

MINISTRY OF HEALTH OF THE KYRGHYZ REPUBLIC
MINISTRY OF EDUCATION AND SCIENCE OF KYRGHYZ REPUBLIC
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Approved by
at a meeting of the Training
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SYLLABUS

Discipline _____ Pathological Anatomy _____

Specialty _____ Morphology _____

Course _____ 2nd & 3rd year _____

Semester _____ 3rd 4th 5th and 6th _____

Credits _____ 4 _____

Total hours for the curriculum __ 120 hours every semester _____

Lectures _____ 34 every semester _____

Practical _____ 50 every semester _____

Independent work _____ 36 every semester _____

Reviewed and approved on the cafedral session of Medical disciplines of the IMU Protocol №
_____ from _____ 2022.

Head of department _____ Almaz Duishenov. _____

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TRAINING AND METODOLOGY COMPLEX

In the discipline “PATHOLOGICAL Anatomy”

Direction: “Pathological Anatomy”

The educational and methodological complex is compiled on the basis of the State educational standard of higher professional education of the Kyrgyz Republic in the specialty “General Medicine” (3 years)

The educational-methodical complex was developed at the department: “Morphology”

Developed by: Dr. Adnan Sami & Dr. Ramsha Rao

Dear Students!

Pathology is the medical science that deals with all aspects of disease, but with special reference to the essential nature, the causes, and the development of abnormal conditions. In this sense, knowledge of pathology is the base of research for the student of medical science and practice. This book contains material in general pathology in the context of modern biology and physiology.

To help the reader in understanding and retaining complex and detailed information, we have illustrated book by photomicrographs and graphic representations of pathologic alterations. Every photograph included in this work illustrates an important morphologic entity.

In this book, we tried to represent the classical morphologic descriptions of disease and contemporary scientific concepts that serves to join tradi-

tional pathology with the modern revolution in biology.

We hope that this book will be useful for you – medical students.

PATHOLOGICAL ANATOMY – is a part of pathology (“pathos”-disease), studying the different aspects of disease. Pathological anatomy studies structural (material) bases of diseases. This study serves as to theory of medicine, as to clinical practice. Due to this pathological anatomy is a scientific-applied subject.

Theoretical, scientific value of pathological anatomy is revealed in study of common natural laws of cell pathology development, pathological processes and disease (i.m. human general pathology). Human general pathology (pathology of cells and morphology of common pathological processes) is a content of general pathological anatomy course.

Clinical value of pathological anatomy is the study of structural bases of human disease, specific features of each disease or creation of patient anatomy (clinical anatomy). It is a content of special pathology.

The study of general and special pathology are correlated because of common pathological processes are the contents as to syndromes, as of human disease. The study of syndromes and disease structural bases is connected with their clinical features. So, clinical-anatomical direction is characteristic feature of modern pathological anatomy.

Disease is considered to be the human organism vital functions disturbances. And structural and functional changes in disease are correlated. Functional changes without the structural changes are absent. This is the base of one of the pathological anatomy principle – entirety and correlation of structure and function.

The study of pathological processes and disease includes:

- the causes of their appearance (etiology),
- mechanisms of development (pathogenesis),
- morphological bases of this mechanisms (morphogenesis),
- different outcomes of disease healing or recovering and its mechanisms (sanogenesis),
- disablement,
- complications,
- death and the mechanisms of death (tanatogenesis).

One of the tasks of PA is the elaboration of study about the diagnose.

PA pays attention on variability of diseases (pathomorphosis) and diseases, the cause of which is a doctor activity (pathology of therapy).

Pathomorphosis reflects:

1. changes in the morbidity and mortality structure,
2. Changes of the clinico-morphological features of disease due to use

of medicines (therapeutic pathomorphosis).

OBJECTS, METHODS AND LEVELS OF PATHOLOGICAL ANATOMY INVESTIGATION

Pathological anatomy takes material for investigation from autopsy, surgical operations, biopsy and experimental investigations.

Autopsy (greek: “autopsia” – to see by proper eyes) gives information about as severe changes, causing the patients death, as initial one, which are revealed microscopically (more frequently). Due to this the study of many diseases stages is possible. Organs and tissues taken at autopsy are studied as macroscopically as microscopically with use of light microscopic methods.

Autopsy determines the causes of patient death, peculiarities of disease course, effectiveness of medicine therapy, diagnostic methods, and makes up the statistics of mortality.

Biopsy (“bios” – life, “opsis” – vision) is diagnostic vital investigation of tissue. Biopsy is performed at all medical departments to prove or to determine clinical diagnose, to receive the information about the course of disease, its character and prognosis, expediency of use and effectiveness of therapy, and gives the information about possible negative effects of drugs.

Biopsy gives the possibility to study initial changes of cells and tissues by electron microscopy, histochemical, histoimmunochemical and enzymological methods, to study those initial changes of disease the clinical manifestations of which are absent due to development of compensatory-adaptive processes.

The modern pathological anatomy methods give the functional valuation of changed structures due to disease, to receive the information about essence and pathogenesis of pathological process, as about degree of compensation of disturbed functions.

So, biopsy material is one of the main object of investigation as for practical, as for theoretical pathological anatomy.

Experiment is very important to clear pathogenesis and morphogenesis of disease. To make up the adequate model of human disease is very difficult, but the patterns of many diseases have been formed. They help to understand deeply the pathogenesis and morphogenesis of diseases. The experimental patterns give possibility to study the action of some drugs, to make up the methods of surgical operations. So, modern pathological anatomy is clinical pathology.

The study of structural bases of disease is performed at different levels: *organism, systemic, organ, tissue, cellular, subcellular, molecular*.

Organism level allows to see the disease of entire organism, its different manifestations and correlation of organs and systems.

Systemic level – the level of study of any system of organs and tissues, combined by common functions (for example: system of connective tissue, system of blood, digestive system).

Organ level allows revealing the changes of organs, which are distinguished macroscopically or sometimes only with use of microscopic methods.

Tissue and cellular levels – the levels of study of changed tissues, cells and intercellular mater with use of microscopic methods.

Sub cellular level – allows to observe using electron microscopic the changes of ultra structures of cells and intercellular matter, which are the primary morphological features of disease.

Molecular level – disease study is possible due to use of some complex methods (electron microscopic, cytochemical and other).

So, deep morphological investigation of disease requires the all modern methods. The tasks of pathological anatomy today determine its particular place among the medical subjects. Simultaneously pathological anatomy is the theory of medicine defined the material base of disease and clinical morphology to determine the diagnose.

Pathological anatomy is based on the principles:

1. Structure and functions.
2. Clinico-anatomical.

The first one allows to determine correlation between pathological anatomy and other theoretical subjects (anatomy, histology, physiology, biochemistry) and to study the bases of pathology. The second principle – clinico-anatomical direction – proves the necessity of pathological anatomy data for other clinical subjects and doctor's practice.

MORPHOLOGY OF CELL INJURY

Depending upon the severity of cell injury degree of damage and residual effects on cell and tissues are variable. In general, morphologic changes in various forms of cell injury can be classified as shown in Table 1 and are discussed below.

