Lecture (1)

Constituents of cells

Histopathology is a branch of pathology which deals with the study of disease in a tissue section.

HIERARCHY OF THE BODY

- **Cell. As a functional unit**
- **Tissue, such as epithelia**
- **Organ, such as kidney**
- **Organ system such as respiratory system**
- **Individual Organism such as human being.**

Main Cell Constituents (Organelles)

1. **Cell membrane-separates cell from external environment; a lipid bilayer**
2. **Cytoplasm-the semifluid contents of the cell; aqueous**
3. **Nucleus-location of the DNA**
4. **Mitochondria-the cell's "power plants"**
5. **Endoplasmic reticulum-site of protein synthesis**

Main Functions of Organelles

Plasma membrane:

1- Because of permeability it controls transport of material across it.
2- Permit diffusion of water and fat soluble components.
3- Fat insoluble components pass through the membrane by forming reversible compounds with membrane proteins.

Endoplasmic reticulum:

1- Due to ribosome, it is involved in protein synthesis, also in glycogen and fat metabolism.
2- Gives mechanical support to cytoplasm.
3- Participate in exchange of materials by active and passive transport.

Golgi complex:

1- Condensation of lipids, carbohydrates hormones takes place in Golgi bodies.
2- Participates in the formation of lysosomes.

Mitochondria:

1- Mainly responsible for transformation of chemical energy into biological energy in the form of ATP compounds.
2- All enzymes involved in Kreb's cycle are present in mitochondria.
3- It is also responsible for transmission of hereditary characters..

Nucleus:

1- Controls all activities of the cell.
2- Biogenesis of ribosome takes place in nucleolus only.
3- Nucleolus take part in cell division.
4- Chromosomes , they play very important role in heredity, mutation and variation

The chemical constituents of cells:

The chemical constituents of cells include

***1- Carbohydrates*:** It is a group of organic compounds containing carbon, hydrogen and water. The ratio of H:O atoms is usually 2:1 as in water. The basic unit of carbohydrates is the single sugar called saccharide. The general formula is $C_x(H_2O)_y$.

***2- Lipid*:** They are organic substances containing C, H and O (O is in a smaller proportion).
There are 4 types of lipids: **fats and oils, phospholipids, waxes and steroids**. They are insoluble in water but are readily soluble in organic solvent e.g. ether, alcohol, acetone.

Functions of lipids

- **As energy source**
- **Storage (triglycerides)**
- **Water proof**
- **Insulation**
- **Protection**
- **Major components of cell membrane (phospholipid)**

3- **Proteins:** The proteins are the cellular macromolecules most abundant constituting 60% of the dry weight of cells. They consist of one or more polypeptide chains and has a molecular weight greater than 10,000 . They have a *unique and diverse structure* . The polypeptide chains fold more or less heavily on themselves inducing a large number of different three-dimensional structures.

Functions

- **Structural components**

e.g. collagen of connective tissues, in cell membrane and cytoplasm.

- **Homeostatic**: soluble proteins act as buffers, stabilizing the pH wherever they occur in the body.
- **Hormonal**: insuline, glucagons (regulation of glucose metabolism)
- **Enzymatic**: most enzymes are proteins e.g. digestive enzymes of gut
- **Transport**: cell membrane protein and protein of membranes of cell organelles (involved in transport of metabolites and ions across membranes), haemoglobin, myoglobin.
- **Protection**: e.g. antibodies

➢ **Movement**: acting, myosin (muscle contraction mechanism)

4- **Nucleotides and nucleic acids:** Two types of nucleic acid are found in living cells: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Most of the DNA is in the nucleus, but while there is some RNA in the nucleus most is in cytoplasm, particular in the ribosomes.

Nucleic acids are long, thread-like macromolecules build up of nucleotides. Nucleotides are arranged to form extremely long molecules: polynucleotides

5- **Water:**

- Water due to its chemical and physical properties takes a very important role in the life of organisms.
- Water is the reagent participating in many metabolic activities of protoplasm.
- Water is the principal solvent for many substances
- The high dielectric constant of water allows the dissociation of substances dissolved in it.

6- **Inorganic ions**:

Important **cations** are: Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}.
Important **anions** are: Cl^-, HCO^{3-}, NO^{3-}, H_2PO^{4-}, SO_4^{2-}, I^-.

The functions of ions:

- As constituents of various chemicals
- As constituents of structures
- As constituents of enzymes
- As metabolic activators
- As constituents of certain pigments
- As determinants of the cation-anion balance in cells

Lecture (2)

The field of pathology is devoted to understanding the causes of disease and the changes in cells, tissues, and organs that are associated with disease and give rise to the presenting signs and symptoms in patients.

There are two important terms that students will encounter throughout their study of pathology and medicine:

• ***Etiology*** refers to the underlying causes and modifying factors that are responsible for the initiation and progression of disease.

• ***Pathogenesis*** refers to the mechanisms of development and progression of disease, which account for the cellular and molecular changes that give rise to the specific functional and structural abnormalities that characterize any particular disease.

Thus, etiology refers to why a disease arises and pathogenesis describes how a disease develops (Fig. 2.1).

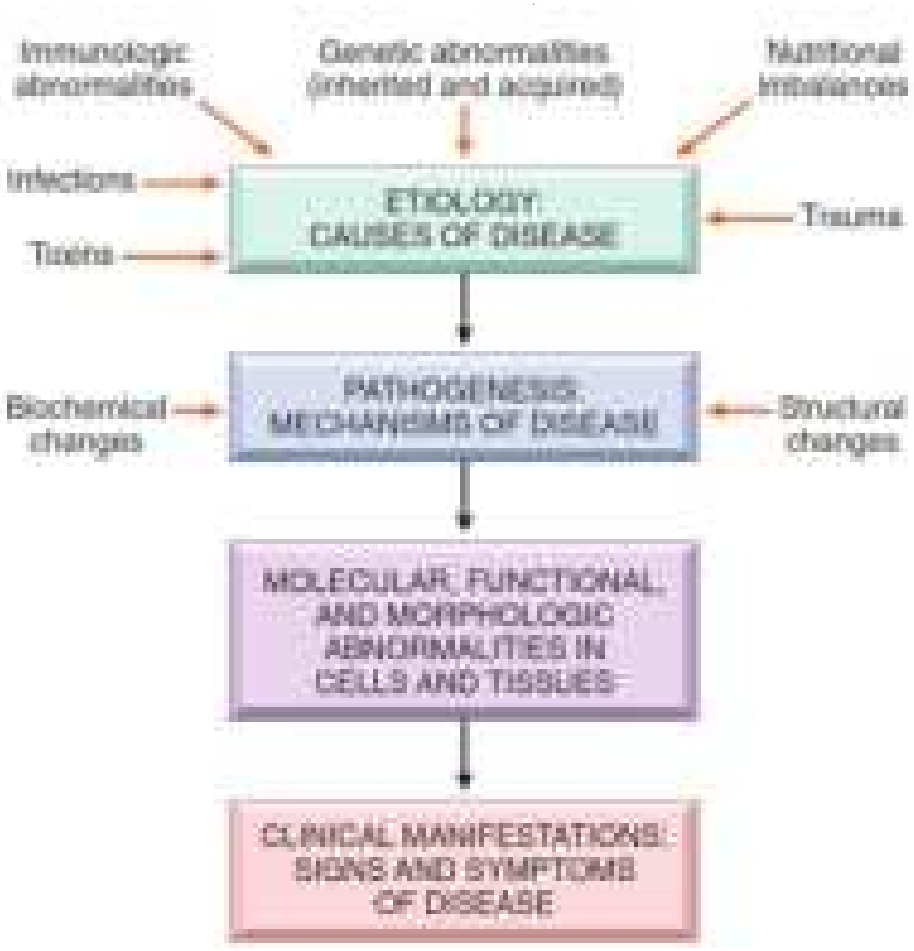


Fig. 2.1 Steps in the evolution of disease.

Cellular Responses to Stress and Noxious Stimuli

Cells actively interact with their environment, constantly adjusting their structure and function to accommodate changing demands and extracellular stresses. The intracellular milieu of cells is normally tightly regulated such that it remains fairly constant, a state referred to as homeostasis.

As cells encounter physiologic stresses (such as increased workload in the heart) or potentially injurious conditions (such as nutrient deprivation), they can undergo *adaptation*, achieving a new steady state and preserving viability and function. If the adaptive capability is exceeded or if the external stress is inherently harmful or excessive, *cell injury* develops (Fig. 2.2).

Within certain limits, injury is *reversible*, and cells return to their stable baseline; however, if the stress is severe, persistent, or rapid in onset, it results in *irreversible injury* and death of the affected cells.

Cell death is one of the most crucial events in the evolution of disease in any tissue or organ. It results from diverse causes, including ischemia (lack of blood flow), infections, toxins, and immune reactions. Cell death also is a normal and essential process in embryogenesis, the development of organs, and the maintenance of tissue homeostasis.

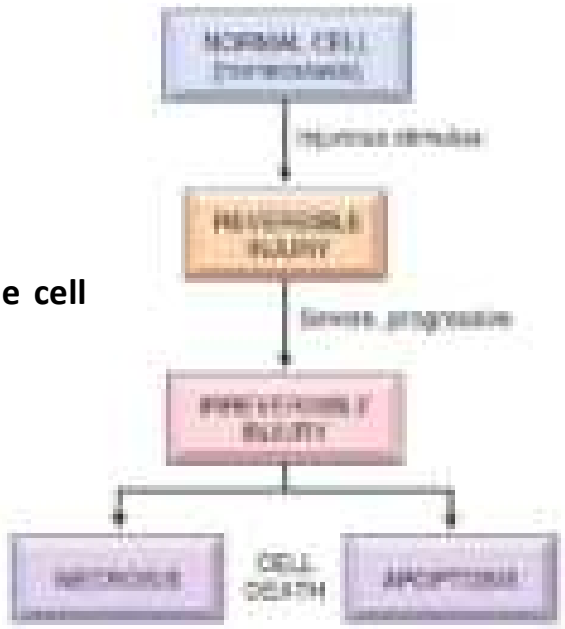


Fig.2.2. Sequences of reversible cell injury and cell death

CAUSES OF CELL INJURY

Most injurious stimuli can be grouped into the following categories.

1) **Hypoxia and ischemia**. Hypoxia, which refers to oxygen deficiency, and ischemia, which means reduced blood supply, are among the most common causes of cell injury.
2) **Toxins**. Potentially toxic agents are encountered daily in the environment; these include air pollutants, insecticides, CO, asbestos, cigarette smoke, ethanol, and drugs.
3) **Infectious agents**. All types of disease-causing pathogens, including viruses, bacteria, fungi, and protozoans, injure cells.
4) **Immunologic reactions**. Although the immune system defends the body against pathogenic microbes, immune reactions also can result in cell and tissue injury.
5) **Genetic abnormalities**. Genetic aberrations can result in pathologic changes as conspicuous as the congenital malformations associated with Down syndrome or as subtle as the single amino acid substitution in hemoglobin giving rise to sickle cell anemia**.**
6) **Nutritional imbalances**. Protein–calorie insufficiency among impoverished populations remains a major cause of cell injury, and specific vitamin deficiencies are not uncommon even in developed countries with high standards of living (Chapter 8). Ironically, excessive dietary intake may result in obesity and also is an important underlying factor in many diseases, such as type 2 diabetes mellitus and atherosclerosis.
7) **Physical agents**. Trauma, extremes of temperature, radiation, electric shock, and sudden changes in atmospheric pressure all have wide-ranging effects on cells
8) **Aging**. Cellular senescence results in a diminished ability of cells to respond to stress and, eventually, the death of cells and of the organism.

Lecture (3)

Cellular responses to injury

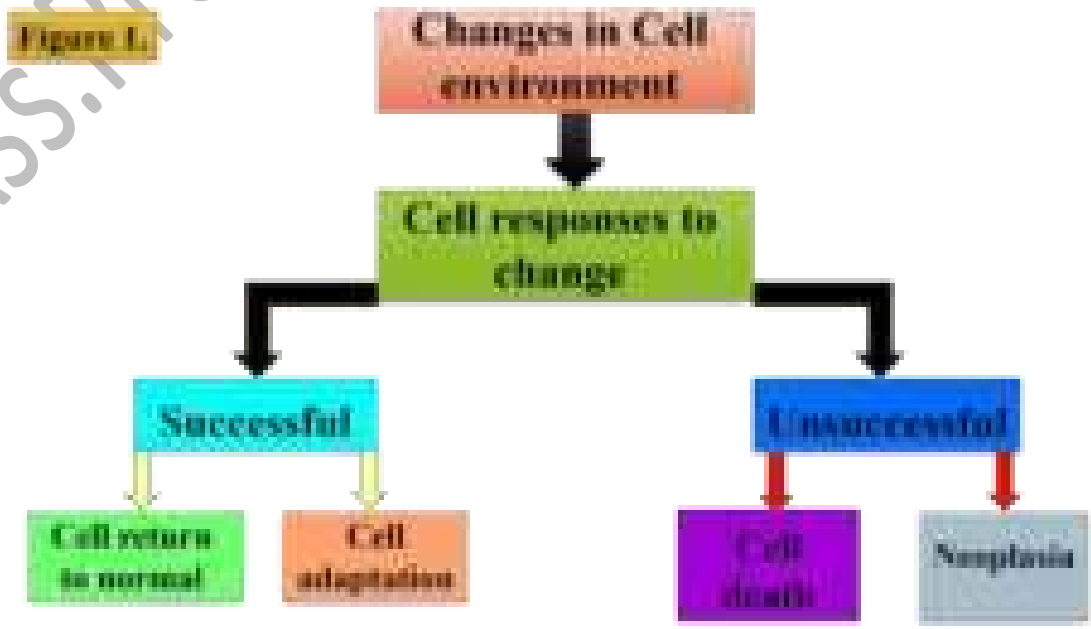
Changing environment demands a considerable degree of cellular adaptation. Adaptations are accompanied by structural changes which are visible microscopically. Many structural changes fall within the normal pattern of growth of tissue. For example the thyroid gland enlarges in pregnancy owing to the action of increased thyroid-stimulating hormones on thyroid epithelial cells. This is an example of physiological cellular adaptation to a stimulus within the normal range

In contrast, certain environmental changing lie outside the normal physiological range and result in cell damage and/or failure of the cells to function adequately: this stimuli are termed *pathological*

If a cell makes a successful adaptation to an environmental change, then it will either return to normal or it may make an adaptive change

If a cell is unable to respond successfully to an environmental change, then the cell may die. There is a further consequence of unsuccessful adaptation to an environmental change, the development of neoplasia

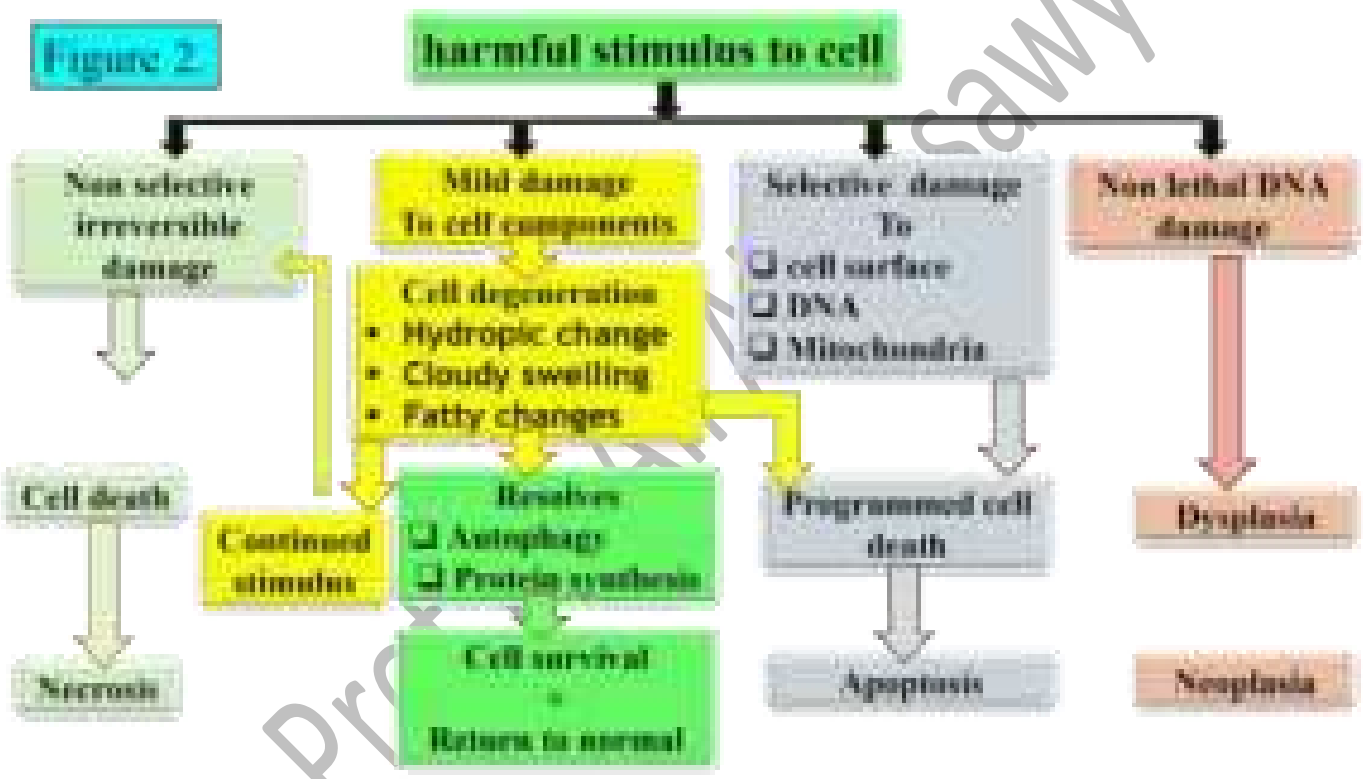
Cells respond to changes in their environment as summarized in Figure 1.



The fate of a cell which is exposed to a harmful stimulus depends

- **In part on the magnitude of the injury, and**
- **In part on how vulnerable the cell is to the injury.**

The common routes and endpoints following cell injury are summarized in Figure 2.



Reversible Injury

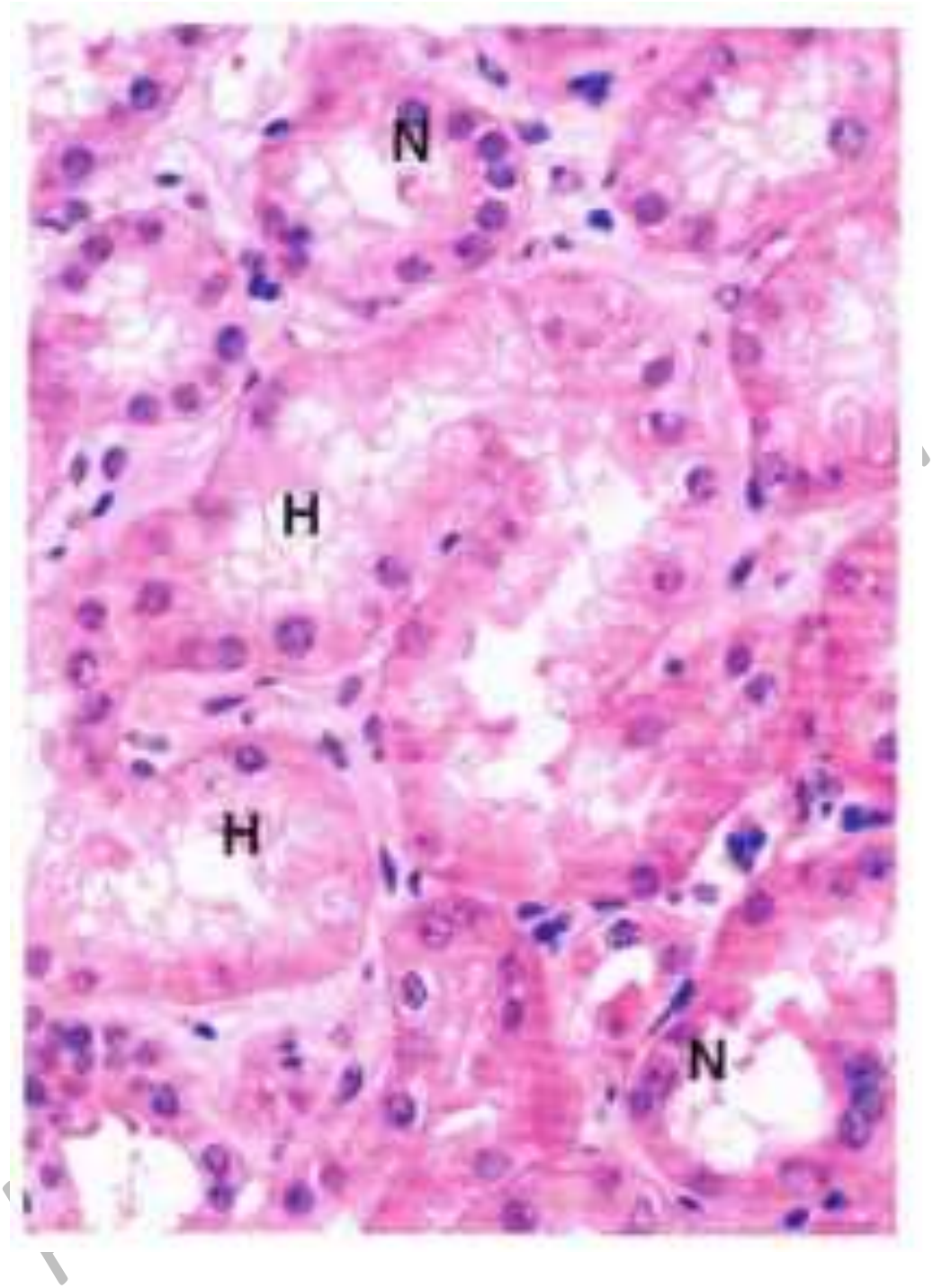
The two main morphologic correlates of reversible cell injury are ***cellular swelling*** and ***fatty change***.

