be checked by anhydrous CuSO_4 .

CLEARING

Clearing is replacement of dehydrating fluid with fluid miscible with both dehydrating fluid and embedding media, e.g. xylene, toluene, chloroform, benzene, etc. Because of its high refractive index it makes the tissue transparent. Its volume also should be 10 times that of tissue. There are many clearing agents. We select one with rapid in action, less damaging to tissue, should not be toxic, and less costly. Important clearing agents are xylene, toluene, chloroform, benzene, etc. Xylene is the most commonly used and excellent clearing agent which is cheaper

but highly flammable. As it makes the tissue hard and brittle it is not used for brain and lymph node. Do not keep the tissue in it >3 hour. Toluene also can be used. Its action is similar to xylene. Toluene is less dangerous and does not harden the tissue but expensive. Chloroform does not cause tissue brittleness. Good for brain and lymph node. But it is expensive and highly toxic. Benzene is highly toxic causing aplastic anemia, therefore it is not used. Cedar wood oil does not cause tissue shrinkage. But impregnation in wax will be delayed.

IMPREGNATION

Impregnation is replacement of clearing fluid in the tissue with embedding media. It fills the interstitium, cavities and spaces by solid media. It makes the tissue sufficiently firm to take thin sections. Impregnation also helps in holding and cutting of small pieces, e.g. paraffin, celloidin, gelatin, resin, etc.

Paraffin Wax

In 1869 Klebs used paraffin wax first time as impregnation media. Still it is the best most commonly used and the best media because it is cheaper, easily available, easy to handle, easy to cut, has low melting point. It is rapid in action, does not alter tissue structure, serial section can be taken. Block does not deteriorate in time. Its melting point can be changed by adding bee wax. 56°C melting point wax is mostly used. Wax is solidified within 5 minutes. Tissue will be kept in it for 1 to 2 hours each in 3 containers. By using automatic processor and agitation we can do it in 2 hours. Volume of wax in the tissue processor must be 25 times of tissue. Muscle and fibers become hard in this.

Other Embedding Medias

Resin is used in electron microscope and for thinner section. Agar is used with wax gelatin is used in frozen section, and small bits as endometrium.

Celloidin does not shrink the tissue. It is used in collapsible cavities like eyeball. But celloidin sections are thicker (10–15 microns), serial sections are not possible. It is slow process, takes 2–5 weeks.

QUESTIONS

1. What is dehydration? What are the chemicals used for this?
2. Why are we using ascending grades of alcohol for dehydration?
3. What is clearing? Which are the chemicals used for this?
4. Why xylene is most commonly used as a clearing agent?
5. What are the advantages and disadvantages of xylene?
6. What is impregnation? What are the common impregnation media?
7. What are the advantages of paraffin wax as impregnation media?



Fig.12.2: Automatic tissue processor

Automatic tissue processor: It has central rotator which carries basket and rotates automatically from one to next beaker containing chemicals. 2 metal beakers contain molten wax. Wax bath temperature must be 3–5°C above melting point. The basket is made to agitate within the solution to speed the process.

The advantages are the processing time will be less as tissue is agitated. More specimens are processed at a time. Quality of processing is better because the exact time will be maintained. We can save man power. But chemicals must be changed frequently. It must be maintained up to marked level. The instrument and its maintenance are expensive. Appropriate tissue time allotted is as follows.

Table. 12.1: Time needed for tissue processing in different chemicals and concentrations

10% Formalin	70% OH	95% OH	100% OH	100% OH	100% OH	100% OH	OH/ Xylene	Xylene	Xylene	Wax	Wax
1 hr	½ hr	½ hr	1 hr	1 hr	1 hr	1 hr	½ hr	1 hr	2 hr	2 ½ hr	4 hr

Agitation

Tissue is processed automatically with agitation. Exchange of fluid is good and rapid if we agitate vertically and horizontally. Agitation must be smooth without tissue breakage.

Processing Temperature

For smooth and speedy processing

If all the fluid temperature is 45°C with vacuum, the whole process can be done in 2 hours.

Embedding and blocking Leuckhart's L shaped brass metal is available in various sizes and adjustable for different shapes.