Table 1. **Classification of morphologic forms of cell injury**

№	Mechanism of cell injury	Nomenclature
1	Reversible cell injury	Retrogressive changes (older term: degeneration)
2	Irreversible cell injury	Cell death (necrosis)
3	Programmed cell death	Apoptosis
4	Residual effects of cell injury	Subcellular alteration
5	Deranged cell metabolism (dystrophy)	Intracellular accumulation of lipid, protein, carbohydrate
6	After-effects of necrosis	Gangrene, pathologic calcification

MORPHOLOGY OF SUB-CELLULAR ALTERATIONS IN CELL INJURY

Certain morphologically distinct alterations at subcellular level are noticeable in both acute and chronic forms of cell injury. These occur at the level of cytoskeleton, lysosomes, endoplasmic reticulum and mitochondria:

1. **CYTOSKELETAL CHANGES.** Components of cytoskeleton may show the following morphologic abnormalities:

i) Defective microtubules:

- In Chediak-Higashi syndrome characterised by poor phagocytic activity of neutrophils

- Poor sperm motility causing sterility

- Immotile cilia syndrome (Kartagener's syndrome) characterised by immotile cilia of respiratory tract and consequent chronic infection due to defective clearance of inhaled bacteria

- Defects in leucocyte function of phagocytes such as migration and chemotaxis

ii) Defective microfilaments:

- In myopathies

- Muscular dystrophies

iii) **Accumulation of intermediate filaments:** Various classes of intermediate filaments (cytokeratin, desmin, vimentin, glial fibrillary acidic protein, and neurofilament) may accumulate in the cytosol. For example:

- Mallory's body or alcoholic hyaline as intracytoplasmic eosinophilic inclusion seen in alcoholic liver disease which is collection of cytokeratin intermediate filaments

- Neurofibrillary tangles, neurites and senile plaques in Alzheimer's disease are composed of neurofilaments and paired helical filaments.

2. **LYSOSOMAL CHANGES.** Lysosomes contain powerful hydrolytic enzymes. Heterophagy and autophagy are the two ways by which lysosomes show morphologic changes of phagocytic function.

i) **Heterophagy.** Phagocytosis (cell eating) and pinocytosis (cell drinking) are the two forms by which material from outside is taken up by the lysosomes of cells such as polymorphs and macrophages to form phagolysosomes. This is termed heterophagy. Microbial agents and foreign particulate material are eliminated by this mechanism.

ii) **Autophagy.** This is the process by which worn out intracellular organelles and other cytoplasmic material form autophagic vacuole that fuses with lysosome to form autophagolysosome.

iii) **Indigestible material.** Some indigestible exogenous particles such as carbon or endogenous substances such as lipofuscin may persist in the lysosomes of the cells for a long time as residual bodies.

iv) **Storage diseases.** As discussed in Chapter 9, a group of lysosomal storage diseases due to hereditary deficiency of enzymes may result in abnormal collection of metabolites in the lysosomes of cells.

3. **S.E.R. HYPERTROPHY.** Hypertrophy of smooth endoplasmic reticulum of liver cells as an adaptive change may occur in response to prolonged use of barbiturates.

4. **MITOCHONDRIAL CHANGES.** Mitochondrial injury plays an important role in cell injury. Morphologic changes of cell injury in mitochondria may be seen in the following conditions:

i) Megamitochondria. Megamitochondria consisting of unusually big mitochondria are seen in alcoholic liver disease and nutritional deficiency conditions.

ii) Alterations in the number of mitochondria may occur. Their number increases in hypertrophy and decreases in atrophy.

iii) Oncocytoma in the salivary glands, thyroid and kidneys consists of tumour cells having very large mitochondria.

iv) Myopathies having defect in mitochondria have abnormal cristae.

DYSTROPHY

Dystrophy – is a pathological process resulting from cells and tissues metabolism disturbances and accompanying with the structural damage.

The causes of dystrophy are:

- disturbances of cell auto regulation
- dysfunction organism transport systems (blood, lymph)
- disturbances of neurohumoral regulation of metabolism

They consider this factor to cause cells enzymes disturbances. Due to this we may say that all dystrophy are enzymopathia.

There are 4 mechanisms of dystrophy development:

1. Infiltration – superfluous entering of the metabolism products from the blood and lymph into cells and intercellular matter and accumulation there. For example: infiltration by glucose of the kidney tubulus epithelium in diabetes.

2. Decomposition – disintegration of cells structures and intercellular matter with accumulation of its products in the cells.

3. Abnormal synthesis – synthesis and accumulation in cells unusual substances, which don't occur there in norm. For example: synthesis of glucose in diabetes; synthesis of amyloid.

4. Transformation – formation of one kind metabolism products instead of another one from common products. For example: synthesis of proteins from the lipids and carbohydrates.

Classification:

1. *According to localization:*

- parenchymatous,
- mesenchymal,
- mixed,

2. *According to metabolism kind disturbances predominance:*

- protein,
- lipid,
- carbo-hydrate,
- mineral,

3. *According to genetic factor influence:*

- congenital (primary),
- acquired (secondary),

4. *According to spreading:*

- local,

- general (total).

PARENCHYMATOUS DYSTROPHIES

Parenchymatous dystrophies develop when changes occur mainly in organ parenchyma (in cells). Intracellular accumulation of substances in abnormal amounts can occur within the cytoplasm (especially lysosomes) or nucleus of the cell. This phenomenon was previously referred to as infiltration, implying thereby that something unusual has infiltrated the cell from outside which is not always the case. Intracellular accumulation of the substance in mild degree causes reversible cell injury while more severe damage results in irreversible cell injury. Such abnormal intracellular accumulations can be divided into 3 groups:

i) Accumulation of constituents of normal cell metabolism produced in excess e.g. accumulations of lipids (fatty change, cholesterol deposits), proteins and carbohydrates.

ii) Accumulation of abnormal substances produced as a result of abnormal metabolism due to lack of some enzymes e.g. storage diseases or inborn errors of metabolism.

iii) Accumulation of pigments e.g. endogenous pigments under special circumstances, and exogenous pigments due to lack of enzymatic mechanisms to degrade the substances or transport them to other sites.

According to the metabolism type disturbances parenchymatous dystrophies are subdivided into protein, lipid and carbohydrate.

Parenchymatous protein dystrophies. Pathologic accumulation of proteins in the cytoplasm of cells may occur in the following conditions.

1. In proteinuria, there is excessive renal tubular reabsorption of proteins by the proximal tubular epithelial cells which show pink hyaline droplets in their cytoplasm. The change is reversible so that with control of proteinuria the protein droplets disappear.

2. The cytoplasm of actively functioning plasma cells shows pink hyaline inclusions called Russell's bodies representing synthesized immunoglobulins.

3. In α_1 -antitrypsin deficiency, the cytoplasm of hepatocytes shows eosinophilic globular deposits of a mutant protein.

4. Mallory's body or alcoholic hyaline in the hepatocytes is intra-

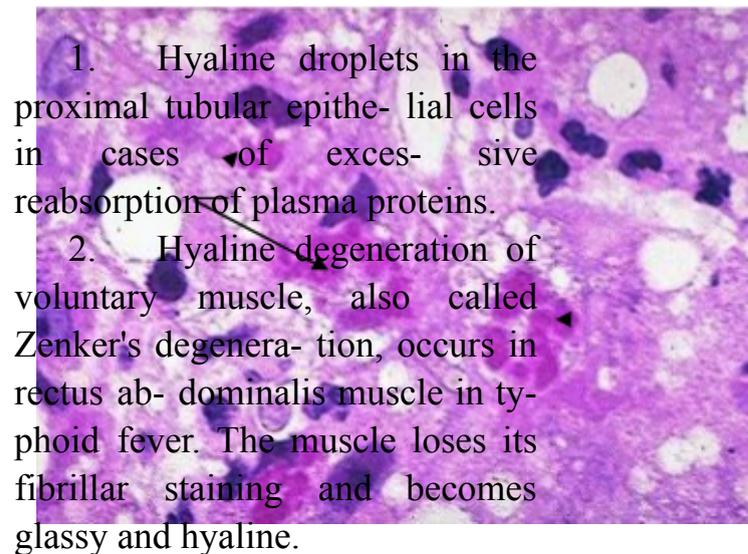
cellular accumulation of intermediate filaments of cytokeratin and appear as amorphous pink masses.

Protein parenchymatous dystrophies are subdivided into:

- a) hyaline-drop,
- b) hydropic,
- c) keratin.

Hyaline-drop dystrophy. The protein droplets (like hyaline) appear in cytoplasm. The word “hyaline” means glassy (hyalos = glass). Hyaline is a descriptive histologic term for glassy, homogeneous, eosinophilic appearance of material in haematoxylin and eosin-stained sections and does not refer to any specific substance. Hyaline change is associated with heterogeneous pathologic conditions and may be intracellular or extracellular.