Cellular swelling is the result of failure of energy-dependent ion pumps in the plasma membrane, leading to an inability to maintain ionic and fluid homeostasis. Fatty change occurs in hypoxic injury and in various forms of toxic or metabolic injury and is manifested by the appearance of small or large lipid vacuoles in the cytoplasm.

Cellular swelling (cloudy swelling)

The first manifestation of almost all forms of injury to cells, is a reversible alteration that may be difficult to appreciate with the light microscope, but it may be more apparent at the level of the whole organ.

When it affects many cells in an organ, it causes some pallor (as a result of compression of capillaries), increased turgor, and increase in weight of the organ. Microscopic examination may reveal small, clear vacuoles within the cytoplasm; these represent distended and pinched-off segments of the endoplasmic reticulum (ER). This pattern of nonlethal injury is sometimes called **hydropic change** or **vacuolar degeneration.**



Micrograph (1) shows a section of kidney deprived of blood flow owing to severe hypotension. Undamaged tubules are seen lined by normal staining epithelial cells N. Some of the tubules are damaged, the cells being pale and vacuolated and exhibiting hydropic degeneration H. Such damage to the renal tubules may lead to acute renal failure. Cloudy swelling and hydropic change reflect failure of membrane ion pumps, because of lack of cellular ATP, allowing the cell to accumulate fluid.

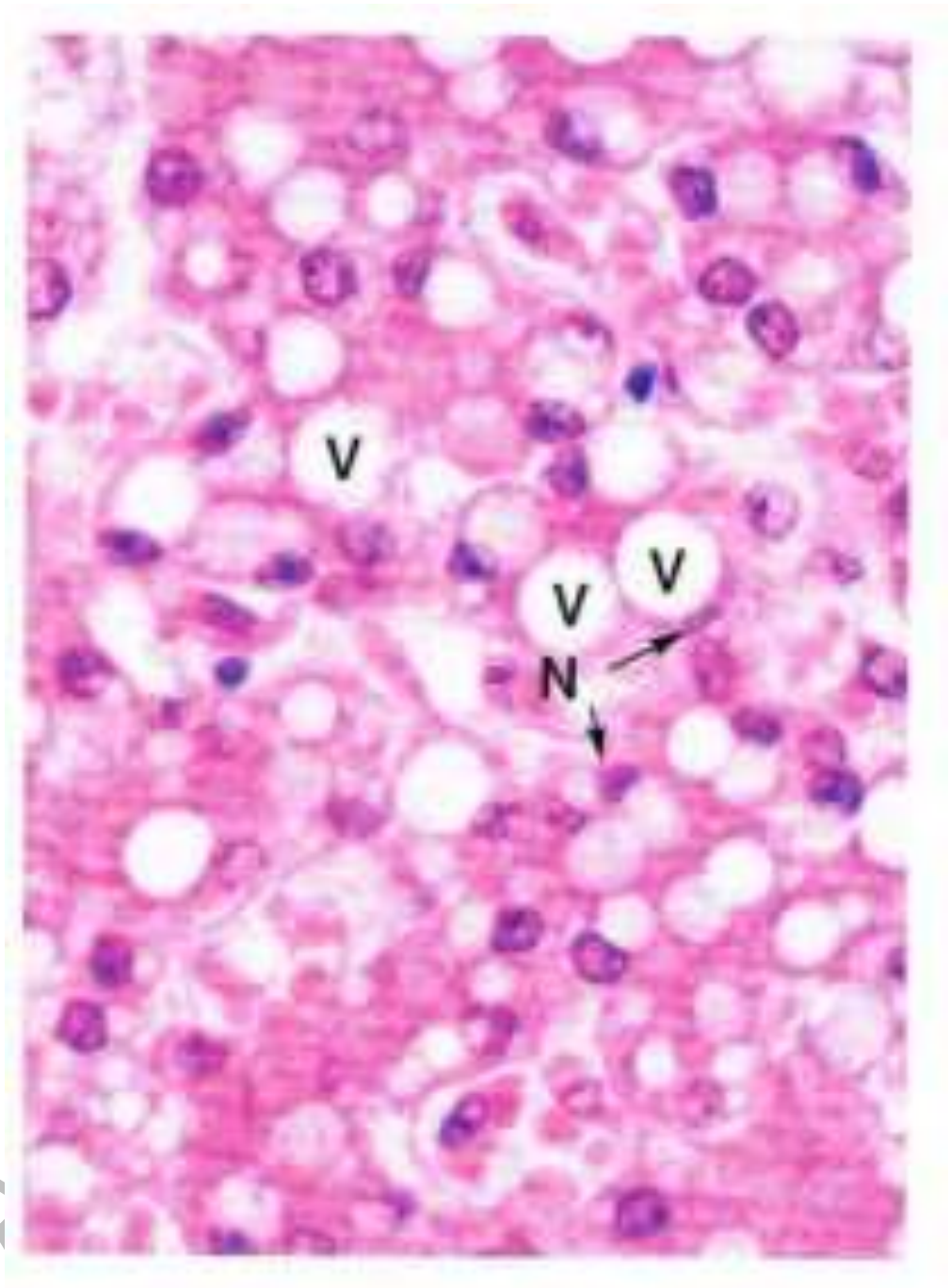
Fatty change

Fatty change is another manifestation of sub-lethal metabolic derangement seen in certain cell types with high energy demand. It is most common in the liver, as in this example, but also occurs in the myocardium and kidney. The common causes of fatty change are

- **toxins (particularly alcohol and halogenated hydrocarbons such as chloroform),**
- **chronic hypoxia,**
- **diabetes mellitus and**
- **Obesity.**

Impaired metabolism of fatty acids leads to accumulation of triglycerides (fat) that form non-membrane-bound vacuoles in cells, which may displace the nucleus from its usual location.

This example of liver from an alcoholic shows large vacuoles V in the hepatocytes with displacement of the nucleus N (Micrograph (2)



Micrograph (2)

Lecture (4)

Necrosis

If a cell develops non-selective, irreversible damage to many cell components then it is unable to respond and dies. Following cell death, a series of morphological changes take place termed *necrosis*.

Necrosis is the type of cell death that is associated with loss of membrane integrity and leakage of cellular contents culminating in dissolution of cells, largely resulting from the degradative action of enzymes on lethally injured cells.

So necrosis may define as: **Series of morphological changes in the lethally injured cell**

The leaked cellular contents often elicit a local host reaction, called *inflammation that* attempts to eliminate the dead cells and start the subsequent repair process.

The enzymes responsible for digestion of the cell may be derived from the lysosomes of the dying cells themselves and from the lysosomes of leukocytes that are recruited as part of the inflammatory reaction to the dead cells.

Necrosis is characterized by changes in the cytoplasm and nuclei of the injured cells

- **Cytoplasmic changes.** Necrotic cells show **increased eosinophilia**
- **Nuclear changes.** Nuclear changes assume one of three patterns, all due to breakdown of DNA and chromatin.
  - ❖ The basophilia of the chromatin may fade **(karyolysis),** presumably secondary to deoxyribonuclease (DNase) activity.
  - ❖ A second pattern is **pyknosis,** characterized by nuclear shrinkage and increased basophilia; the DNA condenses into a solid shrunken mass.
  - ❖ In the third pattern, **karyorrhexis,** the pyknotic nucleus undergoes fragmentation.

In 1 to 2 days, the nucleus in a dead cell may completely disappear. Electron microscopy reveals pro- found nuclear changes culminating in nuclear dissolution.

- **Fates of necrotic cells.** Necrotic cells may persist for some time or may be digested by enzymes and disappear. Dead cells may be replaced by myelin figures, which are either phagocytosed by other cells or further degraded into fatty acids. These fatty acids bind calcium salts, which may result in the dead cells ultimately becoming calcified.

Patterns of Tissue Necrosis

There are several morphologically distinct patterns of tissue necrosis. Most of these types of necrosis have distinct gross appearance; fibrinoid necrosis is detected only by histologic examination.

1- **Coagulative necrosis**: Coagulative necrosis is characteristic of infarcts (areas of ischemic necrosis) in all of the solid organs except the brain.

Coagulative necrosis is the most common and is when the cell is literally "coagulated or clotted". *This usually occurs in conditions of ischemia.* When you look at the coagulative area, you can see all the outlines of the tissue, the basic architecture is there, but the cells are not alive, it is a “ghost town.” Very often the cytoplasm is more eosinophilic (pink) than usual. Most causes of coagulative necrosis are due to loss of blood supply – so, basically, an infarct.

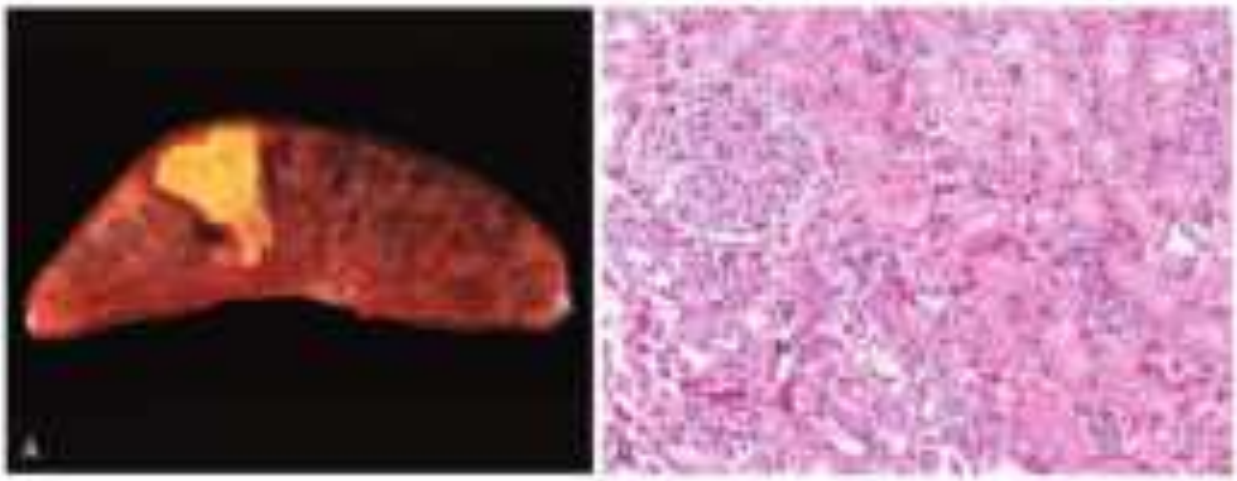


Fig. 4.1 Coagulative necrosis. (A) A wedge-shaped kidney infarct *(yellow)* (B) Microscopic view of the edge of the infarct, with normal kidney *(***N***)* and necrotic cells in the infarct *(***I***)*. The necrotic cells show preserved outlines with loss of nuclei, and an inflammatory infiltrate is present

Causes of coagulative necrosis

Hypoxia (lack of oxygen) is most common condition that causes cell death in a localized area that is perfused by blood vessels. When these vessels fail to deliver oxygen and other important nutrients there is ischemic infarction in that tissue and result in coagulative necrosis. There is reduced blood flow result in reduced oxygen and nutrients to that cells which results in hydrolytic lysis of that cells. When this ischemia

occurs in central nervous system it causes liquefactive necrosis. It is caused by conditions that do not involve trauma, toxin or immune response.

2- Liquefactive necrosis: hypoxic death of cells within the central nervous system often evokes liquefactive necrosis.

Liquefactive necrosis occurs when powerful hydrolytic enzymes play a predominant role over protein denaturation. This occurs in mostly in PUS (those free radicals and proteases!), and also for some reason occurs when there is necrosis in brain. There's so much destruction that you can't even identify the cells that were there in the first place!

Abscesses are classical examples of liquefactive necrosis. The original tissue has been digested away by free radicals and proteases. An abscess contains lakes of degenerate neutrophils with intracellular bacteria.

Necrosis in the BRAIN is almost always liquefactive, even without any neutrophils present. We call brain necrosis **malacia**.

Causes of liquefactive Necrosis:

Liquefactive necrosis is caused by bacterial or fungal infections which produces hydrolytic enzymes and white blood cells which lysis and liquefy the infected tissue and liquefactive necrosis occurs. In brain tissue ischemia leads that inflammatory and lysis of brain tissue.

3- Gangrenous necrosis: It usually refers to the condition of a limb, generally the lower leg that has lost its blood supply and has undergone coagulative necrosis involving multiple tissue layers. When bacterial infection is superimposed, coagulative necrosis is modified by the liquefactive action of the bacteria and the attracted leukocytes (resulting in so-called **wet gangrene**).

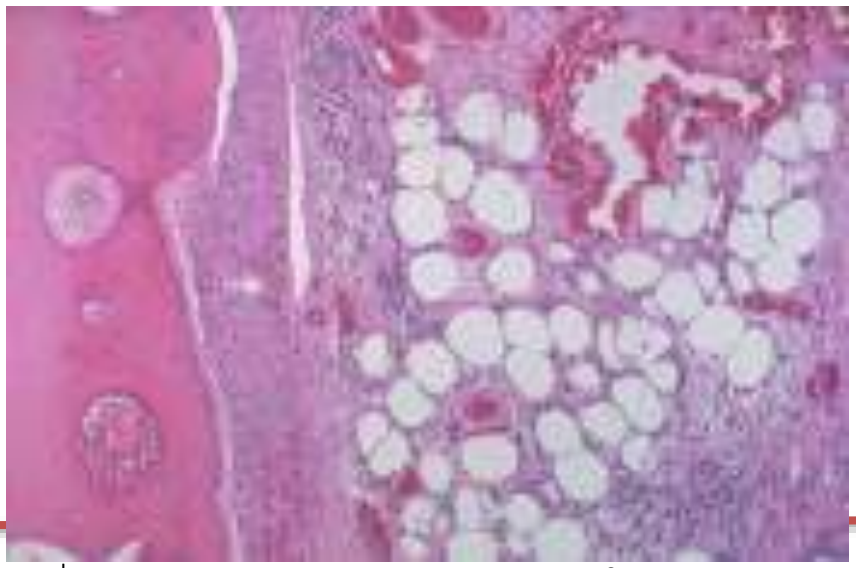


Fig. 4.2. **Gangrenous necrosis** involves the tissues of a body part. The inflammation seen here is extending beneath the skin of a toe to involve soft tissue (fat and connective tissue at the right) and bone (at the left). Because multiple tissues are non-viable, amputation of such areas is necessary.

4- Gaseous necrosis is encountered most often in foci of tuberculous infection.

Gaseous necrosis occurs when the bacteria causing the necrosis are covered with a thick capsule and cannot be easily broken down by neutrophils. The necrotic areas macroscopically have a cheesy-crumbly appearance. Histologically there is destruction of tissue but also many macrophages, and often giant cells.

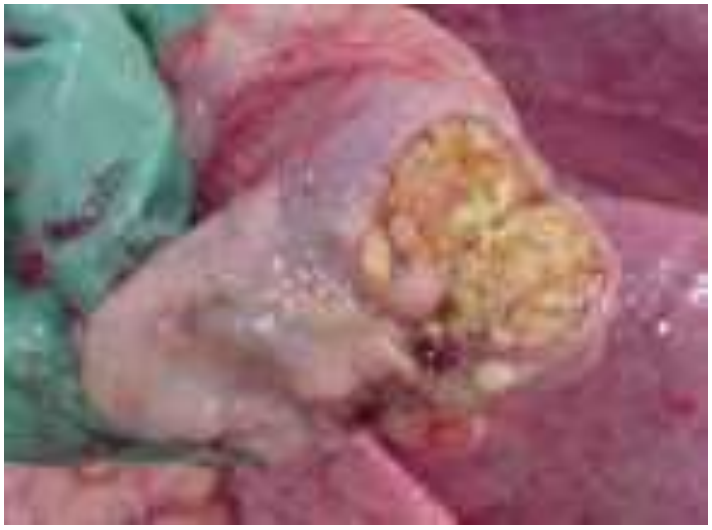


Fig. 4.3. Gaseous necrosis seen grossly in a lymph node from a cow infected with *Mycobacterium bovis*.

Fig. 4.4 This is a case of hepatic pseudotuberculosis. The large white nodule and the numerous smaller nodules are all foci of **gaseous necrosis**.

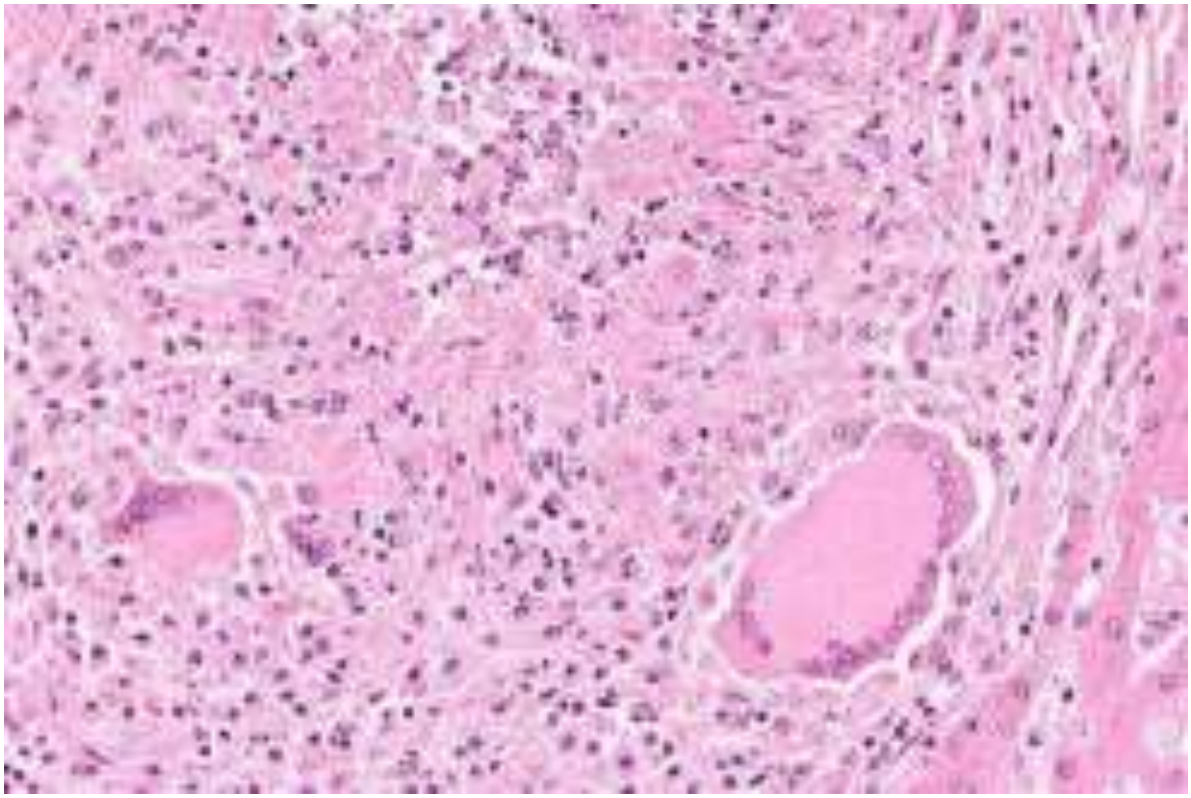


Fig. 4.5. Here is an example of **gaseous necrosis**. You can see some normal tissue at the right edge, this is probably liver. But much of the lesion is taken up by inflammatory cells, including macrophages and giant cells, which are simply macrophages that have fused.