Fig. 12.3: Leuckhart's L block (brass metal)

Procedure

Adjust the Leuckhart's brass mold to desired size on a glass plate. Heat the paraffin wax 2°C above its melting point and fill the mold. Take the capsule from the processor. Open it. Note the laboratory number. Take the tissue in a blunt forceps and decide the plane of cutting. Place the tissue in a mold. Side of the section must be face down and orient carefully. Now keep the block in refrigerator. After few minutes, separate the blocks. Trim the excess wax leaving minimum 2 mm around tissue from all the sides. See that upper and lower surface of the tissue block must be parallel to get ribbon sections. Attach the number paper with hot spatula to the block.

Orientation of Tissue in Block Embedding

Once the section is cut, it must be well oriented for the diagnostic purpose. For that we must embed the tissue in block to cut in representative angle. Tubular structures are embedded to cut

it like cross section. Skin is embedded in right angle. Muscle is embedded to take transverse and longitudinal section.

Vacuum Embedding

We can speed up the procedure in lung, skin, etc. by using –ve pressure.

Celloidin Molding

It is rarely done. It is slow process, sections are thicker, serial sections are not possible. Its only advantage is tissue does not shrink.

Different Molds Available

Peel a way and Tissue-Tek are the other molds available. Peel a way is disposable plastic thin molds of varying in size. Once the plastic is stabilized the walls are peeled off. Tissue-Tek system has a base of stainless steel metal of different sizes placed a plastic mold and fill with wax.

DECALCIFICATION

It is difficult to take microtome section from the calcified tissue. So it is necessary to remove calcium ions from bone or calcified tissue and makes the tissue soft. It is done by keeping the calcified tissue in decalcifying agents important decal lotions used in our laboratory are:

Table 12.2: Table showing the different decalcification lotion

Formalin nitric acid	Formalin formic acid	Neutral EDTA
Nitric acid 10 ml	Formic acid (90%) 10 ml	EDTA salt
Formalin (40%) 10 ml	Formalin (40%) 5 ml	Na OH
Distilled water 80 ml	Distilled water 100 ml	Distilled water 100 ml

Test to completion of decalcification is by palpation and squeezing the specimen. Needling, probing are the other methods to see it. Chemical and radiological methods are rarely used like strong acid, weak acid or chelating agents.

QUESTIONS

1. What are the advantages of automatic tissue processor?
2. How will you embed the tissue?
3. What are the different embedding medias?
4. What are the different molds available?
5. What is decalcification? Give examples of decal lotion.

MICROTOME AND SECTION CUTTING

Microtome is an instrument used to cut thin slices of tissue. There are many types of microtomes. Sliding microtome, freezing microtome, rotary microtome, rocking microtome, Ultra-thin microtome, etc.



Fig. 12.4: Microtomes

Rotary Microtome

Invented by Minot in 1885. It is the most commonly used microtome. We can adjust the section thickness by adjustment. Both reuse knife and disposable knives can be used. It is good for serial section and bigger blocks. This is cheaper and easy to maintain. However, the knife is dangerously kept and only small part of it is used.

Rocking Microtome

It is used to cut paraffin section. It is simple and inexpensive. Tissue blocks moves and struck the fixed knife. Section from hard tissue is difficult in this microtome so many laboratory do not use this microtome.

Knife sharpening

It has two steps.

1. Honing—which removes the nicks. Knife is fitted to its back. Sharpen it by honing on glass or metallic plate. Honing stone is 4 × 18 inch stone mounted on wooden plate. Honing is by moving knife heel to toe in oblique direction. Hone should be lubricated with light oil.
2. Stropping—polishing the sharp edge. Strobe the knife on horse leather from toe to heel in oblique direction. The strops must be free from dust.



Fig. 12.5: Microtome knife with back

BLOCK PREPARATION

Warm the tissue holder. Fix the back side of paraffin block to it by delicate pressure. Put the paraffin block fixed to tissue holder in water for a while. Fit this tissue holder to microtome object holder. Adjust the object holder in a way the whole of the block surface is in cutting plane. By coarse adjustment advance the block until whole plane of tissue is cut. Take a few sections until tissue is seen in the section. Do it with a knife waiting for honing. Now remove the block and keep it on ice cube face down.