Intracellular hyaline is mainly seen in epithelial cells. This dystrophy occurs in kidneys, liver, rarely in myocardium and voluntary muscle. For example:



1. Hyaline droplets in the proximal tubular epithelial cells in cases of excessive reabsorption of plasma proteins.

2. Hyaline degeneration of voluntary muscle, also called Zenker's degeneration, occurs in rectus abdominalis muscle in typhoid fever. The muscle loses its fibrillar staining and becomes glassy and hyaline.

Fig. 1. Alcoholic hyaline in hepatocytes. H and E stained section, x 400.

3. In alcoholic liver cell injury.
4. Nuclear or cytoplasmic hyaline inclusions seen in some viral infections.
5. Russell's bodies representing excessive immunoglobulins in the rough endoplasmic reticulum of the plasma cells.

This form of dystrophy is irreversible. The function of organ is disturbed. Coagulative focal or total cellular necrosis develops.

Hydropic (vacuolar, balloon) dystrophy is characterized by the appearance of vacuoles with cytoplasmic fluid in the cells.

This is the most common and earliest form of cell injury from almost all causes. Other synonyms of cellular swelling used in the past are cloudy swelling (for gross appearance of the affected organ), hydropic change (accumulation of water within the cell), and vacuolar degeneration (due to cytoplasmic vacuolation).

The common causes of cellular swelling include: bacterial toxins, chemicals, poisons, burns, high fever, intravenous administration of hypertonic glucose or saline etc.

Cloudy swelling results from impaired regulation of cellular volume, especially for sodium. This regulation operates on 3 levels: at the plasma membrane itself, at the sodium pump on the plasma membrane, and at the supply of ATP. Injurious agent may interfere with these regulatory mechanisms and result in accumulation of sodium in the cell which, in turn, leads to inflow of water to maintain iso-osmotic conditions and hence cellular swelling occurs.

Grossly, the affected organ such as kidney, liver or heart muscle is enlarged due to swelling. The cut surface bulges outwards and is slightly opaque. Microscopically, it is characterised by the following features:

1. The cells are swollen and the microvasculature compressed. The cellular swelling is due to increased influx of sodium and extracellular water into the cell and escape of potassium.

2. Small clear vacuoles are seen in the cells and hence the term vacuolar degeneration. These vacuoles represent distended cisternae of the endoplasmic reticulum.

3. Ultrastructural changes in hydropic swelling include the following:

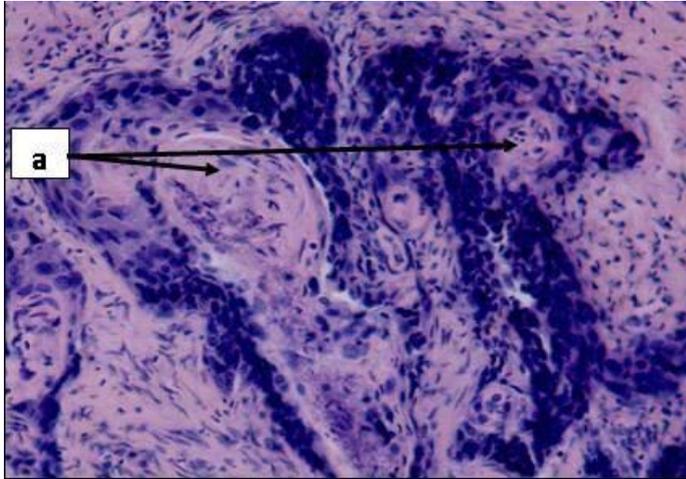
- i) Dilatation of endoplasmic reticulum,
- ii) Detachment of polysomes from the surface of RER,
- iii) Mitochondrial swelling,
- iv) Blebs on the plasma membrane,
- v) Loss of fibrillarity of nucleolus.

It may be mentioned here the hydropic swelling is entirely reversible if the injurious agent is removed.

This kind of dystrophy occurs in epidermis (in smallpox), in kidney (tubular necrosis), in adrenal glands cortex, in hepatocytes and in nervous cells. At the late stages the cells look like balloons, and after that rupture of

cytoplasm happens. This form of dystrophy is irreversible and results in cell death (focal or total colliquative cellular necrosis).

Keratin dystrophy. It is increased formation of keratin matter in ke-



ratinized epithelium (hyperkeratosis, ichtiosis) or keratin matter formation in epithelium, in witch in norm it is absent (leukoplakia) or keratinization in epithelial tumors (pathological formation of keratin “pearls” in squamous cell carcinoma).

Fig. 2. “Squamous pearls” (a) in squamouscellular carcinoma. H and E stained section, x 200.

Parenchymatous fatty (lipid) dystrophy

Fatty change, steatosis or fatty metamorphosis is the intracellular accumulation of neutral fat within parenchymal cells. It includes the older, now abandoned, terms of fatty degeneration and fatty infiltration because fatty change neither necessarily involves degeneration nor infiltration. The deposit is in the cytosol and represents an absolute increase in the intracellular lipids. It is especially common in the liver but may occur in other non-fatty tissues like the heart, kidneys, skeletal muscle and other organs.

One of the main cause of fatty (lipid) dystrophy is the oxygen insufficiency (hypoxia), resulting from cardiovascular diseases, anemia, diseases the lung. Another cause is intoxication.

Heart. In the myocardiocytes lipids appear, then they force out cytoplasm. If this dystrophic process is not severe, externally organ doesn't change. In severe process the organ will be flabby, with increased sizes and in dissection of yellowish color. In endocardium yellow-white striation is visible, particularly in papillary muscles.

Liver. Liver is the most common site for accumulation of fat because it plays central role in fat metabolism. Depending upon the cause and

amount of accumulation, fatty change may be mild and reversible, or severe producing irreversible cell injury and cell death.