5- Fat necrosis refers to focal areas of fat destruction, this occurs in the calamitous abdominal emergency known as acute pancreatitis. Fat necrosis is a form of necrosis characterized by the action upon fat by digestive enzymes. In fat necrosis the enzyme lipase releases fatty acids from triglycerides. The fatty acids then complex with calcium to form soaps. These soaps appear as white chalky deposits. It is usually associated with trauma of the pancreas or acute pancreatitis. It can also

occur in the breast, the salivary glands and neonates after a traumatic delivery

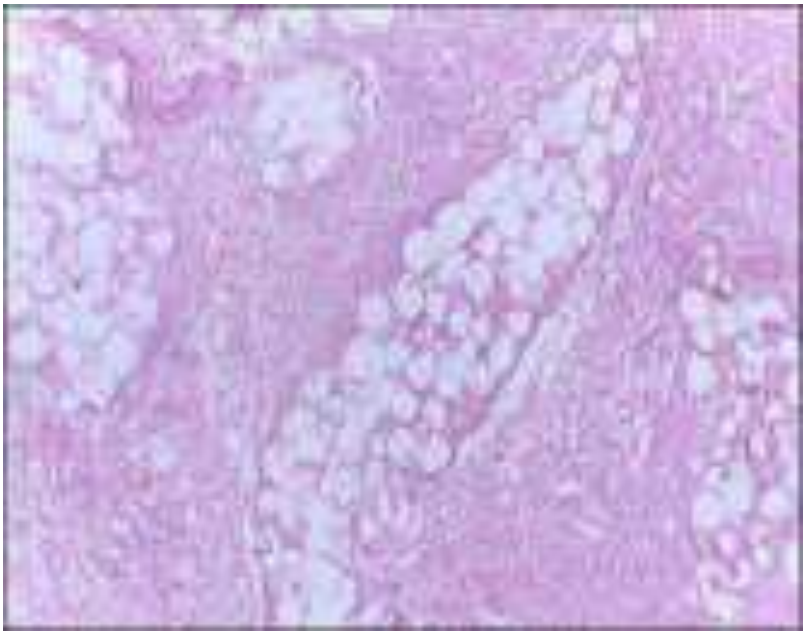


Fig. 4.6. Breast tissue showing **fat necrosis**. H&E

6- Fibrinoid necrosis is a form of necrosis, or tissue death, in which there is accumulation of amorphous, basic, proteinaceous material in the tissue matrix with a staining pattern reminiscent of fibrin. It is associated with conditions such as immune vasculitis (e.g. polyarteritis nodosa), malignant hypertension, preeclampsia, or hyperacute transplant rejection.

In small vessel vasculitis, fibrin plugs frequently occur in the vessel lumen, but the term fibrinoid is usually used to refer to material outside the lumen of a vessel. Fibrinoid necrosis also occurs in the walls of arterioles in malignant hypertension (blood pressure greater than 200/130 mmHg).

Fibrinoid necrosis is a special form of necrosis usually seen in immune reactions involving blood vessels, known as Type III hypersensitivity reactions. This pattern of necrosis typically occurs when complexes of antigens and antibodies are deposited in the walls of arteries. Deposits of these immune complexes, together with fibrin that has leaked out of vessels, result in a bright pink and amorphous appearance in H&E stains, called "fibrinoid" (fibrin-like) by pathologists. Ultimately, in the living patient most necrotic cells and their contents disappear by phagocytosis of the debris and enzymatic digestion by leukocytes. If necrotic cells and cellular debris are not promptly destroyed and reabsorbed, they tend to attract calcium salts and other minerals and to become calcified.

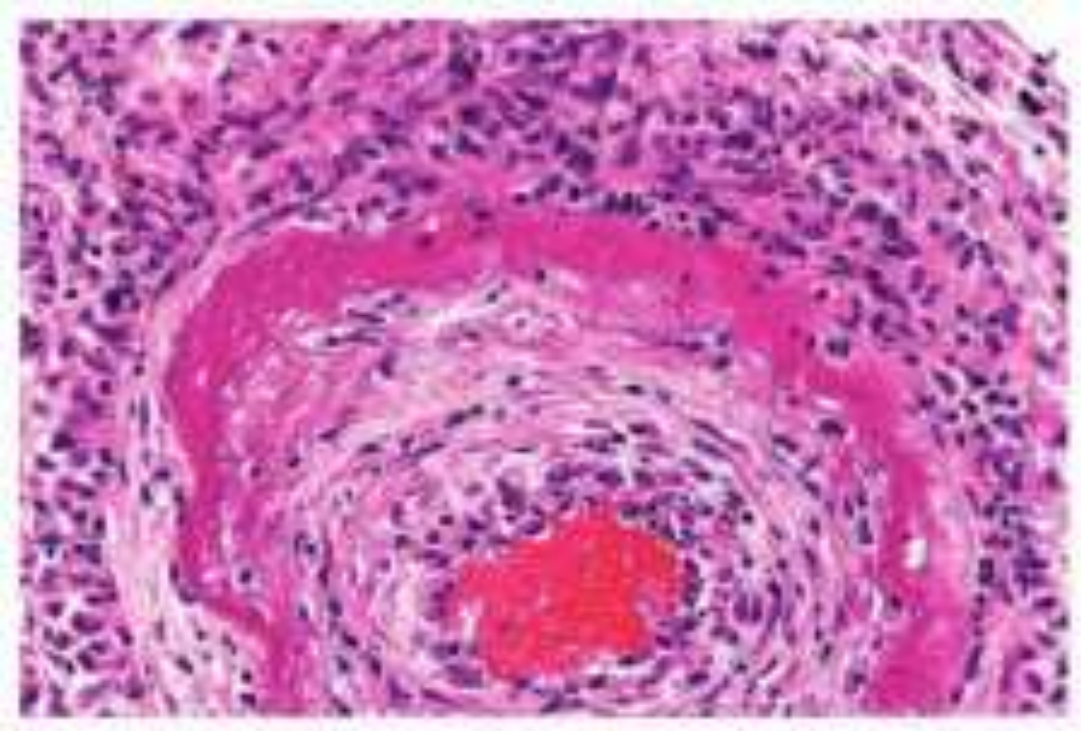


Fig. 4.7. Fibrinoid necrosis in an artery in a patient with polyarteritis nodosa. The wall of the artery shows a circumferential bright pink area of necrosis with protein deposition and inflammation.

Lecture (5)

Apoptosis

Selective damage to certain key cellular components may trigger a process of programmed cell death, termed ***apoptosis***. This is a highly organized process where intracellular signaling systems bring about destruction of the cell. Damage to DNA, cell surface membrane, or mitochondria are potent stimuli for apoptosis. Additionally, some cells that have initially responded by showing signs of cell degeneration (sublethal cell injury) may enter programmed cell death and die through apoptosis.

What is apoptosis or programed death?
Why we require a cell to die naturally?
Apoptosis is a process when cell go to kill itself, So why?

In some time, this decision is necessary

- First reason to eliminate spreading of infection, when cell infected by pathogens.
- Apoptosis require for some development of morphological functionality, the development of hands for example

Apoptosis is a pathway of cell death in which cells activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins. The plasma membrane of the apoptotic cell remains intact, but the membrane altered in such a way that the cell and its fragments become avid targets for **phagocytes**. The dead cell and its fragments are rapidly cleared before cellular contents have leaked out, so *apoptotic cell death does not elicit an inflammatory reaction in the host.*

Causes of Apoptosis

Apoptosis occurs in **many normal situations** and serves to eliminate potentially harmful cells and cells that have outlived their usefulness. It

also occurs as **a pathologic event** when cells are damaged beyond repair, especially when the damage affects the cell's DNA or proteins; in these situations, the irreparably damaged cell is eliminated.

***Apoptosis in Physiologic Situations*:**

Death by apoptosis is a normal phenomenon that serves to eliminate cells that are no longer needed and to maintain a constant number of cells of various types in tissues. It is important in the following physiologic situations:

1. *The programmed destruction of cells during embryogenesis*.
2. *Involution of hormone-dependent tissues upon hormone deprivation,* such as endometrial cell breakdown during the menstrual cycle, and regression of the lactating breast after weaning
3. *Elimination of cells that have served their useful purpose*, such as neutrophils in an acute inflammatory response and lymphocytes at the end of an immune response. In these situations, cells undergo apoptosis because they are deprived of necessary survival signals, such as growth factors.
4. *Elimination of potentially harmful self-reactive lymphocytes,* either before or after they have completed their maturation, in order to prevent reactions against the body's own tissues.
5. *Cell death induced by cytotoxic T lymphocytes,* a defense mechanism against viruses and tumors that serves to kill virus-infected and neoplastic cells.

Apoptosis in Pathologic Conditions

Apoptosis eliminates cells that are genetically altered or injured beyond repair and does so without eliciting a severe host reaction, thereby

keeping the extent of tissue damage to a minimum. Death by apoptosis is responsible for loss of cells in a variety of pathologic states:

1. ***DNA damage****:.* Radiation, cytotoxic anticancer drugs, extremes of temperature, and even hypoxia can damage DNA, either directly or through production of free radicals. If repair mechanisms cannot cope with the injury, the cell triggers intrinsic mechanisms that induce apoptosis. In these situations, elimination of the cell may be a better alternative than risking mutations in the damaged DNA, which may progress to malignant transformation. These injurious stimuli cause apoptosis if the insult is mild, but larger doses of the same stimuli result in necrotic cell death. Inducing apoptosis of cancer cells is a desired effect of chemotherapeutic agents, many of which work by damaging DNA.
2. ***Accumulation of misfolded proteins****:* Improperly folded proteins may arise because of mutations in the genes encoding these proteins or because of extrinsic factors, such as damage caused by free radicals. Excessive accumulation of these proteins in the ER leads to a condition called *ER stress,* which culminates in apoptotic death of cells.
3. ***Cell injury in certain infections****,* particularly viral infections, in which loss of infected cells is largely due to apoptotic death that may be induced by the virus (as in adenovirus and human immunodeficiency virus infections) or by the host immune response (as in viral hepatitis).
4. ***Pathologic atrophy in parenchymal organs after duct obstruc*****tion**. such as occurs in the pancreas, parotid gland, and kidney

Mechanisms of Apoptosis

Apoptosis is regulated by biochemical pathways that control the balance of death-and survival-inducing signals and ultimately the activation of enzymes called *caspases*.

Two distinct pathways converge on caspase activation: the mitochondrial pathway and the death receptor pathway: (**Fig. 5.1**).

1. The mitochondrial (intrinsic) pathway seems to be responsible for apoptosis in most physiologic and pathologic situations.
2. The death receptor (extrinsic) pathway of apoptosis.

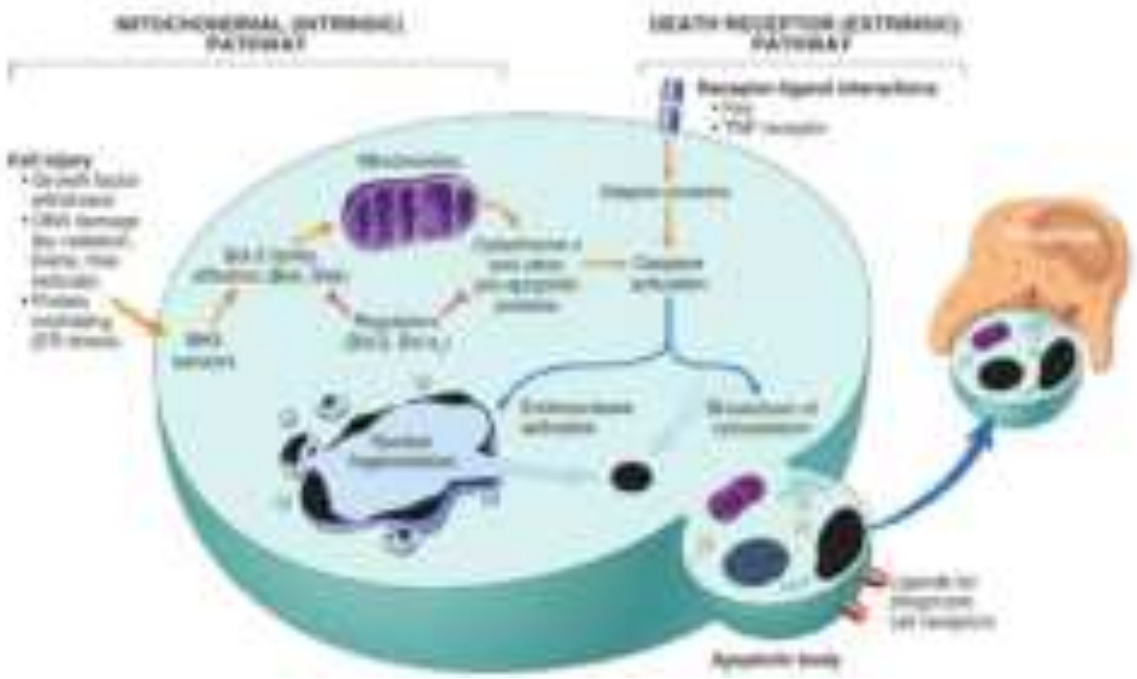


Fig. 5.1. *Mechanisms of Apoptosis*

Differences between apoptosis and necrosis

Apoptosis differs in this respect from necrosis, which characterized by loss of membrane integrity, enzymatic digestion of cells, leakage of cellular contents, and frequently a host reaction (**Fig. 5.2**).

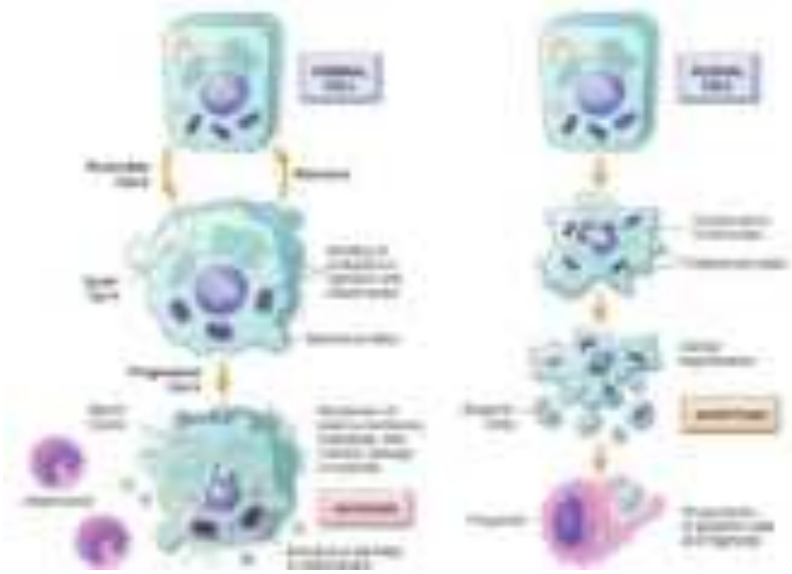


Fig. 5.2. Cellular features of =necrosis (*left*) and apoptosis (*right*).

Table (1) Features of Necrosis and Apoptosis (comparison)

Feature	Necrosis	Apoptosis
Cell Size	Enlarged(swelling)	Reduced(shrinkage)
Nucleus	Pyknosis karyorrehxis Karyolysis	Fragmentation into nucleosome size fragments
Plasma membrane	Disrupted	Intact; altered structure , especially orientation of lipids
Cellular contents	Enzymatic digestion: may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiological or pathological role	Invariable pathologic (culmination of irreversible cell injury)	Often physiologic; means of eliminating unwanted cells; may be pathologic after some forms of cell injury ; especially DNA and protein damage

Lecture (6)

CELLULAR ADAPTATIONS TO STRESS

Adaptations are reversible changes in the number, size, phenotype, metabolic activity, or functions of cells in response to changes in their environment. *Physiologic adaptations* usually represent responses of cells to normal stimulation by hormones or endogenous chemical mediators (e.g., the hormone-induced enlargement of the breast and uterus during pregnancy). *Pathologic adaptations* are responses to stress that allow cells to modulate their structure and function and thus escape injury. Such adaptations can take several distinct forms

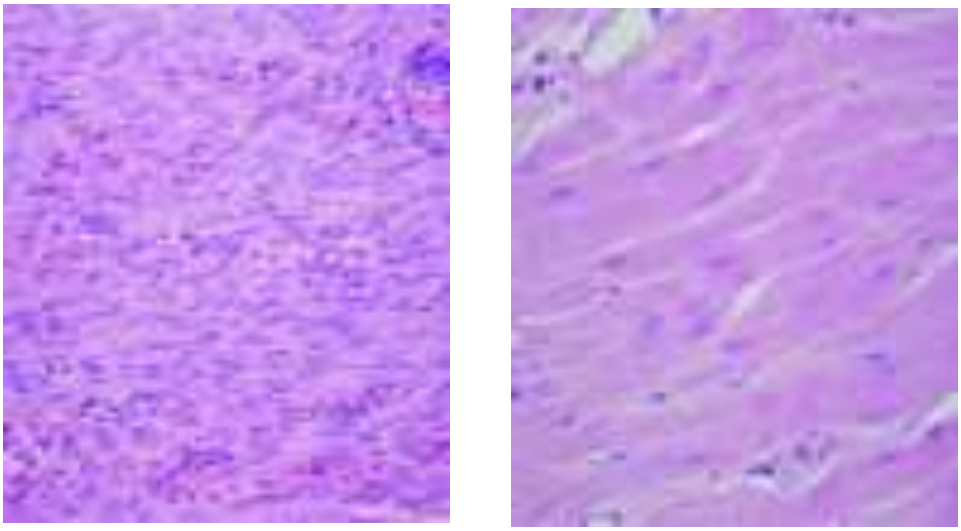
Hypertrophy and hyperplasia

Hypertrophy is an increase in the size of cells resulting in increase in the size of the organ. In contrast, **hyperplasia** is characterized by an increase in cell number because of proliferation of differentiated cells and replacement by tissue stem cells. Stated another way, in pure hypertrophy there are **no new cells**, just bigger cells containing increased amounts of structural proteins and organelles.

Hyperplasia is an adaptive response in cells capable of replication, whereas **hypertrophy** occurs when cells have a **limited capacity to divide**. Hypertrophy and hyperplasia also can occur together, and obviously both result in an enlarged (*hypertrophic*) organ.

Hypertrophy *can be* ***physiologic*** *or* ***pathologic*** and is caused either by increased functional demand or by growth factor or hormonal stimulation.

- The massive physiologic enlargement of the uterus during pregnancy occurs as a consequence of estrogen stimulated smooth muscle hypertrophy and smooth muscle hyperplasia (Fig. 3–1).
- An example of pathologic cellular hypertrophy is the cardiac enlargement that occurs with hypertension or aortic valve disease.



A **B**

Physiologic hypertrophy of the uterus during pregnancy. A, Small spindle-shaped uterine smooth muscle cells from a normal uterus. B, Large, plump hypertrophied smooth muscle cells from a gravid uterus; compare with A. (B and C, Same magnification.)

Hyperplasia

Hyperplasia takes place if the tissue contains cell populations capable of replication; it may occur concurrently with hypertrophy and often in response to the same stimuli.

Hyperplasia can be physiologic or pathologic. In both situations, cellular proliferation is stimulated by growth factors that are produced by a variety of cell types.

- The two types of *physiologic hyperplasia* are (1) *hormonal hyperplasia,* exemplified by the proliferation of the glandular epithelium of the female breast at puberty and during pregnancy, and (2) *compensatory hyperplasia,* in which residual tissue grows after removal or loss of part of an organ. For example, when part of a liver is resected, mitotic activity in the remaining cells begins as early as 12 hours later, eventually restoring the liver to its normal weight. The stimuli for hyperplasia in this setting are polypeptide growth factors produced by uninjured hepatocytes as well as nonparenchymal cells in the liver

- Most forms of *pathologic hyperplasia* are caused by excessive hormonal or growth factor stimulation. For example, after a normal menstrual period there is a burst of uterine epithelial proliferation that is normally tightly regulated by stimulation through pituitary hormones and ovarian estrogen and by inhibition through progesterone. However, a disturbed balance between estrogen and progesterone causes endometrial hyperplasia, which is a common cause of abnormal menstrual bleeding.
- Hyperplasia also is an important response of connective tissue cells in wound healing, in which proliferating fibroblasts and blood vessels aid in repair. In this process, growth factors are produced by white blood cells (leukocytes) responding to the injury and by cells in the extracellular matrix. Stimulation by growth factors also is involved in the hyperplasia that is associated with certain viral infections; for example, papillomaviruses cause skin warts and mucosal lesions composed of masses of hyperplastic epithelium. Here the growth

factors may be encoded by viral genes or by the genes of the infected host cells.