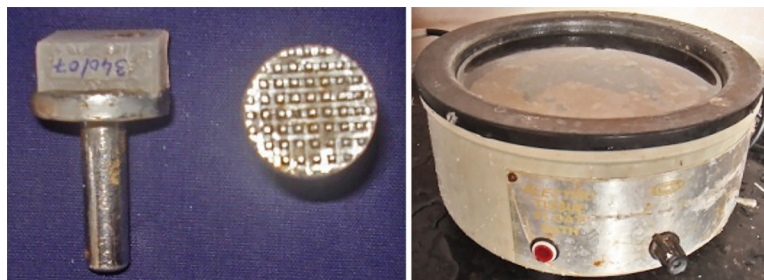


Fig. 12.6: Tissue holder and water bath

Take glass slides, number it with diamond pencil and arrange them in order. Coat the slide with adhesive mix, i.e. Mayer's egg albumin. (50 ml of egg white, 50 ml of glycerol, 100 mg of thymol) Make ready thermostatically controlled water bath and set it to 45°C.

SECTION CUTTING

Set the microtome for desired thickness 3–6 microns. Remove the block from the ice and clamp it firmly on microtome object holder. Move it manually till the surface of the wax touches knife edge. Make the block cool by touching it with ice cubes. By this consistency between wax and tissue will be uniform. Make smooth strokes for soft tissue or quick strokes for hard tissue and get ribbon of 2–3 inches from the section.

Gently raise the section with brush, cut 2–3 inch in length. Float the ribbon on the surface of water bath. The cut section will spread uniformly in hot water bath. Take the appropriate adhesive coated slide, immerse it vertically in the water bath, and bring it to the end of ribbon so that it adheres to the slide. Raise the slide as it touches the ribbon. Section adheres to the slide. This is called floating out. Keep the slide vertically to drain water for 2–5 minutes after that keep it in the hot air oven at 56–60°C for 2 hours. It can also be kept on hot plates at 50°C for 30 minutes.

Difficulties in section cutting section may not be proper due to various causes like improper fixation of tissues. It may be due to faulty processing, dehydration, etc. rarely it is related to knife and microtome.

QUESTIONS

1. What are the different microtomes available?
2. How will you sharp the knife?
3. How do you prepare blocks for cutting?
4. Explain the procedure of section cutting.

FROZEN SECTION

This technique is used to demonstrate fat and enzymes. It is also used for rapid diagnosis. Tissue is placed on a tissue holder,

which is fixed to handle at center of microtome. This handle is attached to CO₂ cylinder. As the CO₂ gas passed on the tissue, it will be frozen. Tissue will be cut about 10 micron thickness by a knife. This knife is fixed and moving horizontally. On each knife movement tissue holder will move upwards. We can take any number of sections. This technique is replaced by cryostat because knife and tissue are not at the same temperature.

Advantages

- Diagnosis is possible within minutes
- We can demonstrate fat and enzymes
- Instrument and maintenance is economical.

Limitations

- Sections are thicker
- Serial sections are not possible
- Microscopy is not as clear as paraffin embedded tissue
- Knife and tissue are not at same temperature, so the section will not be good.

Cryostat

It is a refrigerated cabinet where microtome is operated. The temperature of 0 to 30°C can be adjusted. Microtome is operated with freezing temperature. We can diagnose within minutes and the quality of section is better than frozen section.

Staining (dyeing) if unstained slide is observed under microscope we can make out only nucleus and cell boundary. Cell constituents can be seen clearly if it is stained. Direct staining is staining with single dye like toluidine blue. If we use 2 contrast stains we can see the cell contents still better. Hematoxylin and eosin is the most commonly used stain. It gives good contrast and helps in diagnosis of most of the slide. It is cost effective. Hematoxylin stains nucleus and eosin stains cytoplasm.

Hematoxylin A natural dye extracted from heart wood of hematoxylin campechianum tree from Mexico. The dye extracted from this tree is used in dye industry and histopathology.