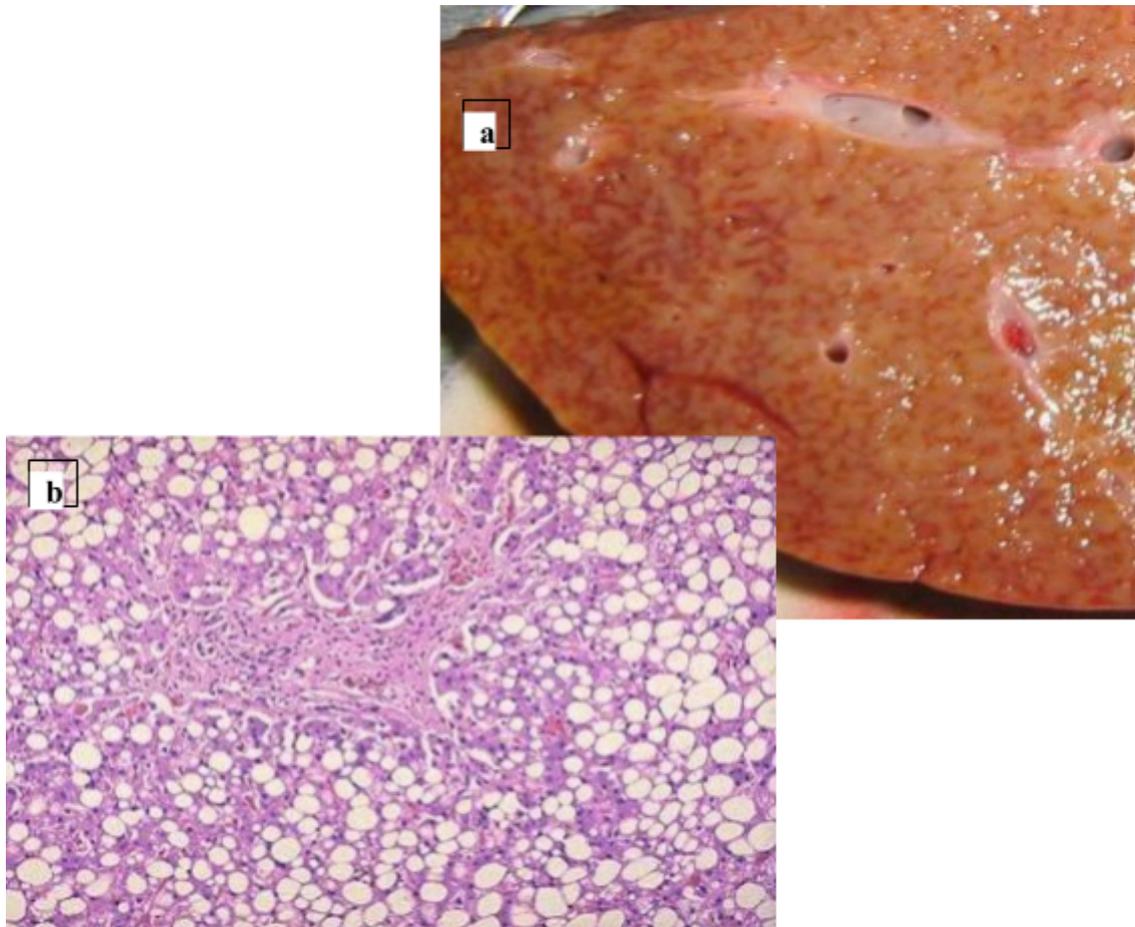


Fig. 3. Fatty (lipid) dystrophy of the liver (a – grossly, b – microscopically, H and E stained section, x 200).

The most common causes of fatty liver include the following:

- i) excess alcohol consumption (most commonly);
- ii) starvation;
- iii) malnutrition;
- iv) obesity;
- v) diabetes mellitus;
- vi) chronic illnesses (e.g. tuberculosis);
- vii) late pregnancy;
- viii) hypoxia (e.g. anaemia, cardiac failure);
- ix) hepatotoxins (e.g. carbon tetrachloride, chloroform ether, aflatox- ins and other poisons);
- x) certain drugs (e.g. administration of oestrogen, steroids, tetra-
cycline etc); and

xi) Reye's syndrome.

Pathogenesis of fatty liver depends upon the stage at which the individual etiologic agent acts in the normal fat transport and metabolism

Normally, lipids are derived from 2 sources:

- From diet as chylomicrons (containing triglycerides and phospholipids) and as free fatty acids; and
- From adipose tissue as free fatty acids.

Free fatty acids from either of these sources enter the liver cell. Normally, a small part of fatty acids is synthesised from acetate in the liver cells. Most of fatty acid is esterified to triglycerides by the action of α -glycerophosphate and only a small part is changed into cholesterol, phospholipids and ketone bodies. Intra-cellular triglycerides require 'lipid acceptor protein' to form lipoprotein, the form in which lipids are normally excreted from the liver cells.

Intracellular accumulation of triglycerides leading to fatty liver can occur due to defect at one or more of the following 6 steps in the normal fat metabolism:

1. Increased entry of free fatty acids into the liver.
2. Increased synthesis of fatty acids by the liver.
3. Decreased conversion of fatty acids into ketone bodies resulting in increased esterification of fatty acids to triglycerides.
4. Increased α -glycerophosphate causing increased esterification of fatty acids to triglycerides.
5. Decreased synthesis of 'lipid acceptor protein' resulting in decreased formation of lipoprotein from triglycerides.
6. Block in the excretion of lipoprotein from the liver into plasma.

In most cases of fatty liver, one of the above mechanisms is operating. But in the case of liver cell injury by chronic alcoholism, many factors are implicated viz.:

- increased lipolysis,
- increased free fatty acid synthesis,
- decreased triglyceride utilisation,
- decreased fatty acid oxidation to ketone bodies, and

- block in lipoprotein excretion.

Even a severe form of liver cell dysfunction may be reversible. For example, an alcoholic who has not developed progressive fibrosis in the

form of cirrhosis, the enlarged fatty liver may return to normal if the person becomes teetotaler.

Grossly, the fatty liver is enlarged with a tense, glistening capsule and rounded margins. The cut surface bulges slightly and is pale-yellow to yellow and is greasy to touch.

Microscopically, there are numerous lipid vacuoles in the cytoplasm of hepatocytes. The vacuoles are initially small and are present around the nucleus (microvesicular). But with progression of the process, the vacuoles become large pushing the nucleus to the periphery of the cells (macrovesicular). At times, the hepatocytes laden with large lipid vacuoles may rupture and lipid vacuoles coalesce to form fatty cysts. Infrequently, lipogranulomas may appear consisting of collections of lymphocytes, macrophages, and some multinucleated giant cells. Fat in the tissue can be demonstrated by frozen section followed by fat stains such as Sudan dyes (Sudan III, IV, Sudan black), oil red O and osmic acid.

Kidney. More frequently epithelium is affected. Kidneys are enlarged, swelling, friable.

Cholesterol. Intracellular deposits of cholesterol and its esters in macrophages may occur when there is hypercholesterolaemia. This turns macrophages into foam cells. The examples are:

1. Fibrofatty plaques of atherosclerosis
2. Clusters of foam cells in tumour-like masses called xanthomas and xanthelasma.

Parenchymatous carbohydrate dystrophies

They are connected with the glycogen and glycoprotein metabolism disturbances. Conditions associated with excessive accumulation of intracellular glycogen are as under:

1. In diabetes mellitus, when absolute or relative insulin insufficiently take place, there is intracellular accumulation of glycogen in different tissues because normal cellular uptake of glucose is impaired. The metabolism of glucose with glycogen formation is disturbed. Hyperglykemia, glucosuria result from this. Glycogen deposits in diabetes mellitus are seen in epithelium of distal portion of proximal convoluted tubule and descending loop of Henle, in the hepatocytes, in beta cells of pancreatic islets, and in cardiac muscle cells. Deposits of glycogen produce clear vacuoles in the cytoplasm of the

affected cells. Bests carmine and periodic acid-Schiff (PAS) staining may be employed to confirm the presence of glycogen in the cells.

2. In glycogen storage diseases or glycogenosis, there is defective metabolism of glycogen due to genetic disorders.

In glycoprotein metabolism disturbances the cells accumulate mucins and mucous like substances. It occurs in inflammatory processes in mucous coats. The number of mucous, producing by mucous cells, increases; mucous changes its physico-chemical properties. It may cause the occlusion of the bronchioles, gland ducts. Sometimes mucous like colloid substances are produced (colloid strum). Histochemical staining reveals the glycoproteins.

STORAGE DISEASES

There are a lot of diseases, which are due to hereditary factors and are connected with metabolism disturbance. These diseases are called storage diseases or enzymopathy.

A few general comments should be made about all storage diseases:

- All the storage diseases occur as a result of autosomal recessive, or sex-(X-) linked recessive genetic transmissions.
- Most of the storage diseases are lysosomal storage diseases. Out of the glycogen storage diseases, only type II (Pompe's disease) is due to lysosomal enzyme deficiency.

According to the type of metabolism disturbance storage diseases have been classified into:

- Proteinoses
- Lipidosis
- Glucogenoses

The type of proteinoses, lipidosis and glucogenoses depends on the defect in the enzyme. The most frequent lipidoses are Gaucher's disease, Niemann-Pick disease.

Gaucher's disease

This is an autosomal recessive disorder in which there is a deficiency of lysosomal enzyme, glucocerebrosidase, which normally cleaves glucose from ceramide. This results in lysosomal accumulation of glucocerebroside (ceramide-glucose) in phagocytes of the body and sometimes in the neurons. The main sources of glucocerebroside in phagocytic cells of the body and sometimes in the neurons are the membrane glycolipids of old leukocytes and erythrocytes, while the deposits in the neurons consist of

gangliosides.

Clinically, there are 3 types of Gaucher's disease:

1. Type 1 or classic form is the adult form of the disease in which there is storage of glycosphingolipids in the phagocytes of the body, principally involving the spleen, liver, bone marrow and lymph nodes. This is the most common type comprising 80% of all cases of Gaucher's disease.
2. Type II is the infantile form in which there is progressive involvement of the central nervous system.
3. Type III is the juvenile form of the disease having features in between type I and type II, i.e. they have systemic involvement like in type I and progressive involvement of the central nervous system (CNS) as in type II.