Atrophy

Shrinkage in the size of the cell by the loss of cell substance is known as atrophy. When a sufficient number of cells are involved, the entire tissue or organ diminishes in size, becoming atrophic. Although atrophic cells may have diminished function, they are not dead.

Causes of atrophy include

1- A decreased workload (e.g., immobilization of a limb to permit healing of a fracture).
2- loss of innervation,
3- Diminished blood supply,
4- inadequate nutrition,
5- loss of endocrine stimulation, and
6- Aging (senile atrophy).

Although some of these stimuli are physiologic (e.g., the loss of hormone stimulation in menopause) and others pathologic (e.g., denervation), the fundamental cellular changes are identical. They represent a retreat by the cell to a smaller size at which survival is still possible; a new equilibrium is achieved between cell size and diminished blood supply, nutrition, or trophic stimulation.

The mechanisms of atrophy consist of a combination of decreased protein synthesis and increased protein degradation in cells. Protein synthesis decreases because of reduced metabolic activity.

- The degradation of cellular proteins occurs mainly by the *ubiquitin-proteasome pathway.* Nutrient deficiency and disuse may activate ubiquitin ligases, which attach multiple copies of the small peptide ubiquitin to cellular proteins and target them for degradation in proteasomes. This pathway is also thought to be responsible for the accelerated proteolysis seen in a variety of catabolic conditions, including the cachexia associated with cancer.
- In many situations, atrophy is also accompanied by increased *autophagy,* with resulting increases in the number of *autophagic vacuoles.* Autophagy ("self-eating") is the process in which the starved cell eats its own components in an attempt to survive. We describe this process later in the chapter.

Metaplasia

Metaplasia is a reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type. In this type of cellular adaptation, a cell type sensitive to a particular stress is replaced by another cell type better able to withstand the adverse environment. Metaplasia is thought to arise by reprogramming of stem cells to differentiate along a new pathway rather than a phenotypic change (transdifferentiation) of already differentiated cells.

Epithelial metaplasia is exemplified by the squamous change that occurs in the respiratory epithelium of habitual cigarette smokers. The normal ciliated columnar epithelial cells of the trachea and bronchi are focally or widely replaced by stratified squamous epithelial cells. The

rugged stratified squamous epithelium may be able to survive the noxious chemicals in cigarette smoke that the more fragile specialized epithelium would not tolerate.

Although the metaplastic squamous epithelium has survival advantages, important protective mechanisms are lost, such as mucus secretion and ciliary clearance of particulate matter.

Epithelial metaplasia is therefore a double-edged sword. Moreover, *the influences that induce metaplastic change, if persistent,*

May predispose to malignant transformation of the epithelium. In fact, squamous metaplasia of the respiratory epithelium often coexists with lung cancers composed of malignant squamous cells. It is thought that cigarette smoking initially causes squamous metaplasia, and cancers arise later in some of these altered foci. Since vitamin A is essential for normal epithelial differentiation, its deficiency may also induce squamous metaplasia in the respiratory epithelium. Metaplasia need not always occur in the direction of columnar to squamous epithelium; in chronic gastric reflux, the normal stratified squamous epithelium of the lower esophagus may undergo metaplastic transformation to gastric or intestinal-type columnar epithelium. Metaplasia may also occur in mesenchymal cells but in these situations it is generally a reaction to some pathologic alteration and not an adaptive response to stress. For example, bone is occasionally formed in soft tissues, particularly in foci of injury.

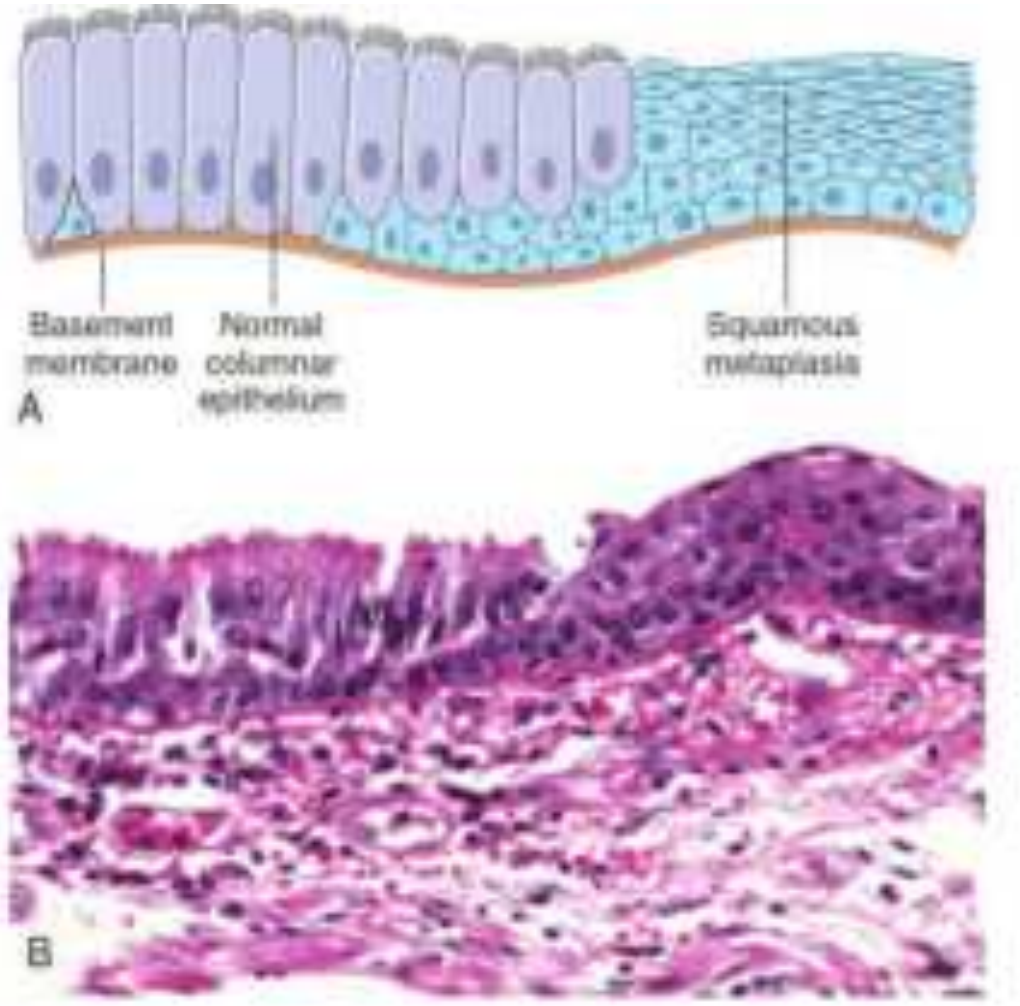


Figure 1–5 Metaplasia of normal columnar (*left*) to squamous epithelium (*right*) in a bronchus, shown schematically (**A**) and histologically (**B**).

Lecture (7)

Inflammation

Inflammation is an almost universal response to tissue damage by a wide range of harmful stimuli including mechanical trauma, tissue necrosis and infection. The purpose of inflammation is to destroy (or contain) the damaging agent, initiate repair processes and return the damaged tissue to useful function.

Inflammation is somewhat arbitrarily divided into ***acute*** and ***chronic inflammation*** but in reality the two often form a continuum. Many causes of tissue damage provoke an acute inflammatory response but some types of insult may bring about a typical chronic inflammatory reaction from the outset (e.g. viral infections, foreign body reactions and fungal infections). Acute inflammation may ***resolve*** or ***heal by scarring*** but may also progress to chronic inflammation and it is common for a mixed acute and chronic response to coexist.

Causes of Inflammation:

Any irritant may act as a causes of inflammation, these irritant may be divided into two groups:

1=**Living irritant**: it is either pathogenic or disease producing by microorganism or by animal parasite. In both of them act as irritant mainly by virtue of chemical poisons which they produce. The pathological bacteria usually excite an acute reaction as a result of which both the cell and the fluid part of the tissue. Some produce a more chronic form of reaction, characterized by proliferation of the tissue cell e.g. T.B. and syphilis.

2= **Non – living irritant**: it's divided into **physical** and **chemical**. Physical irritant like Trauma, present foreign body, the action of under heat or

cold, burns and frost bite, presser of light, electricity, or radiation from radium….etc. Chemical irritant include strong acid and alkali and poisons.

Inflammation Classification;-

There are various way of classification of inflammation condition.

a- **The intensity and duration of the action**, inflammation categorized as; acute or chronic and intermediate stage called sub-acute.
b- Also can classification according **to the nature of the causal agent** e.g. traumatic pericarditis.
c- or **according to the exudative or other change which are present** e.g. fibrinous purities , superlative nephritis …etc .

Acute inflammation

Definition: Acute inflammation is a short-term process occurring in response to tissue injury, usually appearing within minutes or hours.

There are three major and interrelated components of acute inflammation:

1- **Vascular dilatation**: Relaxation of vascular smooth muscle leading to engorgement of tissue with blood (*hyperaemia*).
2- **Endothelial activation:**
    a. increased endothelial permeability allows plasma proteins to pass into tissues
    b. expression of adhesion molecules on the endothelial surface mediates neutrophil adherence
    c. Production of factors which cause vascular dilatation.
3- **Neutrophil activation**
    a. expression of adhesion molecules causes neutrophils to adhere to endothelium

b. increased motility allows emigration from vessels into surrounding tissues

c. Increased capacity for bacterial killing.

Clinical features of acute inflammatory

The vascular and exudative phenomena of acute inflammation are responsible for the clinical features and were described by Celsus in the first century AD. The cardinal signs of Celsus are:

- Redness {rubor)
- Swelling {tumor)
- Heat {calor)
- Pain {dolor)

Galen later added:

- Loss of function (functio laesa)

The nomenclature:

The nomenclature used to describe inflammation in different tissues employs the tissue name (or its Greek or Latin equivalent) and the suffix -itis\ ,there are notable exceptions in traditional clinical usage. For example, inflammation of the pleura is usually termed pleurisy, while inflammation of subcutaneous tissues as a result of infection is usually termed acute cellulitis.

Tissue	Acute inflammation	Typical causes
Meninges	Meningitis	Bacterial and viral infections
Brain	Encephalitis	viral infections
Lung	Pneumonia	Bacterial infections,
Pleura	Pleurisy	Bacterial and viral infections
Pericardium	Pericarditis	Bacterial and viral infections myocardial infarction
Oesophagus	Oesophagitis	Gastric acid reflux, fungal infections
Stomach	Gastritis	Alcohol abuse, Helicobacter pylori infection
Colon	Colitis	Bacterial infections ,Ulcerative colitis
Rectum	Proctitis	Ulcerative colitis
Appendix	Appendicitis	Faecal obstruction
Liver	Hepatitis	Alcohol abuse, viral infections
Gallbladder	Cholecystitis	Bacterial infections, chemical irritation
Pancreas	Pancreatitis	Pancreatic enzyme release
Urinary bladder	Cystitis	Bacterial infections
Bone	Osteomyelitis	Bacterial infections
Subcutaneous tissues	Cellulitis	Bacterial infections
Joints	Arthritis	Bacterial and viral infections, immune complex deposition
Arteries	Arteritis	Immune complex deposition

Morphological types of acute inflammation

While the basic process of acute inflammation is the same in all tissues, there are frequently qualitative differences in the inflammatory response seen under different circumstances. Terms describing these variations are widely used in clinical practice and are summarized below:

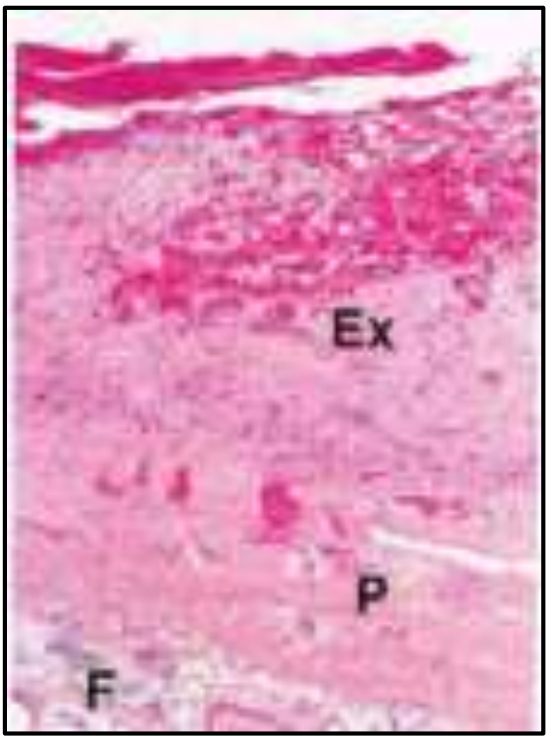
- **Suppurative inflammation (purulent inflammation)**
- **Fibrinous inflammation**
- **Serous inflammation**

Suppurative inflammation (purulent inflammation): refers to acute inflammation in which the acute inflammatory exudate is particularly rich in neutrophil leucocytes.

Suppurative inflammation is most commonly seen as a result of infection by bacteria where the mixture of neutrophils (viable and dead), necrotic tissue, and tissue fluid in the acute inflammatory exudate form a semi-liquid material referred to as pus.Bacteria which produce purulent inflammation are described as pyogenic bacteria. Pyogenic bacteria include Staphylococci, some Streptococci (S. pyogenes, S. pneumoniae), Escherichia coli and the Neisseriae.

Acute inflammation in the meninges surrounding the brain illustrates an example of an exudate in which very little fibrin is formed. In acute meningitis, the exudate is almost entirely composed of oedema fluid and neutrophils. Macroscopically, this appears as a creamy thick fluid and the term acute purulent inflammation is often used to describe In the micrograph, note the densely cellular exudate (Ex) lying on the surface of the brain (B) in the subarachnoid space.

Fibrinous inflammation : refers to a pattern of acute inflammation where the acute inflammatory exudate has a high plasma protein content. Fibrinogen derived from plasma is converted to fibrin, which is deposited in tissues. This pattern is particularly associated with membrane-lined cavities such as the pleura, pericardium and peritoneum, where the fibrin strands form a mat-like sheet causing adhesion between adjacent surfaces.



Acute pericarditis

In this low magnification photomicrograph, the exudate Ex is well established on the epicardial aspect of the pericardium P. No myocardium is seen in this micrograph, but epicardial fat F is readily identifiable. Acute pericarditis most commonly occurs secondary to death of underlying cardiac muscle (myocardial infarction). The acute inflammatory exudate is made up of dense masses of pink-staining fibrin with comparatively few neutrophils.

The process of acute inflammation is designed to neutralise injurious agents and to restore the tissue to useful function.

There are four main outcomes of acute inflammation if the patient survives:

- ❖ Resolution,
- ❖ Healing by fibrosis,
- ❖ Abscess formation, and
- ❖ Progression to chronic inflammation.

Three factors determine which of these outcomes occurs:

- ❖ The severity of tissue damage
- ❖ The capacity of specialized cells within the damaged tissue to divide and replace themselves, a process termed regeneration

❖ The type of agent which has caused the tissue damage.

Lecture (8)

Mechanism of early acute inflammation

Acute inflammation may develop over minutes or hours depending on the type and severity of the tissue damage and generally lasts hours to days. Vascular dilatation, increased vascular permeability and neutrophil activation and migration are interdependent processes and all three are required for the full response. Immediately after the tissue damage has occurred, there may be a brief phase of constriction of arterioles but this is followed within seconds by arteriolar dilatation, which leads to increased blood flow to the area.

At much the same time, gaps form between endothelial cells of the capillaries allowing protein-rich plasma to leak into the tissue. The dilated capillaries become engorged with red cells and blood flow slows and then stops. The slowing of blood flow brings neutrophils into contact with the endothelial cells, which have been busy inserting adhesion molecules into their plasma membranes.

As the neutrophils come into contact with the endothelium, *adhesion molecules* on the neutrophil plasma membrane bind to their complementary receptors on the endothelial cells and become stuck. Activation of the neutrophils plays a role here so that activated neutrophils are more likely to stick. Meanwhile in the tissues, the plasma-derived proteins undergo various changes. The *complement cascade* is initiated (alternative pathway) forming components with a wide range of activities.

Immunoglobulins bind to any causative organisms immobilising them, and forming immune complexes that further activate complement (classical pathway). *Fibrinogen* is cleaved to form monomers which then polymerise to form a network of *fibrin* that impedes the movement of any pathogenic organisms present, and also provides a framework for the movement of neutrophils. The increased fluid in the tissue causes an increased flow of lymph to carry immune complexes and antigenic

material to the lymph nodes where a specific immune response is initiated over a matter of days.

The neutrophils pass through the basement membrane of the endothelium and move along a concentration gradient of chemotactic factors. When they arrive at the site of injury, the activated neutrophils phagocytose necrotic tissue debris and any pathogenic organisms. The activation of the neutrophils makes them more efficient at phagocytosis and bacterial killing. Opsonisation of bacteria by complement and immunoglobulins renders them more readily phagocytosed.

This entire process is orchestrated by a plethora of chemical mediators derived from injured tissues, bacteria, plasma proteins and leucocytes. The most important of these mediators are indicated at their sites of action. Note that some mediators have multiple actions. Following micrographs illustrates the sequence of events during the initial phases of the acute inflammatory response.

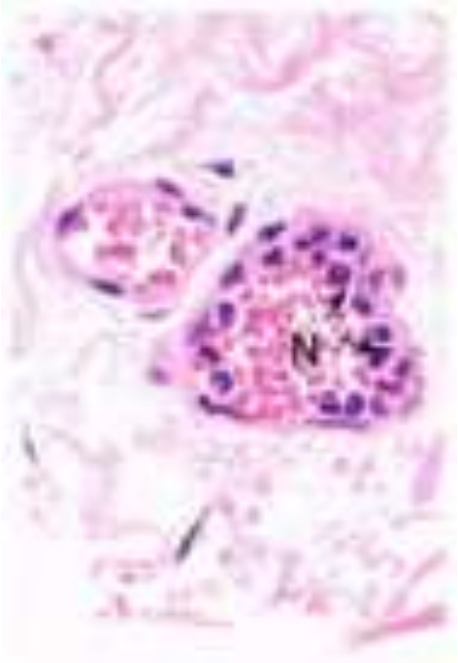


Fig.1. two small capillaries are shown. Both vessels are dilated , in the larger, Neutrophils N line up around the periphery of the vessel, a process termed pavementation. These neutrophils are adherent to the endothelium. The surrounding fibrous connective tissue contains clear spaces owing to the accumulation of fluid {oedema) between the collagen bundles.

Mechanism of early acute inflammation

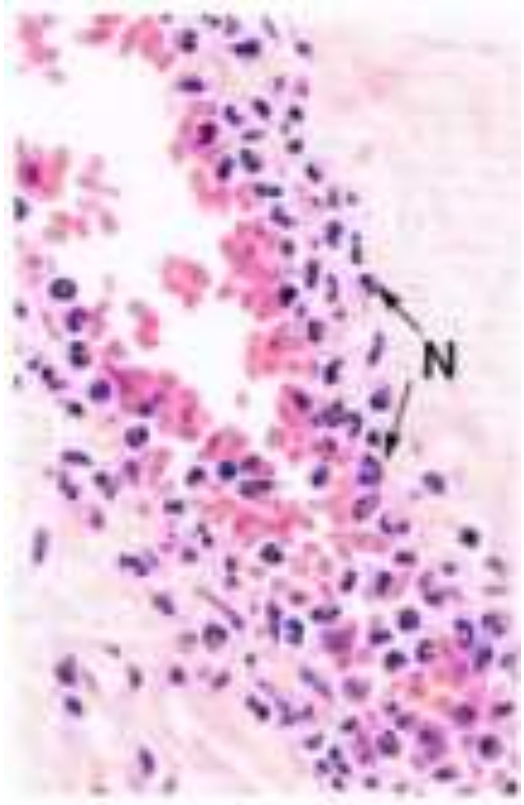


Fig.2. the neutrophils pass through the vessel wall by extending their pseudopodia between adjacent endothelial cells. The neutrophils then penetrate the endothelial basement membrane and move into the perivascular connective tissue as shown in micrograph.