Fig. 12.7: Cryostat

Stains may be acidic, basic or neutral. Different cell components have different affinities to dye. Stain either adsorb or chemically react with cell content. Special stains are chemical react with cell contents, e.g. hemosiderin with Perl's stain, polysaccharide with PAS.

Metachromasia is the color or hue that is different from that of the stain itself, e.g. toluidine blue staining with mast cells. Orthochromatic is shades of their own color.

Ripening or Oxidation

It is conversion inactive natural extract like hematoxylin to active principle (hematin) by oxidation. It is essential that correct amount of oxidation occurs.

Mordants

Mordants are the substances which bind the dye to tissue. For example, salts of ammonia, iron, chromium, copper, etc. Alum of double salt like potash alum, iron alum, etc. Most commonly used mordant is alum.

Accentuators

They increase color intensity and selectivity of dyes and various mechanisms like reducing surface tension, e.g. phenol, KOH.

Differentiation

It is a process of selective removal of excess dye or selective production of certain color with specific pH value. In acidic differentiation we use HCL, acetic acid, etc. Oxidizing and mordant differentiation are rarely used.

Bluing

After staining with hematoxylin the slide is kept in running tap water till it becomes deep blue in color. This is called bluing. It takes 5 to 10 minutes for bluing. Bluing makes the nuclear morphology more clear.

Harris' Hematoxylin

Take 1000 ml of distilled water and add 100 g of ammonium alum by shaking. Heat and bring it to 60°C. Add 5 g of hematoxylin in 50 ml of ethyl alcohol and boil it. Remove from flame and add 2.5 g of Hg oxide. Mix them properly. Heat it



Fig. 12.8: Manual staining arranged

for 3 minutes. Sample it regularly. When properly ripened deep purple solution is ready, cool it. Add 30 ml of glacial acetic acid, 50 ml of ethyl alcohol and filter it. It will be stable for 6 months.

H AND E STAINING PROCEDURE

- Take the slide from incubator and put it in xylene bath for 3 minutes
- Transfer it to second xylene bath for 3 more minutes
- Immerse in absolute alcohol for 2 minutes
- Immerse in 95% alcohol for 2 minutes
- Immerse in running tap water for 1 minute
- Put it 4–8 minutes in Harris's hematoxylin
- Differentiate in 1% acid alcohol for 3–10 seconds
- Rinse the slide in tap water
- Keep it for bluing in running tap water (till it become blue in 5–10 minute)
- Put it in eosin yellow for 1 minute
- Dehydrate by passing in absolute alcohol 15 sec in 4 baths
- Pass in 2–3 bath of xylene
- Mount the slide in DPX
- Result nucleus is blue in color and cytoplasm is pink in color. Pap stain is explained in cytology section.

Automated strainers are available. It is easy and it is giving more accurate result. More slides can be stained at a time. We can better utilize free time. But initial cost and maintenance may be costly. So it is preferred in institutions.



Fig. 12.9: Automated strainer

QUESTIONS

1. Why should we stain a slide?
2. What is oxidation? What are mordants?
3. What is differentiation? How to do it?
4. Explain the procedure of H and E staining
5. Explain the procedure of Papanicolaou staining

Special Stains

To see some of the intracellular and extracellular components, it is necessary to use special stains. These dyes have affinity for a particular compound. There are hundreds of special stains available. Nowadays, use of special stains has been decreased with the advent of immunohistochemistry. Still special stains are in use as they are more economical and widely available. Some of the common special stains we use are as follows.

Table. 12.3: Table showing different special stains for different tissue and color appearance

Demonstration of Amyloid	Stain used Congo red Polarized light Methyl violet Crystal violet Iodine PAS	Appearance Pink to red Apple green birefringence Pink red Purple red Brown Magenta pink
Lipid	Oil red O Sudan III	Orange red Orange red
Collagen	von Gieson's stain	Red
Elastic fiber	Verhoeff's stain	Black
Iron	Prussian blue	Blue
Reticulin fibers	Reticulin	Brown
Mucin and glycogen	PAS	Magenta
Calcium	von Kossa stain	Brown/black
Melanin	Masson Fontana	Black