Morphology

- In addition to involvement of different organs and systems (splenomegaly, hepatomegaly, lymphadenopathy, bone marrow and cerebral involvement), a few other features include pancytopenia, or thrombocytopenia secondary to hypersplenism, bone pains and pathological fractures.
- Microscopically, large number of characteristically distended and enlarged macrophages called *Gaucher cells* are found in the spleen, liver, bone marrow and lymph nodes, and in the case of neuronal involvement, in the Virchow-Robin space. The cytoplasm of these cells is abundant, granular and fibrillar resembling crumpled tissue paper. They have mostly a single nucleus but occasionally may have two or three nuclei. These cells often show erythrophagocytosis and are rich in acid phosphatase.

Niemann-pick disease

- This is also an autosomal recessive disorder characterized by accumulation of sphingomyelin and cholesterol.
- The majority of the cases (about 80%) have deficiency of sphingomyelinase, which is required for cleavage of sphingomyelin, while a few cases probably result from deficiency of an activator protein.
- The condition presents in infancy and is characterized by hepatosplenomegaly, lymphadenopathy and physical and mental underdevelopment.
- About a quarter of patients present with familial amaurotic idiocy with characteristic cherry-red spots in the macula of the retina.
- The storage of sphingomyelin and cholesterol occurs within the lysosomes, particularly in the cells of mononuclear phagocyte system.
- The cells of Niemann-Pick disease are somewhat smaller than Gaucher cells and their cytoplasm is not wrinkled but is foamy and vacuolated

which stains positively with fat stains. These cells are located in the spleen, liver, lymph nodes, bone marrow, lungs, intestine and brain.

The most frequent glycogen storage diseases or glycogenoses are Pompe's disease, Mc Ardle's disease and Gierke disease. There is a defective metabolism of glycogen due to genetic disorders.

Pompe's Disease - is also an autosomal recessive disorder due to deficiency of a lysosomal enzyme, acid mahaase. Its deficiency results in accumulation of glycogen in many tissues, most often in the heart and skeletal muscles leading to cardio-megaly and hypotonia.

Mc Ardle's Disease -the condition occurs due to deficiency of muscle phosphorylase resulting in accumulation of glycogen in the muscle (deficiency of liver phosphorylase). The disease is common in 2nd – 4th decades of life and is characterized by painful muscle cramps, especially after exercise, and detection of myoglobinuria in half of the cases.

Gierke Disease - is inherited as autosomal recessive disorder due to deficiency of enzyme glucose-6-phosphatase. In the absence of glucose-6-phosphatase, excess of normal type of glycogen accumulates in the liver and also results in hypoglycemia due to reduced formation of free glucose from glycogen. As a result, fat is metabolized for energy requirement leading to hyperlipoproteinemia and ketosis. Other change due to deranged glucose metabolism is hyperuricemia. The disease manifests clinically in infancy with failure to grow and stunted growth. Most prominent feature is enormous hepatomegaly with intracytoplasmic and intranuclear parenchymatous glycogen. The kidneys are also enlarged and show intracytoplasmic glycogen in tubular epithelial cells. Other features include gout, skin xanthomas and bleeding tendencies due to platelet dysfunction.

The outcome of storage diseases is unfavorable because of its insufficiency of the respective organ.

MESENCHYMAL DYSTROPHIES

It is pathological process which damages the stroma of organs and blood vessels walls. According to the metabolism type disturbances mesenchymal dystrophies are subdivided into protein, lipid and carbohydrate.

Protein mesenchymal dystrophies include mucoid swelling, fibrinoid swelling, hyalinosis and amyloidosis. First 3 forms are the stages of connective tissue damage. The base of this process is the entering of blood plasma

components through vessels wall and accumulation of them in intercellular matter.

Mucoid swelling is reversible damage of connective tissue. The glycoproteins, glycoproteins, glucosaminoglycans and blood plasma proteins are accumulated in intercellular mater; a volume of fluid increases too. For this kind of dystrophy metachromasia is characteristic histological sign. It means the ability to give unordinary colour during staining. Microsigns are swelling of collagen fibers, increasing of their sizes. Macroscopic signs are almost absent. This process is reversible and in case of pathogen factor removing the structure of tissue restores. If pathogen factor action continues, the fibrinoid swelling develops.

Fibrinoid swelling – more deep and irreversible damage of connective tissue, accompanying with the fibrinoid formation. This is complex substance, which consists of fibrin, blood plasma proteins, products of collagen fibers destruction, nucleoproteins. The collagen fibers lose their structure. Metachromazia is not observed. Fibrinoid swelling may be generalized (in systemic diseases of the connective tissue) and localized (in chronic inflammation). The final stage of this process is fibrinoid necrosis, sclerosis or hyalinosis. Externally organs are almost not changed.

Hyalinosis (*extracellular hyaline, hyaline degeneration*) is seen in connective tissues. Hyaline is pathological close (dense) substance like hyaline cartilage, covering articular surfaces. It consists of fibrillar proteins: fibrin, blood plasma proteins, immunoglobulins, and lipids.

There are 2 types of hyalinosis: hyalinosis of vessels and hyalinosis of connective tissue.

i) Hyalinosis of vessels damage mainly small arteries and arterioles. Due to accumulation of hyaline in the wall blood vessels look like dense tubules (“glass tubes”) with narrowing lumen. It results in blood supply disturbances in tissues and organs.

There are 3 kinds of vascular hyaline according to its nature:

1. Simple – contains the plasma proteins (occurs in arterial hypertension).
2. Lipohyaline – contains lipids (occurs in diabetes).
3. Complex – contains immune components, fibrin, and damaged structures of the vascular wall (in diseases of immune system).

ii) Hyalinosis of connective tissue follows to fibrinoid swelling and is accompanied with the fibres structures destruction. Such changes occur in rheumatic disease, in the bottom of chronic stomach ulcer, in old scars.

Some examples of extracellular hyaline changes are:

1. Hyaline degeneration in the uterus leiomyomas.
2. Hyalinized old scar of fibrocollagenous tissues.
3. Hyaline arteriosclerosis is renal vessels in hypertension and diabetes mellitus.
4. Hyalinized glomeruli in chronic glomerulonephritis.
5. Corpora amyloidea are rounded masses of concentric hyaline laminae seen in the prostate, brain and spinal cord in the elderly persons, and in old infarcts of the lung.

Amyloidosis is a kind of mesenchymal protein dystrophy. Amyloid is a hyaline glycoprotein material with a microfibril structure that stains under Congo red dye. It is stored systemically or locally in the extracellular space, giving the respective tissue a glassy, wax-like appearance.

Amyloidoses represent a group of disorders of varying etiology that involve the deposit of amyloid in tissue. The following forms are differentiated according to the site:

– Systemic amyloidoses involve amyloid deposits in several organs and/or tissues.

– Localized amyloidoses involve amyloid deposits in one organ and/or tissue.

Structure of Amyloid

These components are common to all types of amyloid:

– Fibril protein with a B-pleated structure (B-pleated fibrils). This protein varies with the underlying disease and its name is used to identify the respective type of amyloid.

– Amyloid P component arises from SAP (serum amyloid P component, which is physiologic serum protein, a component of the glomerular basement membrane).

– Heparan sulfate proteoglycans are proteoglycans of the basement membrane type.

All components have antigenic properties.

Substances used for detecting amyloid:

- Lugol's solution normally detects starches (hence the name "amyloid").
- Amyloid is positive under Congo red.

– Amyloid shows green birefringence under polarized light.

The reasons of amyloidosis development are unknown. Theories of amyloidosis are:

1. The immunological theory (amyloidosis is the result of antibody- antigen interaction).
2. The theory of cellular local synthesis (amyloid is produced by mesenchyma descended cells).
3. The mutational theory (the synthesis of amyloid is the result of genes cell mutation which produce amyloid).