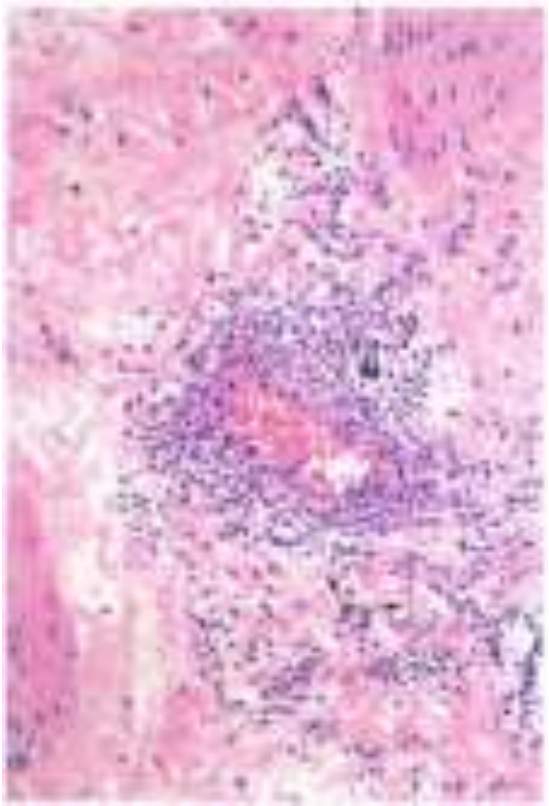


Fig.3. Once in the extravascular tissues N, the neutrophils are attracted to the site of tissue damage by **chemotactic agents** and migrate actively towards higher concentrations of these agents (chemotaxis); this is shown in micrograph .

Mechanism of early acute inflammation

Chemical mediators, control this process.

Vasodilatation is mediated by prostaglandins and nitric oxide. Increased vascular permeability is controlled by substances such as the vasoactive amines, serotonin and histamine, complement components C5a and C3a, leukotrienes C4, D4, and E+ platelet activating factor (PAF) and substance P. Leucocyte activation and chemotaxis are influenced by C5a, leukotriene B4, various chemokines and bacterial products.

Acute inflammatory exudate

The quality of an acute inflammatory exudate varies depending on

- the state and nature of the injured tissue and
- the type of noxious agent involved.

Following micrographs show established acute inflammatory exudates differing from one another in the number of neutrophils N and amount of fibrin F present; fibrin strands stain bright pink with H&E staining.

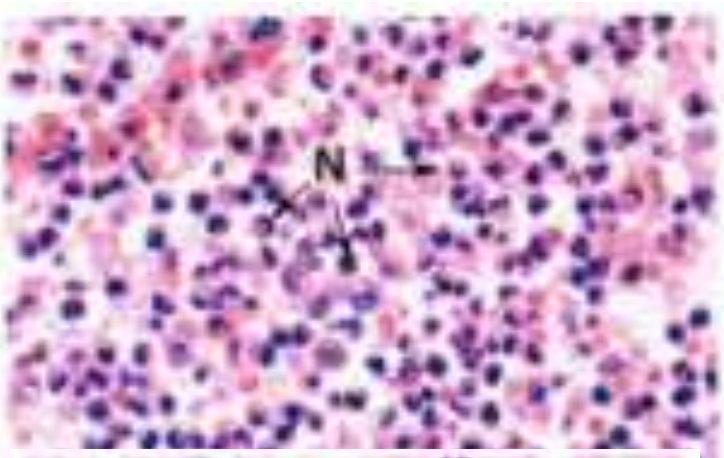


Fig.4. Micrograph shows an acute inflammatory exudate in which neutrophils are the main component, usually the case when the damaging stimulus is a bacterial infection; this neutrophil-rich exudate is commonly called 'pus' and this pattern is therefore often called **acute purulent inflammation**.

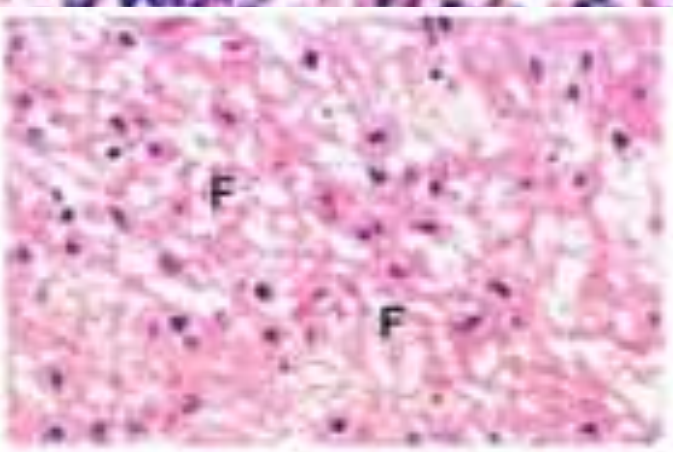


Fig.5. Micrograph shows an acute inflammatory exudate in which fibrin is the main component (fibrinous exudate); this occurs most commonly on serosal surfaces.

Mechanism of early acute inflammation

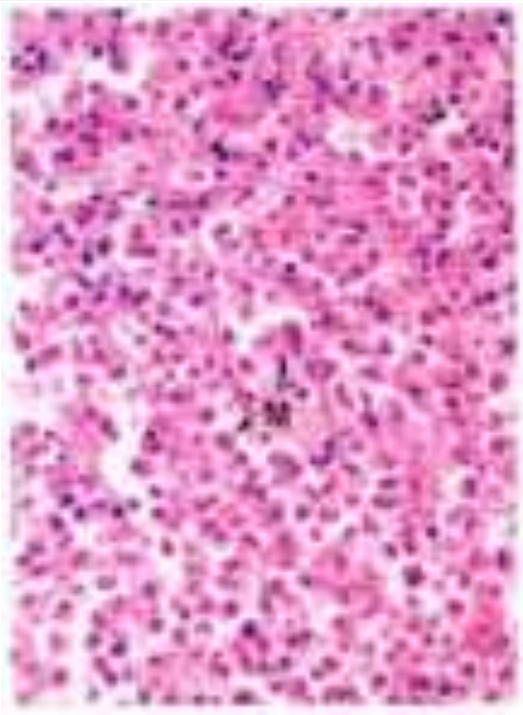


Fig.6. Micrograph shows a mixed neutrophilic/fibrinous exudate at a late stage, with some macrophages entering the picture.

Although the dominant cell type in the early phases of acute inflammation is the neutrophil, within 24 hours macrophages also begin to migrate into the damaged tissue and by 48-72 hours are the predominant cell type. The macrophages are derived from circulating blood monocytes. Macrophages M, a few of which can be seen in micrograph (6), continue the phagocytic work begun by neutrophils and ultimately mop up the degenerate neutrophils and fibrin strands. Unlike neutrophils, macrophages can regenerate their lysosomal enzymes and are capable of sustained activity. The monocytes may also act as antigen-presenting cells to initiate specific immunological responses.

Lecture (9)

Chronic Inflammation

Chronic inflammation is a response of prolonged duration (weeks or months) in which inflammation, tissue injury, and attempts at repair coexist, in varying combinations. It may follow acute inflammation, as described earlier, or may begin insidiously, as a smoldering, sometimes progressive, process without any signs of a preceding acute reaction.

Causes of Chronic Inflammation

Chronic inflammation arises in the following settings:

- **Persistent infections** by microorganisms that are difficult to eradicate, such as mycobacteria and certain viruses, fungi, and parasites. These organisms often evoke an immune reaction called *delayed-type hypersensitivity*. *Autoimmune diseases (*rheumatoid arthritis and multiple sclerosis) and *Allergic diseases (*bronchial asthma)
- **Hypersensitivity diseases.** Chronic inflammation plays an important role in a group of diseases that are caused by excessive and inappropriate activation of the immune system
- **Prolonged exposure to potentially toxic agents, either exogenous or endogenous.** An example of an **exogenous agent** is particulate silica, a non-degradable inanimate material that, when inhaled for prolonged periods, results in an inflammatory lung disease called ***silicosis*. *Atherosclerosis*** *is a chronic* inflammatory process affecting the arterial wall that is thought to be induced, at least in part, by excessive production and tissue deposition of **endogenous** cholesterol and other lipids.
- Some forms of chronic inflammation may be important in the **pathogenesis of diseases that are not conventionally thought of as inflammatory disorders**. These include neurodegenerative diseases such as Alzheimer disease, metabolic syndrome and the associated

type 2 diabetes, and certain cancers in which inflammatory reactions promote tumor development.

Morphologic Features

In contrast to acute inflammation, which is manifested by vascular changes, edema, and predominantly neutrophilic infiltration, chronic inflammation is characterized by the following:

- **Infiltration with mononuclear cells,** which include macrophages, lymphocytes, and plasma cells.
- **Tissue destruction,** induced by the persistent offending agent or by the inflammatory cells
- **Attempts at healing** by connective tissue replacement of damaged tissue, accomplished by *angiogenesis* (proliferation of small blood vessels) and, in particular, *fibrosis*.

Cells and Mediators of Chronic Inflammation

The combination of leukocyte infiltration, tissue damage, and fibrosis that characterize chronic inflammation is the result of the local activation of several cell types and the production of mediators.

1- Role of Macrophages

The dominant cells in most chronic inflammatory reactions are macrophages, which contribute to the reaction by secreting cytokines and growth factors that act on various cells, by destroying foreign invaders and tissues, and by activating other cells, notably T lymphocytes.

Macrophages are professional phagocytes that act as filters for particulate matter, microbes, and senescent cells. They also function as effector cells that eliminate microbes in cellular and humoral immune responses. But they serve many other roles in inflammation and repair.

Macrophages are tissue cells derived from hematopoietic stem cells in the bone marrow and from progenitors in the embryonic yolk sac and fetal liver during early development. Circulating cells of this lineage are known as *monocytes*. Macrophages are normally diffusely scattered in most connective tissues. In addition, they are found in specific locations in organs such as the liver (where they are called Kupffer cells), spleen and lymph nodes (sinus histiocytes), central nervous system (microglial cells), and lungs (alveolar macrophages). Together these cells comprise the *mononuclear phagocyte system*, also known by the older (and inaccurate) name of reticuloendothelial system.

Chronic Inflammation

In inflammatory reactions, progenitors in the bone marrow give rise to monocytes, which enter the blood, migrate into various tissues, and differentiate into macro phages. Entry of blood monocytes into tissues is governed by the same factors that are involved in neutrophil emigration, such as adhesion molecules and chemokines. The half-life of blood monocytes is about 1 day, whereas the life span of tissue macrophages is several months or years.

Thus, macrophages often become the dominant cell population in inflammatory reactions within 48 hours of onset. The macrophages that reside in tissues in the steady state (in the absence of tissue injury or inflammation), such as microglia, Kupffer cells, and alveolar macrophages, arise from the yolk sac or fetal liver very early in embryogenesis, populate the tissues, stay for long periods, and are replenished mainly by the proliferation of resident cells.

There are two major pathways of macrophage activation, **called *classical* and *alternative***.

Which of these two pathways is taken by a given macrophage depends on the nature of the activating signals.

- **Classical macrophage activation** may be induced by microbial products such as endotoxin, which engage *Toll-like receptors* (TLRs) and other sensors, and by T cell–derived signals, importantly the cytokine IFN-γ, in immune responses. Classically activated (also called M1) macrophages produce NO and ROS and upregulate lysosomal enzymes, all of which enhance their ability to kill ingested organisms, and secrete cytokines that stimulate inflammation. These macrophages are important in host defense against microbes and in many inflammatory reactions.
- **Alternative macrophage activation** is induced by cytokines other than IFN-γ, such as IL-4 and IL-13, produced by T lymphocytes and other cells.

These macrophages are not actively microbicidal; instead, the principal function of alternatively activated (M2) macrophages is in tissue repair. They secrete growth factors that promote angiogenesis, activate fibroblasts, and stimulate collagen synthesis.

It seems plausible that in response to most injurious stimuli, the first activation pathway is the classical one, designed to destroy the offending agents, and this is followed by alternative activation, which initiates tissue repair. However, such a precise sequence is not well documented in most inflammatory reactions. In addition, although the concept of M1 and M2

macrophages provides a useful framework for understanding macrophage heterogeneity, numerous other subpopulations have been described and the M1 and M2 subsets are not fixed.

The products of activated macrophages eliminate injurious agents such as microbes and initiate the process of repair, but are also responsible for much of the tissue injury in chronic inflammation. Several functions of macrophages are central to the development and persistence of chronic inflammation and the accompanying tissue injury.

- **Macrophages secrete mediators of inflammation,** such as cytokines (TNF, IL-1, chemokines, and others) and eicosanoids. Thus, macrophages are central to the initiation and propagation of inflammatory reactions.
- Macrophages **display antigens to T lymphocytes and respond to signals from T cells,** thus setting up a feedback loop that is essential for defense against many microbes by cell-mediated immune responses.

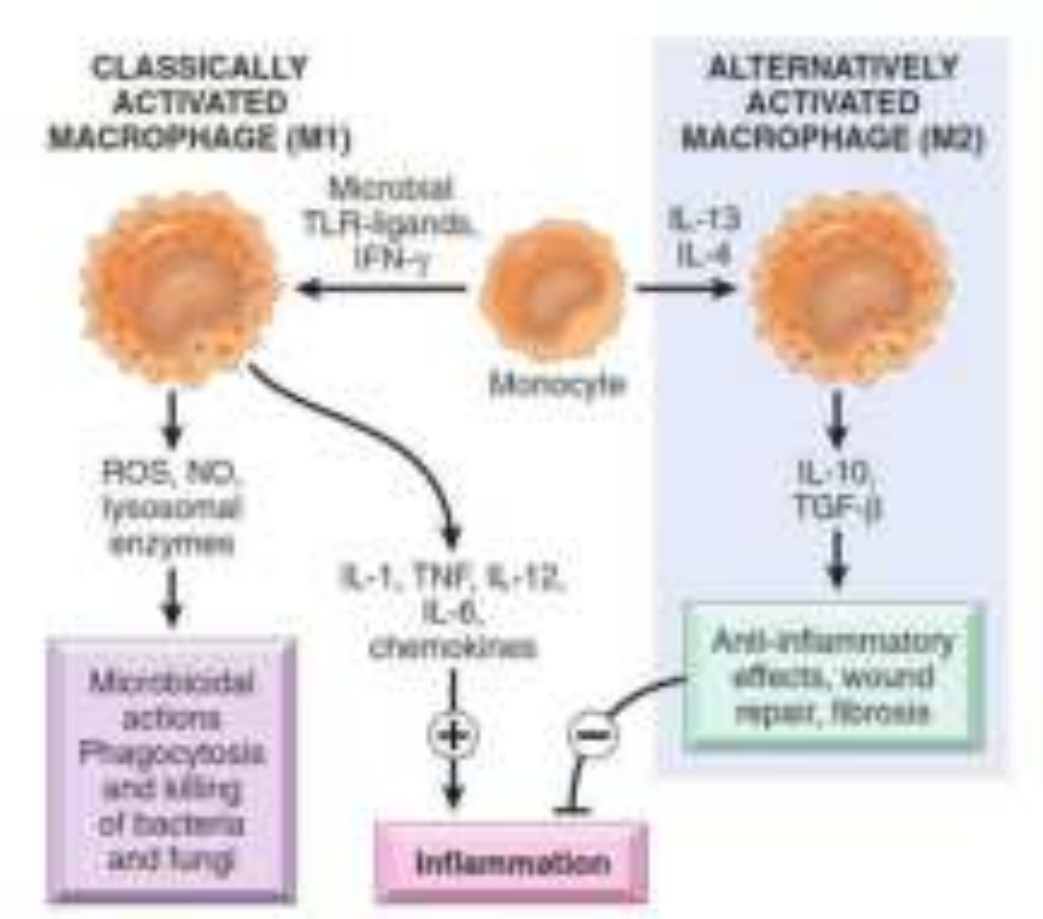


Fig. 1. Classical and alternative macrophage activation.

Chronic Inflammation

Role of Lymphocytes

Microbes and other environmental antigens activate T and B-lymphocytes, which amplify and propagate chronic inflammation. Although the major function of these lymphocytes is as the mediators of adaptive immunity, which provides defense against infectious pathogens these cells, are often present in chronic inflammation and, when they are activated, the inflammation tends to be persistent and severe. Some of the strongest chronic inflammatory reactions, such as granulomatous inflammation are dependent on lymphocyte responses.

Lymphocytes may be the dominant population in the chronic inflammation seen in autoimmune and other hypersensitivity diseases. By virtue of their ability to secrete cytokines, CD4+ T lymphocytes promote inflammation and influence the nature of the inflammatory reaction.

2- Other Cells in Chronic Inflammation

Other cell types may be prominent in chronic inflammation induced by particular stimuli.

- **Eosinophils** are abundant in immune reactions mediated by IgE and in parasitic infections**.**
- **Mast cells** are widely distributed in connective tissues and participate in both acute and chronic inflammatory reactions. Mast cells arise from precursors in the bone marrow.
- Although **neutrophils** are characteristic of acute inflammation, many forms of chronic inflammation, lasting for months, continue to show large numbers of neutrophils, induced either by persistent microbes or by cytokines and other mediators produced by activated macrophages and T lymphocytes. In chronic bacterial infection of bone (osteomyelitis), a neutrophilic exudate can persist for many months. Neutrophils also are important in the chronic damage induced in lungs by smoking and other irritant stimuli. This pattern of inflammation has been called *acute on chronic*.

Chronic Inflammation

Granulomatous Inflammation

Granulomatous inflammation is a form of chronic inflammation characterized by collections of activated macrophages, often with T lymphocytes, and sometimes associated with central necrosis.

There are two types of granulomas, which differ in their pathogenesis.

- **Immune granulomas** are caused by a variety of agents that are capable of inducing a persistent T cell–mediated immune response.
- **Foreign body granulomas** are seen in response to relatively inert foreign bodies, in the absence of T cell–mediated immune responses

Lecture (10)

Tissue Repair: Regeneration, Healing, and Fibrosis

Critical to the survival of an organism is the ability to repair the damage caused by toxic insults and inflammation.

Repair refers to the restoration of tissue architecture and function after an injury. It occurs by two types of reactions (Fig. 1).

Some tissues are able to replace the damaged components and essentially return to a normal state; this process is called ***regeneration***.

If the injured tissues are incapable of complete restitution, or if the supporting structures of the tissue are severely damaged, repair occurs by laying down of connective (fibrous) tissue, a process termed ***healing*** that results in ***scar formation***.

Although the fibrous scar is not normal, it provides enough structural stability that the injured tissue is usually able to function. After many common types of injury, both regeneration and scar formation contribute in varying degrees to the ultimate repair.

The term ***fibrosis*** is most often used to describe the extensive deposition of collagen that occurs in the lungs, liver, kidney, and other organs as a consequence of chronic inflammation, or in the myocardium after extensive ischemic necrosis (infarction). If fibrosis develops in a tissue space occupied by an inflammatory exudate it is called ***organization*** (as in organizing pneumonia affecting the lung).

Repair involves the proliferation of various cells, and close interactions between cells and the extracellular matrix (ECM). Therefore,

an understanding of the process of repair requires some knowledge of the control of cell proliferation and the functions of the ECM.

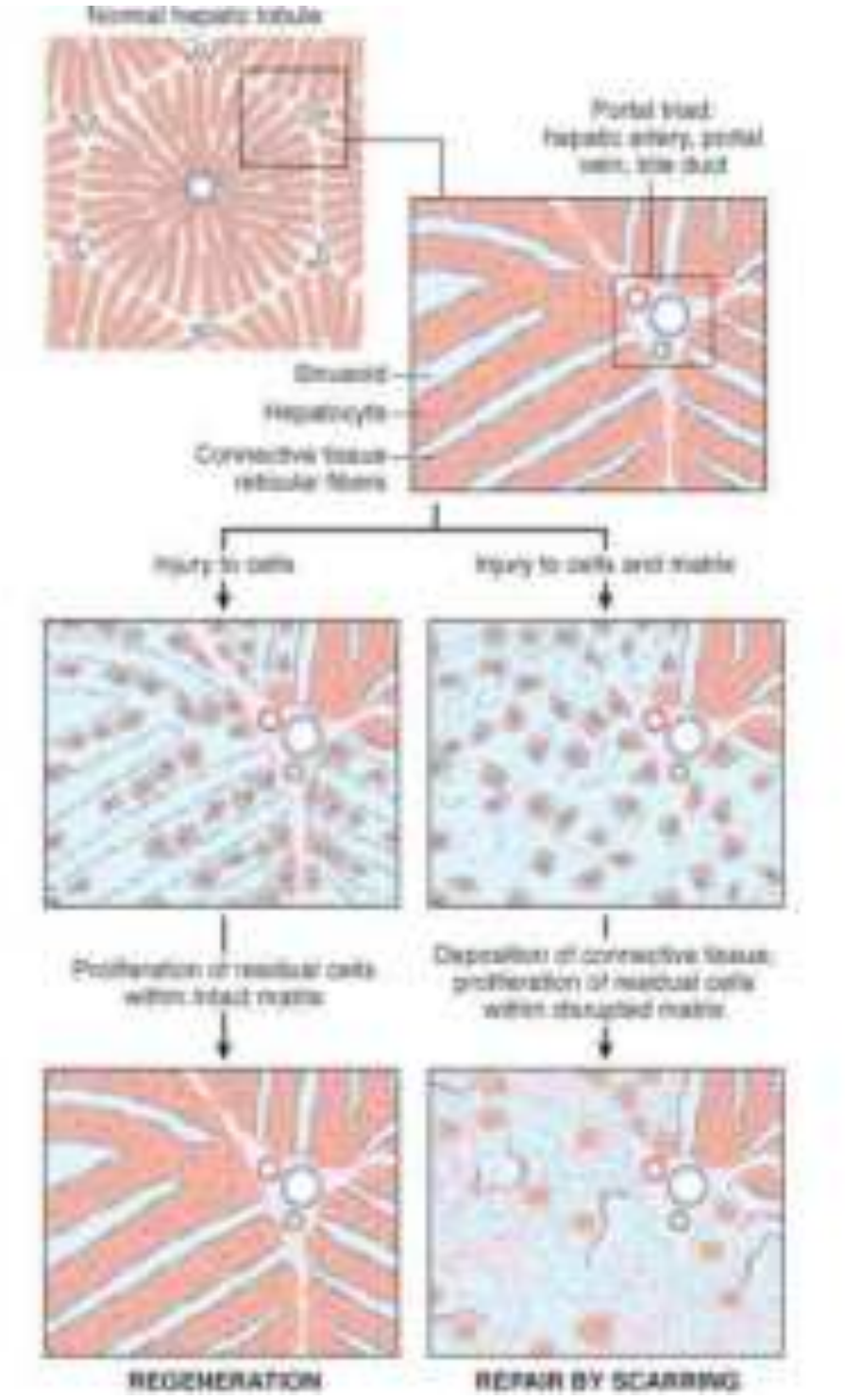


Fig. 1.

Cell proliferation is regulated by cyclins that, when complexed with cyclin-dependent kinases (CDKs), regulate the phosphorylation of proteins involved in cell cycle progression leading to DNA replication and mitosis. The cell cycle is tightly regulated by stimulators and inhibitors, and contains intrinsic checkpoint controls to prevent replication of abnormal cells.

Tissues are divided into labile, stable and permanent, according to the proliferative capacity of their cells. Continuously dividing tissues (labile tissues) contain stem cells that differentiate to replenish lost cells and maintain tissue homeostasis.

Proliferative Capacities of Tissues

The ability of tissues to repair themselves is critically influenced by their intrinsic proliferative capacity. Based on this criterion, the tissues of the body are divided into three groups.

Continuously Dividing Tissues (also known as *labile tissues*)

Cells of these tissues are continuously being lost and replaced by maturation from stem cells and by proliferation of mature cells. Labile cells include hematopoietic cells in the bone marrow and the majority of surface epithelia, such as the stratified squamous surfaces of the skin, oral cavity, vagina, and cervix; the cuboidal epithelia of the ducts draining exocrine organs (e.g., salivary glands, pancreas, biliary tract); the columnar epithelium of the gastrointestinal tract, uterus, and fallopian tubes; and the transitional epithelium of the urinary tract. These tissues can readily regenerate after injury as long as the pool of stem cells is preserved.

Stable Tissues

Cells of these tissues are quiescent (in the G_0 stage of the cell cycle) and have only minimal replicative activity in their normal state. However, these cells are capable of proliferating in response to injury or loss of tissue mass. Stable cells constitute the most solid tissues, such as liver, kidney, and pancreas. They also include endothelial cells, fibroblasts, and smooth muscle cells; the proliferation of these cells is particularly important in wound healing. With the exception of liver, stable tissues have a limited capacity to regenerate after injury.

Permanent Tissues

The cells of these tissues are considered to be terminally differentiated and non-proliferative in postnatal life. The majority of neurons and cardiac muscle cells belong to this category. Thus, injury to brain or heart is irreversible and results in a scar, because myocytes do not divide. Limited stem cell replication and differentiation occurs in some areas of the adult brain, and there is some evidence that heart muscle cells may proliferate after myocardial necrosis. Nevertheless, whatever proliferative capacity may exist in these tissues, it is insufficient to produce tissue regeneration after injury. Skeletal muscle is usually classified as a permanent tissue, but satellite cells attached to the endomysial sheath provide some regenerative capacity for this tissue. In permanent tissues, repair is typically dominated by scar formation.

With the exception of tissues composed primarily of non-dividing permanent cells (e.g., cardiac muscle and nerve), most mature tissues contain variable proportions of three cell types: continuously dividing

cells, quiescent cells that can return to the cell cycle, and non-dividing cells.

Extracellular Matrix and Tissue Repair.

The ECM consists of: the interstitial matrix between cells, made up of collagens and several glycoproteins; and basement membranes underlying epithelia and surrounding vessels, made up of nonfibrillar collagen and laminin. The ECM serves several important functions:

- It provides mechanical support to tissues; this is the role of collagens and elastin.
- It acts as a substrate for cell growth and the formation of tissue microenvironments.
- It regulates cell proliferation and differentiation; proteoglycans bind growth factors and display them at high concentration, and fibronectin and laminin stimulate cells via cellular integrin receptors.

An intact ECM is required for tissue regeneration, and if the ECM is damaged, repair can only be accomplished by scar formation.

Neoplasia

Lect. 11 & 12

Nomenclature
Etiology
Benign Tumors
Malignant Tumors
Characteristics of Benign and Malignant Neoplasms
Metastasis
Criteria used for ctyopathological diagnosis of cancer

Before we discuss the features of cancer cells and the mechanisms of carcinogenesis, it is useful to summarize the fundamental and shared characteristics of cancers:

- **Cancer is a genetic disorder caused by DNA mutations.**
- **Genetic alterations in cancer cells are heritable, being passed to daughter cells upon cell division. As a result, cells harboring these alterations are subject to Darwinian selection.**
- **Mutations and epigenetic alterations impart to cancer cells a set of properties that are referred to collectively as *cancer hallmarks***

Nomenclature:

Neoplasia literally means "new growth." Neoplastic cells are said to be *transformed* because they continue to replicate, apparently oblivious to the regulatory influences that control normal cell growth. Neoplasms therefore enjoy a certain degree of autonomy and tend to increase in size regardless of their local environment. Their autonomy is by no means complete, however. Some neoplasms require endocrine support, and such dependencies sometimes can be exploited therapeutically. All neoplasms depend on the host for their nutrition and blood supply.

In common medical usage, a neoplasm often is referred to as a ***tumor,*** and the study of tumors is called ***oncology*** (from *oncos,* "tumor," and *logos,* "study of").

Among tumors, the division of neoplasms into **benign** and **malignant** categories is based on **a judgment of a tumor's potential clinical behavior**.

• ***A tumor is said to be benign*** when its microscopic and gross characteristics are considered to be relatively **innocent,** implying that it will remain localized and is amenable to local surgical removal; the patient generally survives.

• **Malignant tumors** are collectively referred to as *cancers,* derived from the Latin word for "crab"—that is, they adhere to any part that they seize in an obstinate manner, similar to a crab's behavior. *Malignant,* as applied to a neoplasm, implies that the lesion can invade and destroy adjacent structures and spread to distant sites (**metastasize**) to cause death.

All tumors, benign and malignant, have two basic components:

1- **The *parenchyma*,** made up of transformed or neoplastic cells, and
2- The supporting, host derived, non-neoplastic ***stroma,*** made up of connective tissue, blood vessels, and host-derived inflammatory cells.

The parenchyma of the neoplasm largely determines its biologic behavior, and it is this component from which the tumor derives its name.

The stroma is crucial to the growth of the neoplasm, since it carries the blood supply and provides support for the growth of parenchymal cells.

Etiology:-

The chief etiological factors is the following heading.

1- **Developmental abnormalities**.
2- **Parasites**.
3- **Irritation**.
4- **Hormones**.

Benign Tumors:

In general, benign tumors are designated by attaching the **suffix** -***oma*** to the cell type from which the tumor arises. As example

A benign tumor arising in fibrous tissue is a ***fibroma***;

A benign cartilaginous tumor is a ***chondroma***.

The nomenclature of **benign epithelial tumors** is more complex. They are classified sometimes on **the basis of their microscopic pattern** and others are classified by **their cells of origin**.

1- Benign epithelial tumors:

Adenoma is generally applied to benign **epithelial neoplasms producing gland patterns** and to neoplasms derived from glands but not necessarily exhibiting glandular patterns. A benign epithelial neoplasm arising from renal tubule cells and growing in gland-like patterns is termed an **adenoma**, as is a mass of benign epithelial cells that produces no glandular patterns but has its origin in the adrenal cortex.

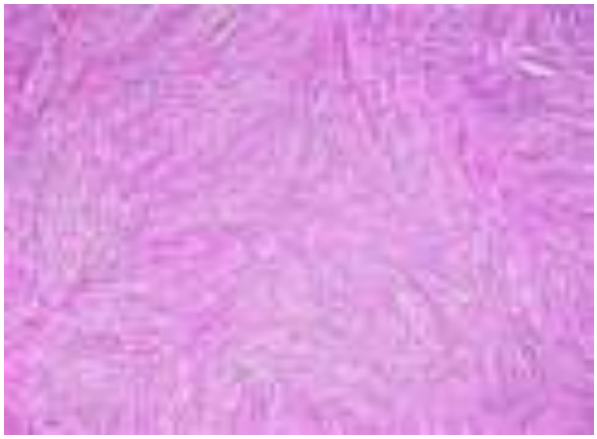
Papillomas are benign epithelial neoplasms, growing on any surface, that produce microscopic or macroscopic finger-like fronds. A *polyp* is a mass that projects above a mucosal surface, as in the gut, to form a macroscopically visible structure (Fig.1).

Although this term commonly is used for benign tumors, some malignant tumors also may grow as polyps, whereas other polyps (such as nasal polyps) are not neoplastic but inflammatory in origin. ***Cystadenomas*** are hollow cystic masses that typically arise in the ovary.

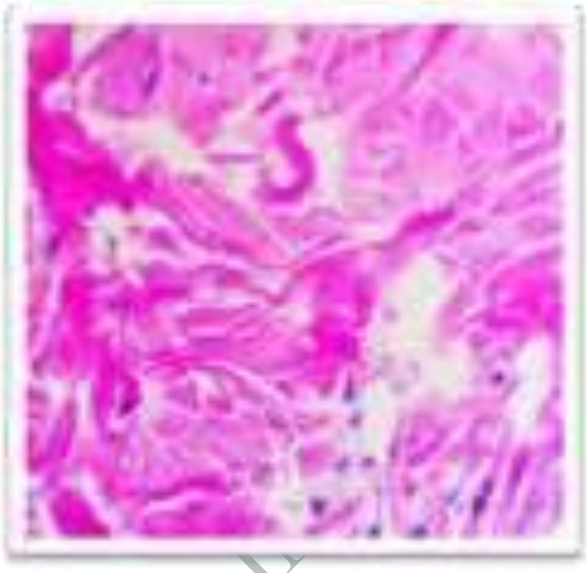
Figure 1 Colonic polyp. This glandular tumor (adenoma) is seen projecting into the colonic lumen. The polyp is attached to the mucosa by a distinct stalk.

Benign connective tissue tumors: include the followings:

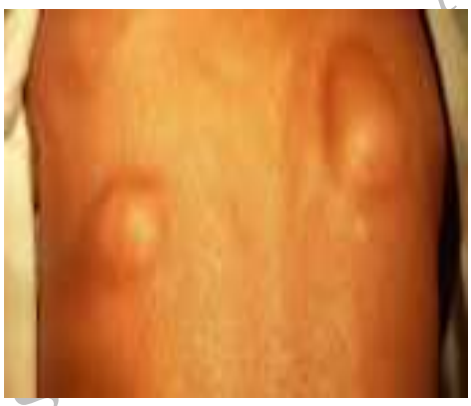
1- Fibroma: it is a neoplasm consisting of white connective tissue fiber and cell, it is hard and soft fibrous and may be round or lobulated by well-define capsule and irregular wavy band of white color or pinker.it can form anywhere in the body and any age either sex.

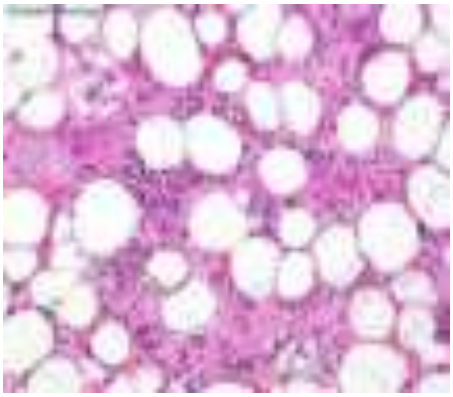


2- **Myxoma**: the structure of which resemble that of embryonic connective tissue, it consist of stellate cell with long interlacing branching process. Most common in the heart, it can be remove by surgical resection.

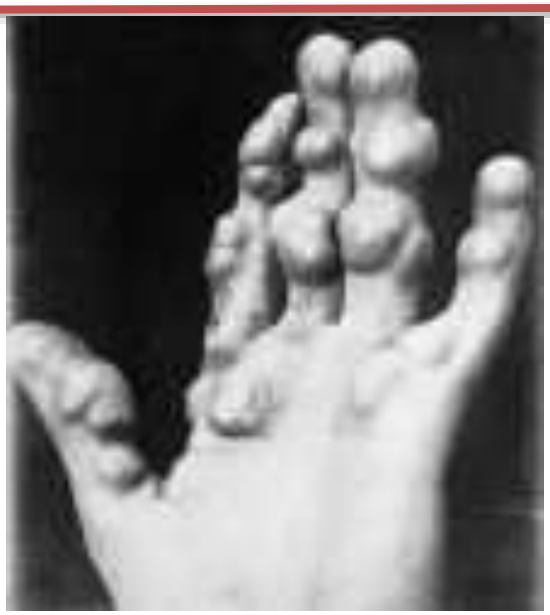


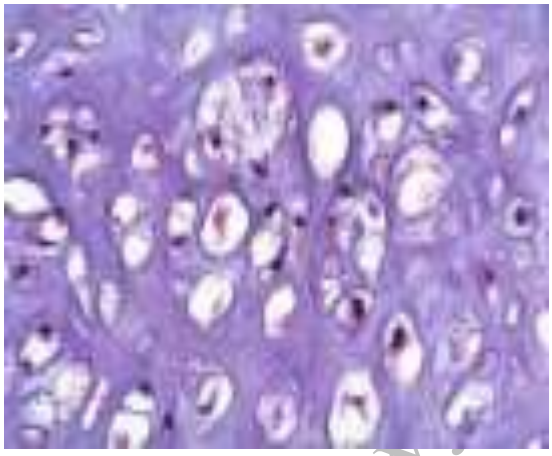
3- **Lipoma** : this is neoplasm consisting from adipose tissue , in general the structure is resemble of the normal , except the fat cell are large and appear to be grouped together into a lobules by strand of fibrous connective tissue . It located in thin fibrous capsule.



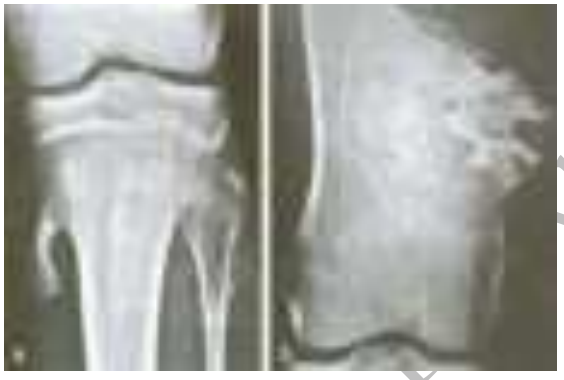


4-**Chondroma**: this tumor consist of island of hyaline cartilage bound together into a solid mass by vascular fibrous tissue and well – defined capsule. It located in soft tissue and synovial of bone.

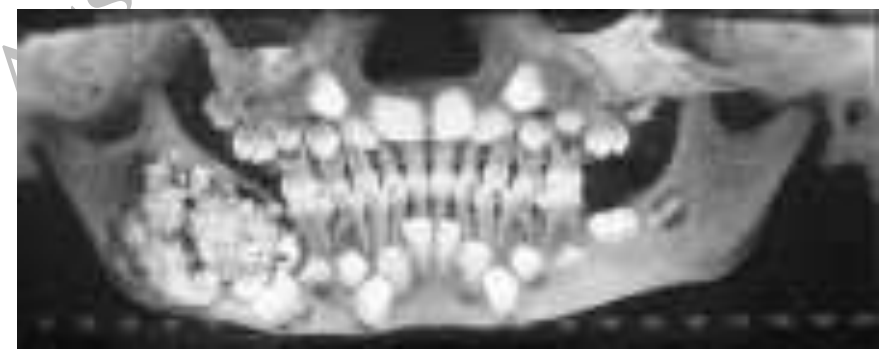




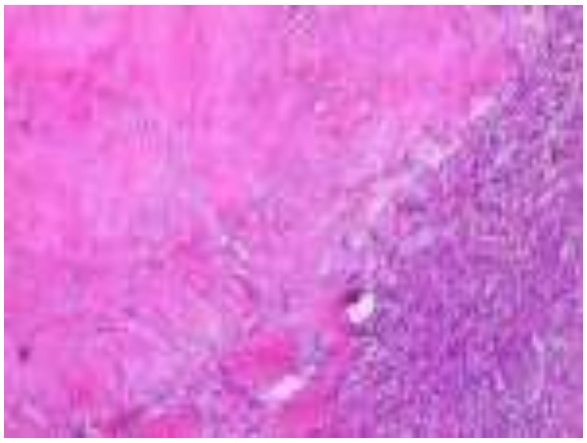
5-**Osteoma** : this tumor is composed of bone tissue .

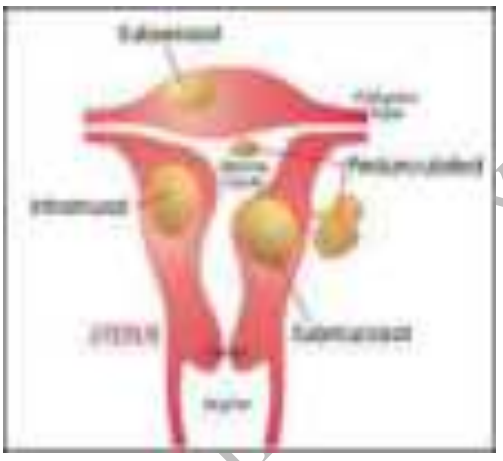


6-**Odontoma**: it a tumor arising from dental tissue, it is small, hard, nodular projection confined to the part of teeth such as crown.

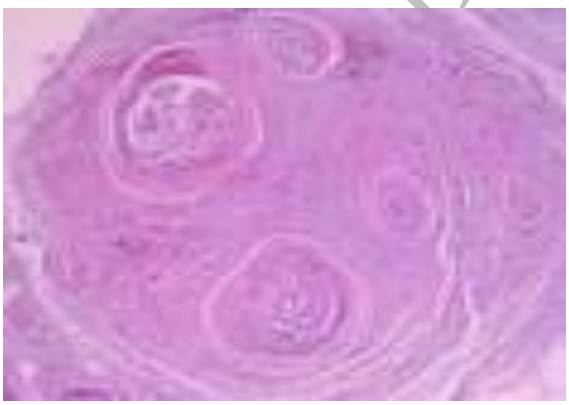


7- **Myoma**: this is tumor composed of muscle tissue, it consist of striated muscle known as **rhabdomyomata**, and in smooth muscle called **leiomyoma** ,the chief site is in uterus, and vagina, intestine and stomach.