Amyloid can store along reticular fibers (perireticular amyloidosis with involvement of spleen, kidneys, liver, intestine, adrenal glands, an intima of small and medium calibre vessels) and along collagenic fibers (pericollagenous amyloidosis with the lesion of large vessels, myocardium, muscles, nerves and skin). Organs affected by amyloidosis are increased in sizes, dense in consistence; they have a tallowy appearance on the cut section.

Types of Amyloid

AL Amyloid, Synonym: light-chain amyloid.

Pathogenesis: AL amyloid is the derivative of variable parts of the immunoglobulin light chains (k chains more often than j chains). An immune stimulus causes proliferation of a plasma cell clone, triggering formation of a monoclonal immunoglobulin with an excess of light chains. The result is incomplete lysosomal breakdown of the immunoglobulin light chains in the macrophages, which in turn causes formation of k and j light chain fragments. These fragments condense to b-clumpy fibrils with a b-pleated sheet structure. Manifestation as systemic amyloidosis: see primary forms of amyloidosis.

AA Amyloid, Synonym: amyloid A.

Pathogenesis: AA amyloid is the derivative of an HDL apolipoprotein (SAA = serum amyloid A precursor protein). In an inflammation, the acute-phase proteins are synthesized in the liver during the acute phase. These include SAA, which is released into the serum and phagocytized by cells of the macrophage system. There, the lysosomes cleave off the amyloid-forming fragments. These fragments condense to b-clumpy fibrils with a b-pleated sheet structure.

Manifestation as systemic amyloidosis: see secondary forms of amyloidosis.

AF Amyloid, Synonym: familial amyloid.

Pathogenesis: AF amyloid is the derivative of transthyretin (the transport protein for thyroxin and retinol). A single-point mutation of transthyretin generates amyloid-forming fragments without prior proteolytic cleavage. These fragments condense to β -pleated fibrils with a β -clumpy sheet structure.

Manifestation as local amyloidosis:

- Kidney amyloidosis.
- Predominant distal polyneuropathies: Amyloid deposits cause nerve damage with sensory deficits and weakening of reflexes.
- Hypertrophic cardiomyopathy: Amyloid deposits between the muscle cells in the myocardium cause fatty hardening of the myocardium.

AE Amyloid, Synonym: endocrine amyloid.

Pathogenesis: AE amyloid comes from endocrine cells. Endocrine cells cause generation of abnormal peptides, which in turn result in incomplete proteolytic breakdown with residual amyloid fragments. These fragments condense to β -clumpy fibrils with a β -pleated sheet structure.

Manifestation as local amyloidosis:

- Islet amyloidosis: Amyloid deposits in the islets of Langerhans in the pancreas displace the endocrine cells (A, B), causing type II diabetes mellitus.
- Calcitonin amyloid: The clumped configuration of cells in a C-cell thyroid carcinoma (C1) form clumpy deposits of AE amyloid in the tumor stroma (C2) and express calcitonin (D).

AS Amyloid, Synonym: amyloid of aging.

Pathogenesis: AS amyloid is produced in abnormally aging brains. Mutation of a gene on chromosome 21 (which normally codes for a proteinase inhibitor) causes coding of an atypical β -amyloid precursor proteins (β -APP). These proteins condense to β -clumpy fibrils with a β -pleated sheet structure.

Manifestation as local amyloidosis:

- Alzheimer’s disease involves focal Amyloid deposits in the brain known as “Amyloid cores” (E, F), some of which are surrounded by distended nerve endings with an abnormal cytoskeleton.
- Down’s syndrome (trisomy 21).

Classification of amyloidosis:

I) by origin:

● *Idiopathic* (synonym: atypical, primary) amyloidosis occur in the absence of a discernible prior disorder or a discernible etiology.

This also includes the paramyloidoses. These disorders are characterized by generalized deposits of amyloid that occur in the setting of lymphoplasmacytic neoplasms. Deposition sites especially include mesenchymal organs less often affected in classic secondary amyloidosis: the tongue, skeletal musculature, and myocardium. The nerves, brain, skin, and lungs are affected less frequently.

● *Hereditary amyloidosis*. The synthesis of abnormal protein is genetically determined.

● *Acquired amyloidosis* (synonym: reactive systemic amyloidosis, secondary). Amyloidosis occurs secondary to such diseases as rheumatoid arthritis, chronic osteomyelitis, chronic abscess of lung, bronchiectatic disease, multiple myeloma, tuberculosis.

● *Senile amyloidosis*. This form develops in elderly persons; it is connected with general disturbances of protein metabolism.

● *Local tumor-like amyloidosis* - amyloid accumulates locally in the form of node.

II) by specificity of fibrillar protein;

III) by extension:

● *Systemic*.

● *Localized form*. It is limited to a single organ or circumscribed area within organs. Some forms of hereditary, senile amyloidosis and local tumor-like amyloidosis are referred to this form. Locations and forms include:

- Brain: cerebral amyloidosis.
- Nerves: polyneuropathic amyloidosis.
- Heart: cardiomyopathic amyloidosis.
- Lung: respiratory-tract amyloidosis.
- Kidney: nephropathic amyloidosis.
- Eye: ocular amyloidosis.

- Endocrine system: AE amyloidosis.
- Skin: lichenoid cutaneous amyloidosis.

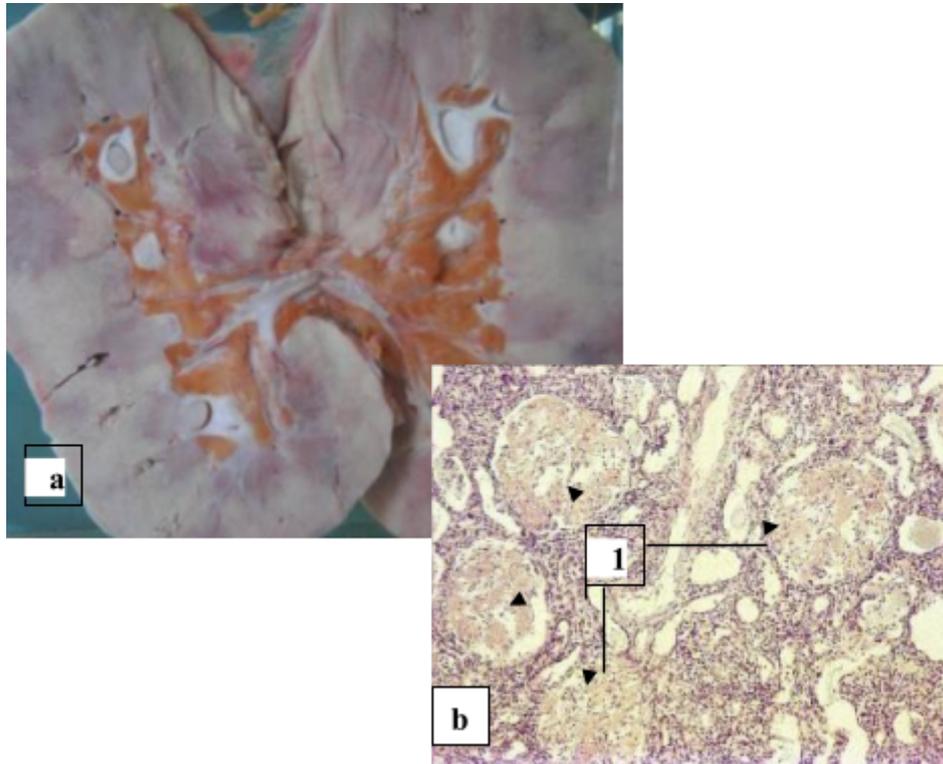


Fig.4. Kidney amyloidosis (a – grossly, b – microscopically, H and E stained section, x 200). 1- deposition of Amyloid in glomeruli.

IV) *by clinical manifestations:*

- cardiopathic;
- epinephropathic (adrenal glands);
- nephropathic;
- neuropathic;
- APUD-amyloidosis;
- hepatopathic,
- mixed.

General amyloidosis is characterized by lesion of adrenal glands, kidneys, liver, nervous system, heart. Amyloid accumulates in lymphatic follicles of the spleen (sago-spleen) or evenly in all pulp (a tallowy spleen). Kidneys become dense, tallowy too. Amyloidosis of kidneys leads to a chronic renal insufficiency.

Clinical presentation of amyloidosis:

AL Amyloidosis

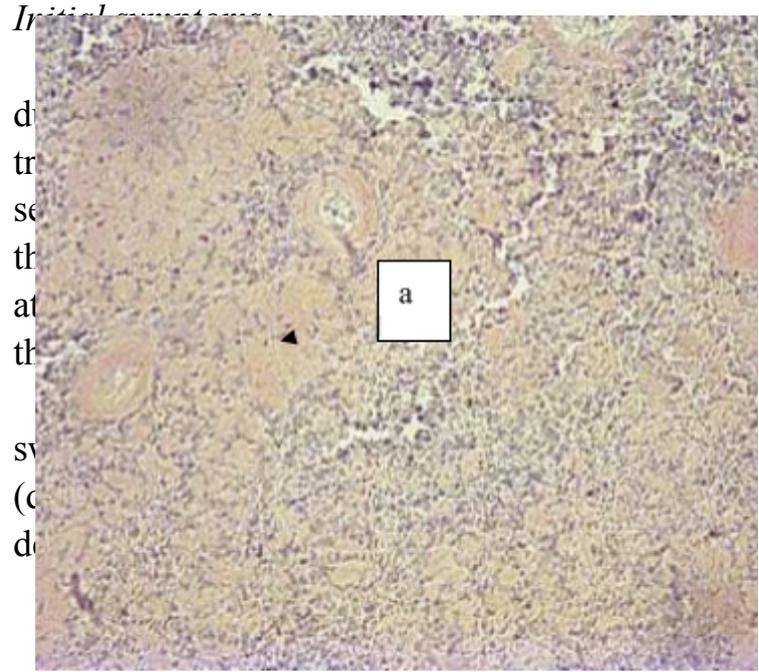


Fig. 5. Amyloidosis of the spleen. H and E stained section, x 200. a – deposition of Amyloid in the spleen pulp.

lesions and purpura (punctuate bleeding sites).

- Joints: Major joints exhibit arthritic symptoms.

Late symptoms:

- Heart: Heart failure not responding to treatment, occasionally accompanied by conduction disorders due to restrictive cardiomyopathy. Amyloid deposits in the myocardium impair its contractility and elasticity, producing reactive thickening of the myocardium (hypertrophy) with ventricular narrowing lacking mechanical efficiency.

- Liver: Hepatomegaly (enlargement of the liver) due to perisinusoidal amyloid deposits (C, D). This causes atrophy of the hepatic cords with strikingly little functional impairment.

- Small bowel: Amyloid deposits in the submucosa and around the arteries cause obstructions, mucosal bleeding, and diarrhea where the autonomous nervous system is involved.

- Nerves: Peripheral neuropathy, usually associated with sensory deficits.

- Lung: Respiratory insufficiency is present only where there are diffuse amyloid deposits in the alveolar and extraalveolar interstitium (the bronchial wall).

AA Amyloidosis

– Kidney: Amyloid deposits occur in the mesangium and coils of the glomeruli (E, F) and/or predominantly in the arterial branches (AA greater than 50%; AL 15%; AF 25%). This leads to impaired renal function in the form of a “nephrotic syndrome” characterized by:

- Proteinuria;
- Hypoproteinemia;
- Hyperlipidemia;
- Edemas, occurring primarily in the lower leg in adults and primarily in the eyelids in children.

– Liver: Hepatomegaly (enlargement of the liver) with negligible functional deficiency.

- Small bowel: Obstructions and mucosal bleeding.

Mesenchymal lipid (fatty) dystrophies result from the disturbances of lipids and cholesterol metabolism. Stromal fatty infiltration is quite different from fatty change previously described. Stromal fatty infiltration is the accumulation of mature adipose cells in the stromal connective tissue in contrast to intracellular deposition of fat in the parenchymal cells in fatty changes. This condition occurs most often in patients with obesity. The two commonly affected organs are the heart and the pancreas. The presence of mature adipose cells in the stroma generally does not cause any dysfunction. In the heart stromal fatty infiltration is associated with increased adipose tissue in the epicardium.

There are 2 types of **General obesity**: *primary* (the cause is unknown) and *secondary* (in diseases of the central nerve and endocrine systems). General obesity is classified into 4 forms: symmetric, upper (mainly in the regions of head, upper limbs), middle (in the region of abdomen), inferior (lower limbs).

There are several types of secondary obesity:

1. Alimentary (a lot of lipids in food).
2. Cerebral.
3. Endocrine (e.g. in Icenko-Kushing syndrome).
4. Hereditary (eg. in Gierke's disease).

According to morphological peculiarities obesity may be:

1. Hypertrophic.

2. Hyperplastic.

In hypertrophic type adipose tissue enlarges due to increased volume of fatty cells, in hyperplastic – due to increase in their number.

Depending on the excess of patient's body weight compared to the norm, 4 degrees of obesity are defined:

1st degree – when 20 - 29% of overweight ;

2nd degree - when 30 - 49% of overweight;

3rd degree – when 50 - 99% of overweight ;

4th degree – when body weight increases up to 100% and more.

Severe reduction in the amount of neutral fat in the whole organism is cachexia.

Local increasing of fat tissue number is termed *lipomatosis* and occurs in Dercum's disease when painful fat nodes appear in the subcutaneous fat of the lower and upper extremities and trunk.

The *cholesterol metabolism disturbance* is the base of atherosclerosis with the damage of blood vessel wall internal coat. It causes the narrowing of arteries lumen.

Mesenchymal carbohydrate dystrophies result from the glycoproteids and glycosaminoglycans metabolism disturbances. When glycoproteids metabolism is disturbed, chromotropic substances are released from the protein bonds. The products of metabolism are accumulated in the intercellular mater. Collagens are replaced by mucous substance, connective and cartilage tissue lose their structure. More frequently this process is connected with endocrine dysfunctions.

Connective tissue mucin is associated with:

- Muroid or myxoid degeneration in some tumors (myxoma).
- Neurofibromas, soft tissue sarcomas, etc.
- Myxomatous change in the dermis in myxedema (mucous edema in hypothyreosis).
- Myxoid change in the synovium in ganglion on a wrist.

The condition results in colliquative necrosis with formation of cavities filled with mucus.

Mucopolysaccharidoses (MPS)

- Disturbance of glycosaminoglycans (GAG) is due to hereditary factors as in a storage disease.

- It is characterized by the deficiency of specific lysosomal enzyme involved in the degradation of mucopolysaccharides or glycosaminoglycans.

- Syndrome of MPS manifests in infancy or early childhood and involves multiple organs and tissues, chiefly connective tissues, liver, spleen, bone marrow, lymph nodes, kidneys, heart and brain.

- The mucopolysaccharides accumulate in mononuclear phagocytic cells, endothelial cells, smooth muscle cells and fibroblasts. The material is finely granular and PAS-positive by light microscopy.

- By electron microscopy, it appears in the swollen lysosomes and can be identified biochemically as mucopolysaccharide.

- The most frequent of them are Pfaundler-Hurler disease, or gargoylism. Its cause is congenital defect of the enzyme determined GAG metabolism. This disease is characterized by irregular skeleton growth, “massive” skull, heart defects, inguinal and umbilical hernias, hepato- and splenomegaly, keratoleukoma (retina opacity).

MIXED DYSTROPHIES

It is pathological process which effects stroma and parenchyma. This kind of dystrophy includes the metabolism disturbances of nucleoproteins, lipoproteins, mineral substances, chromoproteins (pigments).

PIGMENTS are coloured substances present in most living beings including humans. There are 2 broad categories of pigments: endogenous and exogenous.

A. Endogenous pigments (chromoproteins) are either normal constituents of cells or accumulate under special circumstances e.g melanin, ochronosis, haemoprotein-derived pigments and lipofuscin.