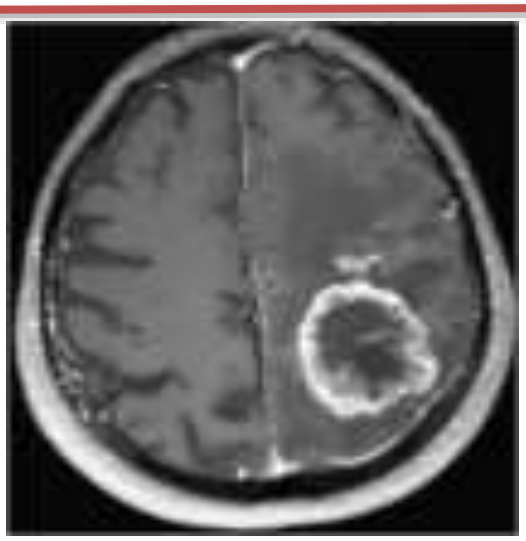


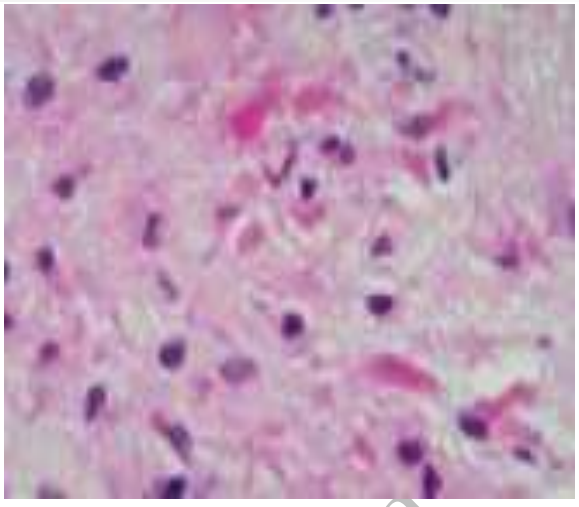


8-**Neuroma**: this is tumor consist of nerve cell and fibers. it is very rare occur . The so-called neuroma is merely of inflammation fibrous tissue which is found at the end of nerve follow neurectomy . It is benign tumor of the nerve tissue, common occur between the 3th and 4th toes. it is referred some time as inter metatarsal , pain and called (Morton's neuroma) .

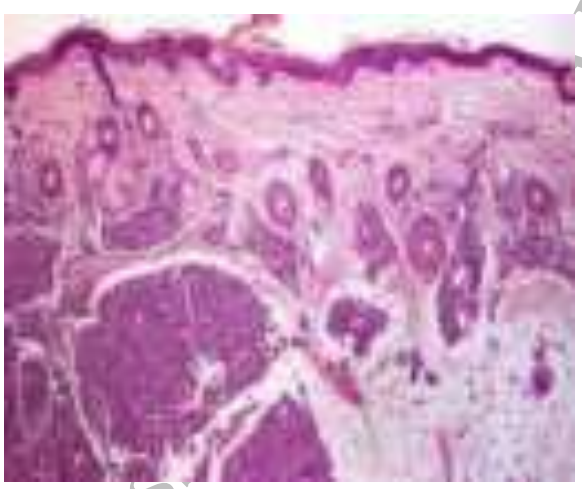


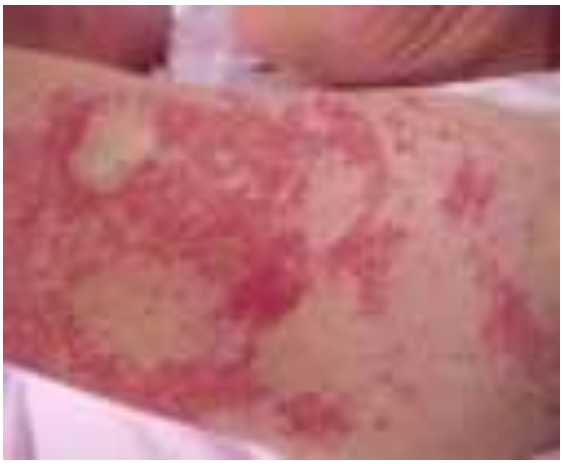
9- **Glioma**: it is neoplasm of connective tissue of the nervous system (neuroglia), it is usually a soft and very vascular growth in which hemorrhage present, the site is brain and spinal cord.





10-**Angioma** : is a tumor of vascular tissue ,consisting of proliferation or dilation blood or lymph vessels , it is two type (haemangioma) for blood vessels and (lymphangioma) for the dilated lymphatic it chiefly occur in skin and subcutaneous tissue .





Malignant Tumors

The nomenclature of malignant tumors essentially follows that of benign tumors, with certain additions and exceptions.

• Malignant neoplasms arising in "solid" mesenchymal tissues or its derivatives are called ***sarcomas,*** whereas those arising from the mesenchymal cells of the blood are called **leukemias** or **lymphomas**. Sarcomas are designated by the cell type of which they are composed, which is presumably their cell of origin. Thus, a cancer of fibrous tissue origin is a ***fibro*sarcoma**, and a malignant neoplasm composed of chondrocytes is a ***chondro*sarcoma.**

• While the epithelia of the body are derived from all three germ cell layers, **malignant neoplasms of epithelial cells** are called ***carcinomas*** regardless of the tissue of origin. Thus, a malignant neoplasm arising in the renal tubular epithelium (mesoderm) is a carcinoma, as are the cancers arising in the skin (ectoderm) and lining epithelium of the gut (endoderm).

• **Carcinomas** are subdivided further. Carcinomas that grow in a glandular pattern are called ***adenocarcinomas***, and those that produce squamous cells are called ***squamous cell carcinomas***. Sometimes the tissue or organ of origin can be identified, as in the designation of renal cell adenocarcinoma. Sometimes the tumor shows little or no differentiation and must be called *poorly differentiated or undifferentiated carcinoma.*

In some unusual instances, however, the tumor cells undergo *divergent differentiation,* creating so called ***mixed tumors***. The best example is **mixed tumor of salivary gland**. These tumors have obvious epithelial components dispersed throughout a **fibromyxoid stroma**, sometimes harboring islands of cartilage or bone (Fig. 2).

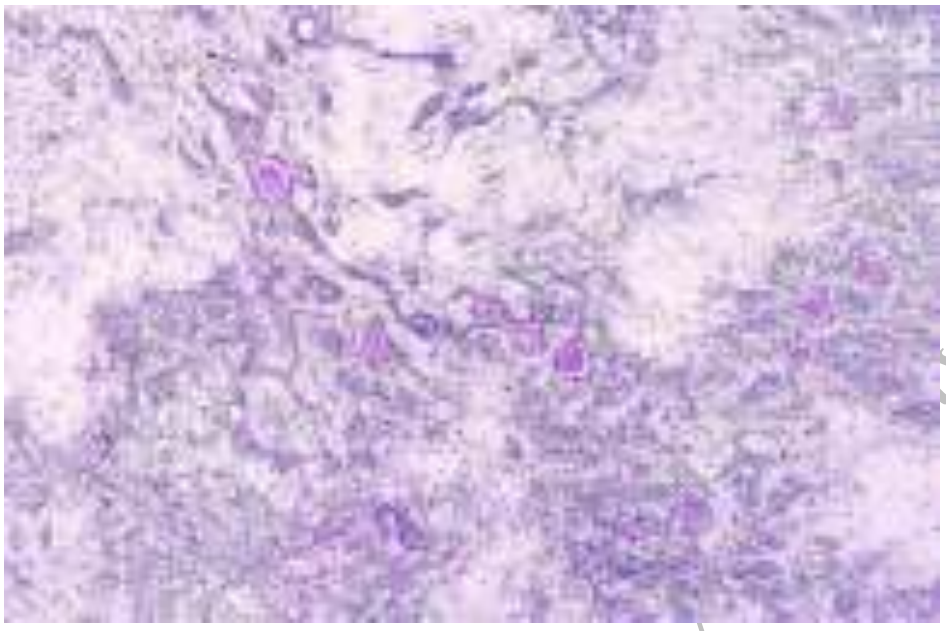


Figure 2. Mixed tumor of the parotid gland contains epithelial cells forming ducts and myxoid stroma that resembles cartilage.

All of these diverse elements are thought to derive from epithelial cells or myoepithelial cells, or both, and the preferred designation for these neoplasms is ***pleomorphic adenoma***.

Fibroadenoma of the female breast is another common mixed tumor.

Teratoma is a special type of mixed tumor that contains recognizable mature or immature cells or tissues representative of more than one germ cell layer and sometimes all three. **Teratomas** originate from totipotential germ cells such as those normally present in the ovary and testis.

The terms **lymphoma** (is a group of blood cancers that develop from lymphocytes),

Mesothelioma, is a type of cancer that develops from the thin layer of tissue that covers many of the internal organs (the mesothelium). The most common area affected is the lining of the lungs and chest wall

Melanoma, is a type of cancer that develops from the pigment-containing cells known as melanocytes.

Seminoma is a germ cell tumor of the testicle or, more rarely, the mediastinum or other extra-gonadal locations

All above are used for malignant neoplasms.

CHARACTERISTICS OF BENIGN AND MALIGNANT NEOPLASMS

There are **four fundamental features** *by which benign and malignant tumors can be distinguished:*

- **Differentiation and anaplasia,**
- **Rate of growth,**
- **Local invasion, and**
- **Metastasis.**

Differences between Benign and Malignant Neoplasm

Benign	Malignant
In well differentiated benign tumors, mitoses are usually rare and are of normal configuration.	wide range of parenchymal cell differentiation, from surprisingly well differentiated to completely undifferentiated
Cells tend not to spread	Cells can spread
Most grow slowly	Usually grow fairly rapidly
Do not invade nearby tissue	Often invade basal membrane that surrounds nearby healthy tissue
Do not metastasize (spread) to other parts of the body	Can spread via bloodstream or lymphatic system, or by sending "fingers" into nearby tissue
Under a pathologist's microscope, shape, chromosomes, and DNA of cells appear normal	Cells have abnormal chromosomes and DNA characterized by large, dark nuclei; may have abnormal shape
Do not secrete hormones or other substances	Can secrete substances that cause fatigue and weight loss (paraneoplastic syndrome)
May not require treatment if not health-threatening	May require aggressive treatment, including surgery, radiation, chemotherapy, and immunotherapy medications
Do not recur after removal.	May recur after removal, sometimes in areas other the original site

Metastasis:

The spread of cancer cells from the place where they first formed to another part of the body. In metastasis, cancer cells break away from the original (primary) tumor, travel through the blood or lymph system, and form a new tumor in other organs or tissues of the body. The new, metastatic tumor is the same type of cancer as the primary tumor. For example, if breast cancer spreads to the lung, the cancer cells in the lung are breast cancer cells, not lung cancer cells.

It is generally distinguished from **cancer invasion**, which is the direct extension and penetration by cancer cells into neighboring tissues

Metastases are secondary implants of a tumor that are discontinuous with the primary tumor and located in remote tissues (Fig. 3).

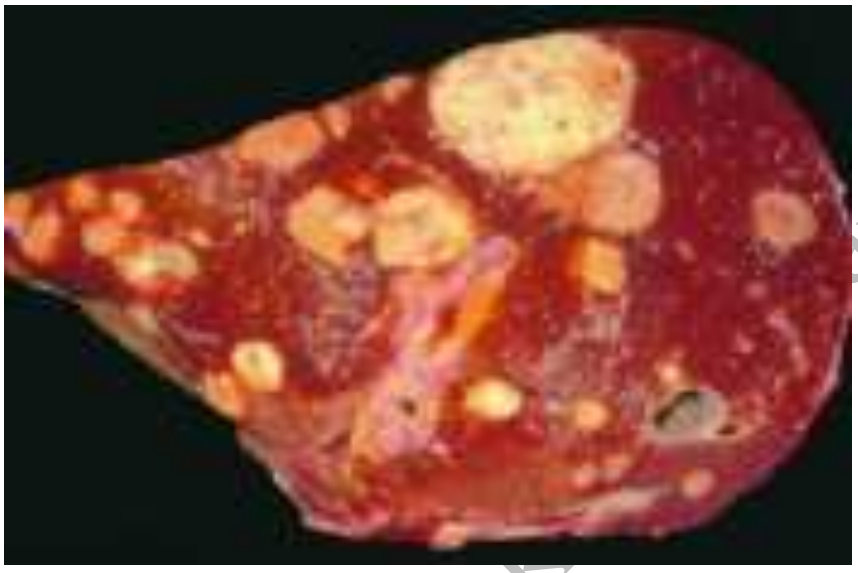


Figure 3. A liver studded with metastatic cancer.

Not all cancers have equivalent ability to metastasize, however. At one extreme are basal cell carcinomas of the skin and most primary tumors of the central nervous system, which **are highly invasive locally but rarely metastasize**. At the other extreme are osteogenic (bone) sarcomas, which usually have **metastasized to the lungs at the time of initial discovery**.

Metastasis occurs by the following three routes:

1- Transcoelomic spread:

The spread of a malignancy into body cavities can occur via penetrating the surface of the peritoneal, pleural, pericardial, or subarachnoid spaces. For example, ovarian tumors can spread transperitoneally to the surface of the liver.

2- Lymphatic spread

Lymphatic spread allows the transport of tumor cells to regional lymph nodes near the primary tumor and ultimately, to other parts of the body. This is called **nodal involvement, positive nodes, or regional disease**.

"Positive nodes" is a term that would be used by medical specialists to describe regional lymph nodes that tested positive for malignancy. It is common medical practice to test by biopsy at least one lymph node near a tumor site when carrying out surgery to examine or remove a tumor. This lymph node is then called **a sentinel lymph node**.

Lymphatic spread is the **most common route of initial metastasis for carcinomas**. In contrast, it is **uncommon for a sarcoma to metastasize via this route**. Localized spread to regional lymph nodes near the primary tumor is not normally counted as a metastasis, although this is a sign of a worse outcome.

The lymphatic system does eventually drain from the thoracic duct and right lymphatic duct into the systemic venous system at the venous angle and into the brachiocephalic veins, and therefore these metastatic cells can also eventually spread through the **haematogenous route**.

Figure 4. Lymph node with almost complete replacement by metastatic melanoma. The brown pigment is focal deposition of melanin

3- Hematogenous spread

This is typical route of metastasis for sarcomas, but it is also the favored route for certain types of carcinoma, such as **renal cell carcinoma** originating in the kidney. Because of their thinner walls, veins are more frequently invaded than are arteries, and metastasis tends to follow the pattern of venous flow. That is, hematogenous spread often follows distinct patterns depending on the location of the primary tumor. For example, colorectal cancer spreads primarily through the portal vein to the liver.

Criteria used for cytopathological diagnosis of cancer:

Laboratory diagnosis: the pathologist studies the section paying special attention to

- The cytological appearance of the tumor cell
- The relation of these cell to the surround tissue especially as regard the invasion of this tissue by the tumor cell.

Biopsy: the biopsy method of frozen section is invaluable particularly in the case of Brest tumor, it has however limited which should be recognized, particularly by the surgeon. The frozen section are twice the thickness of those of the paraffin method. The examination of several block from different part of the tissue is seldom possible the rushed atmosphere of the operation room.

In cytology, nuclear criteria serve as a guide as in order to establish diagnosis of malignant tumor, these criteria include:

1. **Anisokaryosis**: (Nucleo-cytoplasm ratio which is variable or high macrokaryosis), nuclear greater than 10 micron in diameter.
2. **Multi nuclei** in a single a nucleus, especially present variability in size within the same cell.
3. **Elevated mitotic index**, the present of abnormal mitotic figures asymmetric in appropriately aligned.
4. **Prominent nucleoli**, the present of irregular or angular nucleoli, the present of variable nuclear size with in a nucleus, nuclear molding (deformation of nuclear next to each other with in cell) a coarse or

blotchy chromatin pattern large cell carcinoma of the lung, malignant cell exhibit prominent nucleoli. Sarcoma diffuse proliferation of large round to oval cell with pleomorphic vesicular nuclei prominent nucleoli and abundant eosinophilic cytoplasm.

5. **Nuclear fragmentation or budding** can also be observe and correspond to an irregular nuclear shape, variability in cell size can also observed in malignant tumor. However they are not exclusive to these general characteristic of neoplastic cell nucleoli.
6. **Anisocytosis** (variability in cell size), macrocytosis (the present of large cell, hypercellularity , the present of highly cellular sample . and polymorphism , the present of cell of variable shape in sample.
7. **Hyperchromasia**. refer to the dark staining nuclei which is usually due to increase DNA content , in this example of small cell carcinoma of the lung , all of the tumor cell exhibited darkly stained nuclei and the cell have very little cytoplasm , another feature commonly seen in malignant cells increase mitotic activity is also present .
8. **Mitotic are often numerous** in malignant process and reflect increase proliferative activity.

TISSUE PROCESS:

Preservation

Fixation

Fixatives

Methods of Fixation

Sectioning and Mounting

Staining

Types of Staining

An Introduction to Specimen Processing

Microscopic analysis of cells and tissues requires the preparation of very thin, high-quality sections (slices) mounted on glass slides and appropriately stained to demonstrate normal and abnormal structures.

Most fresh tissue is very delicate and easily distorted and damaged, and it is thus impossible to prepare thin sections from it unless it is chemically preserved or “fixed” and supported in some way whilst it is being cut. Broadly, there are two strategies that can be employed to provide this support:

1. We can freeze the tissue and keep it frozen while we cut our sections. These sections are called “frozen sections”.
2. Alternatively, we can infiltrate our tissue specimen with a liquid agent that can subsequently be converted into a solid that has appropriate physical properties, which will allow thin sections to be cut from it. Paraffin wax is such an agent. This produces so-called “paraffin sections”.

Introduction

“Tissue processing” describes the steps required to take an animal or human tissue from fixation to the state where it is completely infiltrated with a suitable histological wax and can be embedded ready for section cutting on the microtome.

Tissue processing can be performed manually (hand processing), but where multiple specimens must be dealt with, it is more convenient and much more efficient to use an automated tissue processing machine (a “tissue processor”). These devices have been available since the 1940’s[1] and have slowly evolved to be safer in use, handle larger specimen numbers, process more quickly, and to produce better quality outcomes. There are two main types of processors: the tissue-transfer (or “dip and dunk”) machines where specimens are transferred from container to container to be processed, and the fluid-transfer (or “enclosed”) types where specimens are held in a single process chamber or retort and fluids are pumped in and out as required. Most modern fluid-transfer processors employ raised temperatures, effective fluid circulation and incorporate vacuum/pressure cycles to enhance processing and reduce processing times.

The importance of tissue processing

Most laboratory supervisors would emphasize to their staff the importance of tissue processing. It is worthwhile to stress that the use of an inappropriate processing schedule or the making of a fundamental mistake (perhaps in replenishing or sequencing of processing reagents) can result in the production of tissue specimens that cannot be sectioned and therefore will not provide any useful microscopic information. This can be disastrous if you are dealing with human diagnostic tissue where the whole of the specimen has been processed (“all in”). There is no spare tissue. There is no diagnosis. There is, however, a patient to whom an explanation has to be provided.

Although mechanical or electrical faults occasionally occur in tissue processors, processing mishaps where tissues are actually compromised mainly occur because of human error. It is important to emphasize the value of proper education and training for those carrying out tissue processing and the need to apply particular care when setting up a processor for any processing run.

Overview of the steps in tissue processing for paraffin sections

1. Obtaining a fresh specimen

Fresh tissue specimens will come from various sources. It should be noted that they can very easily be damaged during removal from the patient or experimental animal. It is important that they are handled carefully and appropriately fixed as soon as possible after dissection. Ideally, fixation should take place at the site of removal, perhaps in the

operating theatre, or, if this is not possible, immediately following transport to the laboratory.

2. Fixation

The specimen is placed in a liquid fixing agent (fixative) such as formaldehyde solution (formalin). This will slowly penetrate the tissue causing chemical and physical changes that will harden and preserve the tissue and protect it against subsequent processing steps.[2] There are a limited number of reagents that can be used for fixation as they must possess particular properties that make them suitable for this purpose. For example, tissue components must retain some chemical reactivity so that specific staining techniques can be applied subsequently.[3] Formalin, usually as a phosphate-buffered solution, is the most popular fixative for preserving tissues that will be processed to prepare paraffin sections. Ideally, specimens should remain in fixative for long enough for the fixative to penetrate into every part of the tissue and then for an additional period to allow the chemical reactions of fixation to reach equilibrium (fixation time). Generally, this will mean that the specimen should fix for between 6 and 24 hours. Most laboratories will use a fixative step as the first station on their processor.

Following fixation, the specimens may require further dissection to select appropriate areas for examination. Specimens that are to be processed will be placed in suitably labeled cassettes (small perforated baskets) to segregate them from other specimens. The duration of the processing schedule used to process the specimens will depend on the type and dimensions of the largest and smallest specimens, the particular processor employed, the solvents chosen, the solvent temperatures, and other factors. The following example is based on a six-hour schedule suitable for use on a Leica Peloris™ rapid tissue processor.

3. Dehydration

Because melted paraffin wax is hydrophobic (immiscible with water), most of the water in a specimen must be removed before it can be infiltrated with wax. This process is commonly carried out by immersing specimens in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached. Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. A series of increasing concentrations is used to avoid excessive distortion of the tissue.

A typical dehydration sequence for specimens not more than 4mm thick would be:

1. 70% ethanol 15 min
2. 90% ethanol 15 min
3. 100% ethanol 15 min
4. 100% ethanol 15 min

5. 100% ethanol 30 min
6. 100% ethanol 45 min

At this point, all but a tiny residue of tightly bound (molecular) water should have been removed from the specimen.

4. Clearing

Unfortunately, although the tissue is now essentially water-free, we still cannot infiltrate it with wax because wax and ethanol are largely immiscible. We, therefore, have to use an intermediate solvent that is fully miscible with both ethanol and paraffin wax. This solvent will displace the ethanol in the tissue, then this, in turn, will be displaced by molten paraffin wax. This stage in the process is called “clearing” and the reagent used is called a “clearing agent”. The term “clearing” was chosen because many (but not all) clearing agents impart an optical clarity or transparency to the tissue due to their relatively high refractive index. Another important role of the clearing agent is to remove a substantial amount of fat from the tissue, which otherwise presents a barrier to wax infiltration.

A popular clearing agent is xylene, and multiple changes are required to completely displace ethanol.

A typical clearing sequence for specimens not more than 4mm thick would be:

1. xylene 20 min
2. xylene 20 min
3. xylene 45 min

5. Wax infiltration

The tissue can now be infiltrated with a suitable histological wax. Although many different reagents have been evaluated and used for this purpose over many years, the paraffin wax-based histological waxes are the most popular. A typical wax is liquid at 60°C and can be infiltrated into tissue at this temperature then allowed to cool to 20°C, where it solidifies to a consistency that allows sections to be consistently cut. These waxes are mixtures of purified paraffin wax and various additives that may include resins such as styrene or polyethylene. It should be appreciated that these wax formulations have very particular physical properties which allow tissues infiltrated with the wax to be sectioned at a thickness down to at least 2 µm, to form ribbons as the sections are cut on the microtome, and to retain sufficient elasticity to flatten fully during flotation on a warm water bath.

A typical infiltration sequence for specimens not more than 4mm thick would be:

1. wax 30 min

2. wax 30 min
3. wax 45 min

6. Embedding or blocking out

Now that the specimen is thoroughly infiltrated with wax, it must be formed into a "block" which can be clamped into a microtome for section cutting. This step is carried out using an "embedding centre" where a mold is filled with molten wax and the specimen placed into it. The specimen is very carefully orientated in the mold because its placement will determine the "plane of section", an important consideration in both diagnostic and research histology. A cassette is placed on top of the mold, topped up with more wax, and the whole thing is placed on a cold plate to solidify. When this is completed, the block with its attached cassette can be removed from the mold and is ready for microtomy. It should be noted that, if tissue processing is properly carried out, the wax blocks containing the tissue specimens are very stable and represent an important source of archival material.

"Xylene-free" processing

Although xylene is used widely as a clearing agent for tissue, processing it is a toxic reagent. Some laboratories prefer to use less-toxic alternatives such as isopropanol or other xylene substitutes. For this method to be successful, higher wax temperatures are required so that isopropanol can be eliminated from specimens during infiltration.

High-quality tissue processing is critical for accurate diagnosis

The combined effects of fixation and processing are to harden the tissue, and it is inevitable that shrinkage will also occur. It has been estimated that tissues shrink as much as 20% or more by the time they are infiltrated with wax[4]. Notwithstanding these effects, sections prepared from optimally processed tissues will consistently show excellent morphological detail, which allows comparisons to be made between specimens and accurate histopathological diagnoses to be determined.

In theory and practice, the paraffin blocks that will be easiest to section contain relatively homogenous tissue of uniform soft consistency (such as kidney), which, when infiltrated with wax, have a consistency similar to that of solidified wax alone (not containing tissue). Tissues of a dense or fibrous nature or a specimen where both hard and soft tissue are present in discrete layers can pose more of a challenge because parts of them are not so well supported by the solidified wax. Differential shrinkage of the various elements in these blocks during fixation and processing contributes to the problems that might be experienced when they are being sectioned.

Steps to Better Processing and Embedding

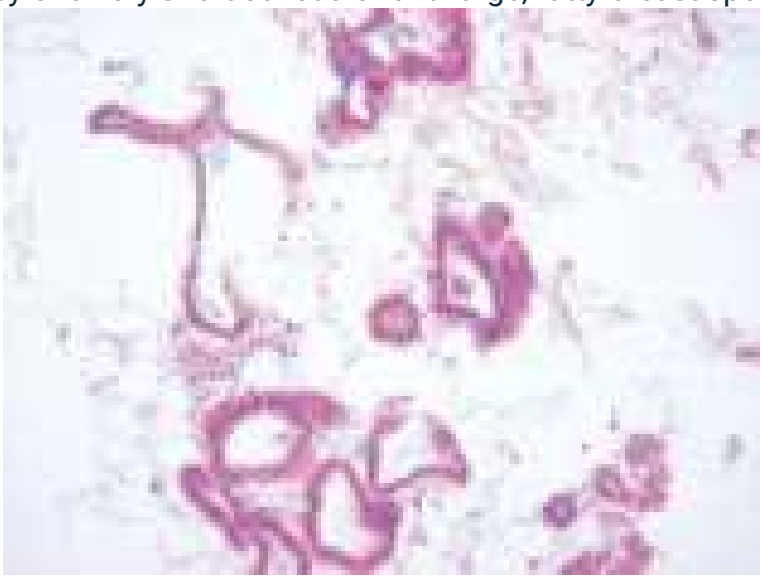
From patient to pathologist, preparing tissue specimens for histological examination requires care, skill and sound procedures. This guide provides practical advice on best-practice techniques and simple ways to avoid common errors.

Tips for better tissue processing and embedding are highlighted in this guide. We hope each step provides a valuable reminder of good histology practice and helps with troubleshooting when unacceptable results do occur.

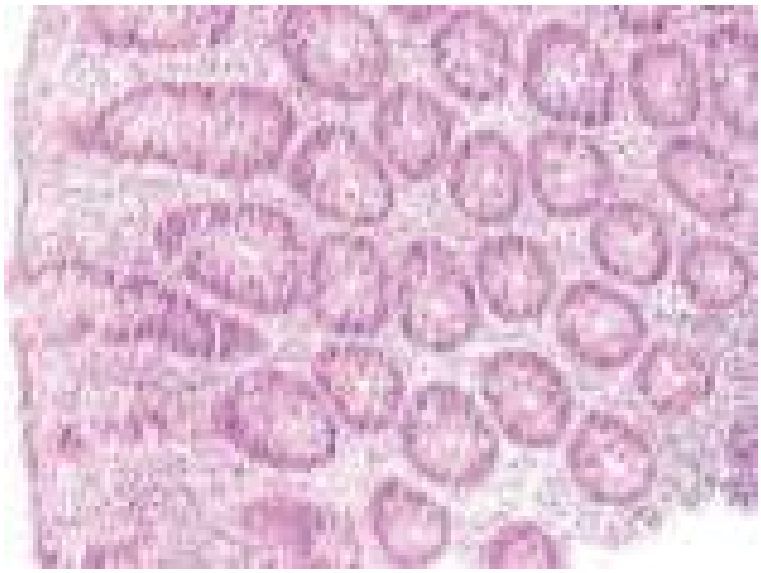
Use an Appropriate Schedule

An appropriate schedule is chosen for the tissue type and size.

An inappropriate schedule is chosen. For example, a very long schedule for a small endoscopic biopsy or a very short schedule for a large, fatty breast specimen.



This micrograph of a small area of subcutaneous tissue from a large, fatty specimen shows the effects of under-processing. The fibro-fatty tissue is poorly supported and therefore fragmented while the epithelial tissue of the glands shows a lack of nuclear definition and peculiar staining due to retained solvent (H&E).



This endoscopic biopsy has been over-processed and has become very brittle. As a consequence, many fine cracks are visible throughout the section. Poor microtomy technique will exacerbate the problem (H&E).

Provide Additional Fixation

For optimal processing and good morphology, tissue should be well fixed before processing. Where specimens are incompletely fixed, additional formalin fixation is provided in the processing schedule.

Incompletely fixed specimens go directly into alcohol producing zonal fixation formalin fixation for the outside of the specimen, alcohol fixation for deeper areas).

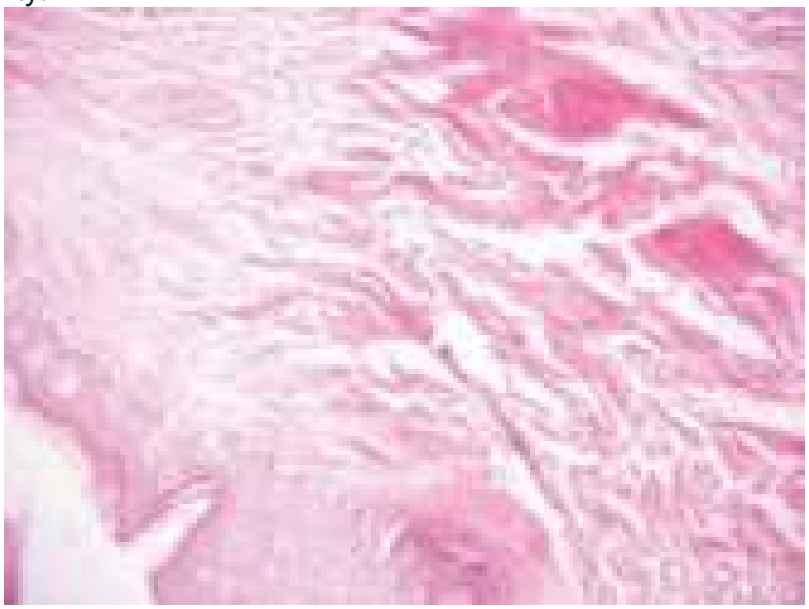
This micrograph shows the effects of zonal fixation on a section of a marrow aspirate (H&E). In the upper left portion, the red cells are intact whereas in the lower part, they are hemolyzed.

This micrograph shows a low power view of liver stained with a trichrome stain. The staining result in the outer zone of the specimen is different to that of the inner.

Maintain Reagent Quality

Processing reagents are replaced strictly according to established guidelines (ideally using are agent management system in an advanced tissue processor such as Leica Biosystems PELORIS).

Guidelines for the placement of processing reagents are ignored, meaning that ineffective, contaminated or diluted reagents are used (e.g. “out-of-threshold” warnings from the PELORIS reagent management system are ignored). This can cause poor processing quality.



In this section – from a large skin specimen – the poor preservation of the dense collagen is due to inadequate processing. In this case, we believe it was due to the use of heavily contaminated reagent swell “out-of-threshold”.

Use High-Quality Wax

High-quality wax is used for infiltration and especially for embedding (blocking out) to ensure high-quality blocks that are easy to cut.

Cheap, poor quality wax from little-known sources is used for infiltration and embedding. Poor quality wax produces blocks that are difficult to cut.

A ribbon of sections was slowly cut from this block while the block was cold. The sections show considerable compression despite the low temperature used. Here the poor quality wax failed to properly support the tissue.

Avoid Hazardous Reagent

Where possible, xylene-free protocols are used (such as those available when using Leica Biosystems PELORIS). This provides a safer laboratory environment without compromising processing quality.

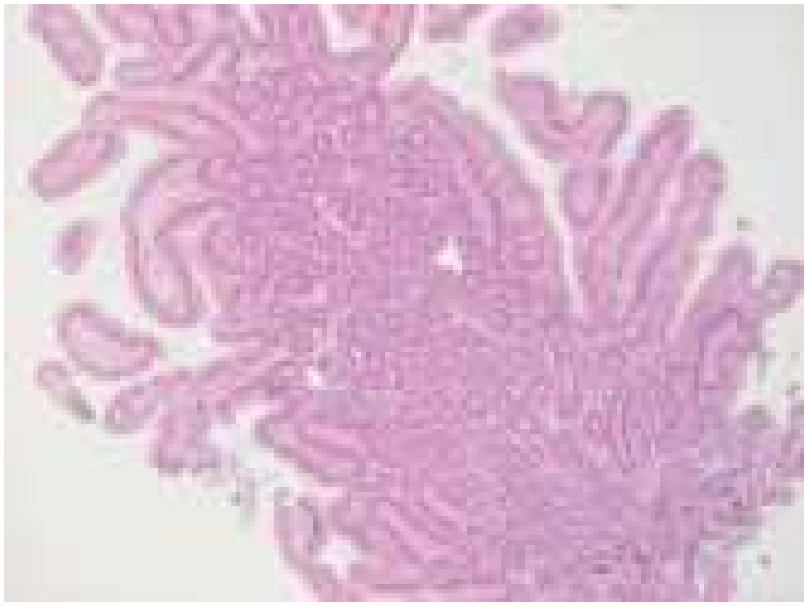
No consideration is given to the health effects of xylene use. The possibility of using alternatives has not been considered.

Xylene-free processing can improve laboratory safety while maintaining quality.

Orientate Specimens Carefully

Specimens are carefully orientated. Competent grossing ensures flat surfaces on most specimens. Staff performing embedding have ready access to each specimen description and are appropriately trained.

Orientation is incorrect. This can result in loss of tissue as re-embedding is required. Some poorly prepared specimens require extensive trimming on the microtome to obtain a full-face section.


This endoscopic biopsy has been orientated incorrectly and shows only the superficial level of the mucosa.

Choose an Appropriate Mold

A mold of suitable size is always chosen for each specimen.

The same mold size is used for every specimen. Often the tissue touches the edge of the mold.

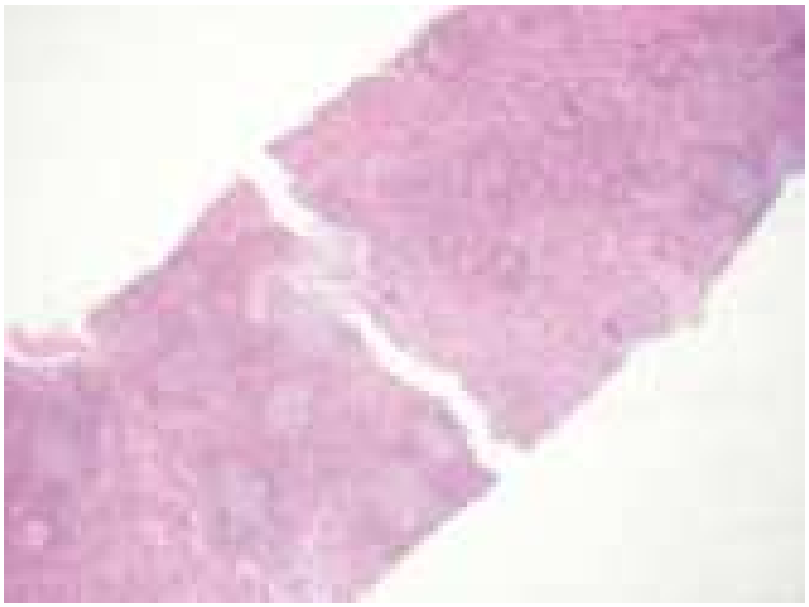
The mold used for this specimen was too small. The specimen is in contact with the edges of the block and may therefore be difficult to section.

Molds of different sizes are available for a variety of specimen sizes.

Handle Specimens Gently

Specimens are handled gently during embedding.

Specimens are handled forcefully during embedding to make them lie flat in the mold. Some tissue can be fractured by this process.

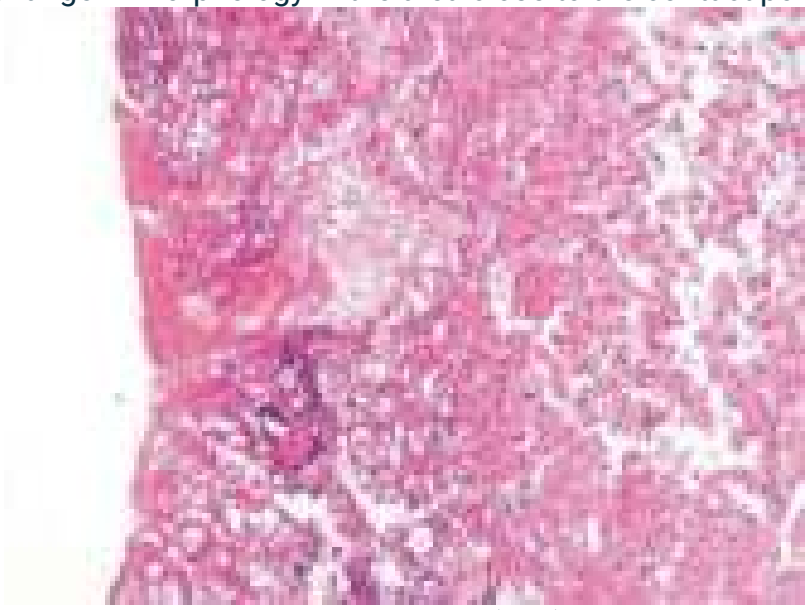


An H&E stained section of spleen was fractured during embedding in an attempt to make the specimen lie flat on the base of the mold.

Avoid Excessive Heat

Before handling tissue, forceps are heated to the point where the wax just melts.

Forceps are heated well beyond the melting point of wax. This can cause local heat damage and a change in morphology in the area close to the contact point.

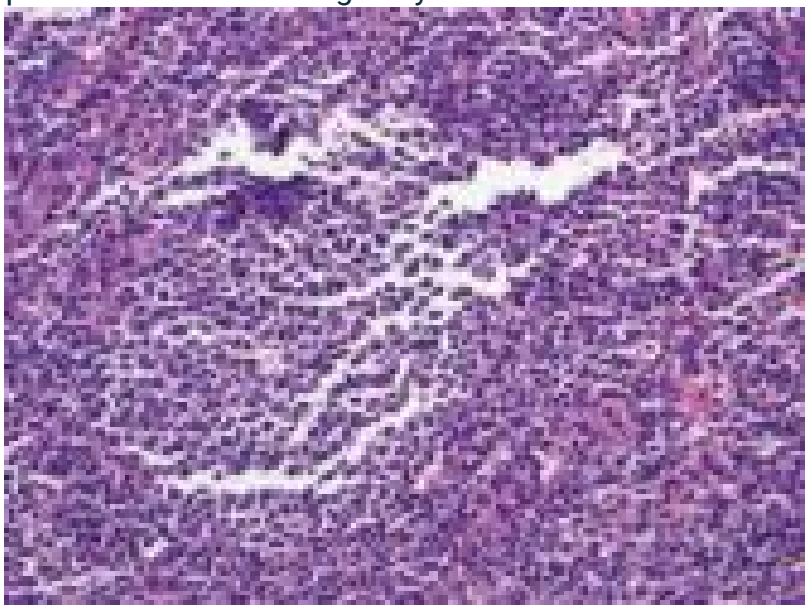


This micrograph shows the surface of a section of liver (H&E). Extreme local damage (making the tissue almost unrecognizable) has been caused by the application of heat to the tissue during embedding.

Check Temperatures Regularly

The temperature of the embedding center hot plate and wax reservoir is regularly checked.

The temperature of the embedding center hot plate is never checked. Even at this stage of processing, specimens can be damaged by excessive local heat.



This lymph node was damaged by over-heating of the embedding center hot plate. Note the shriveled, pyknotic nuclei and extensive cracking. Cracking like this can also be caused by flotation on a water bath that is too warm or by drying on a hot plate without sufficient draining.

Do Not Over-fill Molds

Molds are filled to an optimum level and do not overflow.

Molds are over-filled, requiring scraping of the back and edges of the cassette prior to microtomy. Over-filled blocks may sit unevenly in the microtome chuck, causing instability that may lead to the tissue becoming damaged during microtomy.