B. Exogenous pigments are those which are introduced into the body from outside such as by inhalation, ingestion or inoculation.

A. ENDOGENOUS PIGMENTS

According to the pigment structure endogenous pigments are classified into:

1. Proteinogenic (tyrosinogenic) pigments.
2. Haemoprotein-derived (haemoglobinogenic) pigments.
3. Lipidogenic (lipid) pigments.

PROTEINOGENIC (TYROSINOGENIC) PIGMENTS

MELANIN is the proteinogenic brown-black pigment normally present in the hair, skin, choroid of the eye, meninges and adrenal medulla. It is synthesised in the melanocytes and dendritic cells, both of which are present in the basal cells of the epidermis and is stored in the form of cytoplasmic granules in the phagocytic cells called the melanophorocyte, present in the underlying dermis. Melanocytes possess the enzyme tyrosinase necessary for synthesis of melanin from tyrosine. However, sometimes tyrosinase is present but is not active and hence no melanin pigment is visible. In such cases, the presence of tyrosinase can be detected by incubation of tissue section in the solution of dihydroxy phenyl alanine (DOPA). If the enzyme presents, dark pigment is identified in pigment cells. This test is called as DOPA reaction and is particularly useful in differentiating amelanotic melanoma from other anaplastic tumours. Various disorders of melanin pigmentation cause generalised and localised hyperpigmentation and hypopigmentation.

Generalised hyperpigmentation:

- a) In Addison's disease, there is generalised hyperpigmentation of the skin, especially in areas exposed to light, and of buccal mucosa.
- b) Chloasma observed during pregnancy is the hyperpigmentation on the skin of face, nipples, and genitalia and occurs under the influence of oestrogen. A similar appearance may be observed in women taking oral contraceptives.
- c) In chronic arsenical poisoning, there is characteristic rain-drop pigmentation of the skin.

Focal hyperpigmentation:

- a) Cafe-au-lait spots are pigmented patches seen in neurofibromatosis and Albright's syndrome.
- b) Peutz-Jeghers' syndrome is characterized by focal perioral pigmentation.
- c) Melanosis coli is pigmentation of the mucosa of the colon.
- d) Melanotic tumours, both benign such as pigmented naevi, and malignant such as melanoma, are associated with increased melanogenesis.
- e) Lentigo is a pre-malignant condition in which there is focal hyperpigmentation on the skin of hands, face, neck, and arms.

Generalised hypopigmentation: albinism is an extreme degree of

generalized hypopigmentation in which tyrosinase activity of the melanocytes is genetically defective and no melanin is formed. Albinos have blond hair, poor vision and severe photophobia. They are highly sensitive to sunlight. Chronic sun exposure may lead to precancerous lesions and squamous and basal cell cancers of the skin in such individuals.

Localised hypopigmentation:

- a) Leucoderma is a form of partial albinism and is an inherited disorder.
- b) Vitiligo is local hypopigmentation of the skin and is more common. It may have familial tendency.
- c) Acquired focal hypopigmentation can result from various causes such as leprosy, healing of wounds, DLE, radiation dermatitis etc.

ADRENOCHROME is a pigment of dark brown color. It is situated in a form of small kernels in the cells of adrenal glands medullar. It can be found in pheochromocytoma.

THE PIGMENT OF ENTEROCHROMAFFIN CELLS GRANULES is a tryptophan derivative. The excessive amounts of this pigment are found in tumors of enterochromaffin cells which are called carcinoid tumors.

OCHRONOSIS

Ochronosis is an autosomal recessive disorder in which there is deficiency of an oxidase enzyme required for breakdown of homogentisic acid which then accumulates in the tissues and is excreted in the urine. The pigment is melanin-like and is deposited both intra-cellularly and inter-cellularly. Most commonly affected tissues are the cartilages, capsules of joints, ligaments and tendons. In almost all the cases, alkaptonuria is present in which homogentisic acid is excreted by the kidneys. The urine of these patients, if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid.

HAEMOPROTEIN-DERIVED PIGMENTS

Haemoproteins are the most important endogenous pigments derived from haemoglobin, cytochromes and their breakdown products. For an understanding of disorders of haemoproteins, it is essential to have knowledge of normal iron metabolism and its transport. In disordered iron metabolism and transport, haemoprotein-derived pigments accumulate in the body. These pigments are haemosiderin, haematin, bilirubin, and porphyrins.

Hemoprotein-derived pigments include normal (ferritin, haemosyderin, bilirubin) and pathological (haematoidin, haematin, porphirin).

Ferritin is iron complexed to apoferritin and can be identified by electron microscopy. It is located in liver, spleen, bone marrow and lymph nodes. There are two forms of ferritin: inactive and active (formed due to lack of oxygen, characterized by vasoparalytic and hypotensive actions). Active ferritin is adrenalin antagonist). The pathological condition characterized by increasing of ferritin level in blood and tissues is haemosiderosis. Ferritinaemia is a cause of shock irreversibility.

Haemosiderin, which is formed by aggregates of ferritin and is identifiable by light microscopy as golden- yellow to brown, granular pigment, especially within the mononuclear phagocytes of the bone marrow, spleen and liver where breakdown of senescent red cells takes place. Haemosiderin is ferric iron that can be demonstrated by Prussian blue reaction. In this reaction, colourless potassium ferrocyanide reacts with ferric ions of haemosiderin to form deep blue ferric-ferrocyanide

Excessive storage of haemosiderin occurs in situations when there is increased breakdown of red cells, or systemic overload of iron due to primary (idiopathic, hereditary) haemochromatosis, and secondary (acquired) causes such as in thalassaemia, sideroblastic anaemia, alcoholic cirrhosis, multiple blood transfusions etc.

Accordingly, the effects of haemosiderin excess are as under:

a) **Local haemosiderosis.** This develops whenever there is haemorrhage into the tissues. With lysis of red cells, haemoglobin is liberated which is taken up by macrophages where it is degraded and stored as haemosiderin. The changing colours of a bruise or a black eye are caused by the pigments like biliverdin and bilirubin which are formed during transformation of haemoglobin into haemosiderin. Another example of local haemosiderosis is brown induration in the lungs as a result of small haemorrhages as occur in mitral stenosis and left ventricular failure. Microscopy reveals the presence of 'heart failure cells' which are haemosiderin-laden alveolar macrophages.

b) **Generalised haemosiderosis.** Systemic overload with iron may result in generalised haemosiderosis. There can be two types of patterns:

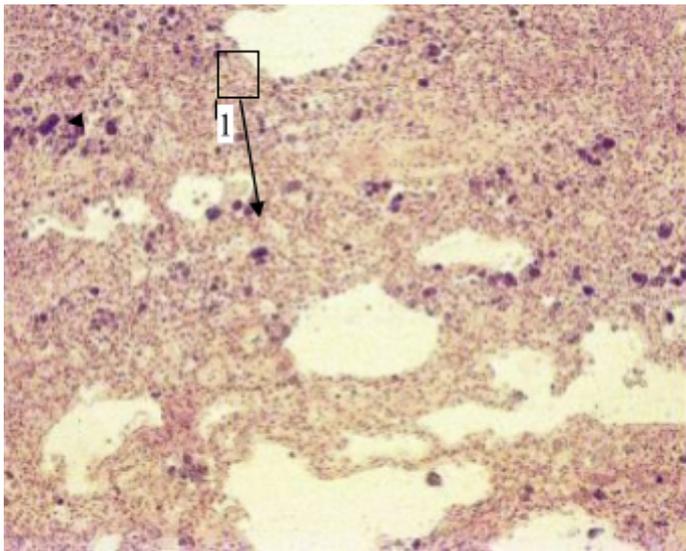


Fig.6. Haemosiderosis of the lungs. H and E stained section, x 200.

1 – storage of haemosiderin.

– *Parenchymatous deposition* of haemosiderin occurs in the parenchymal cells of the liver, pancreas, kidney, and heart. The causes for parenchymatous haemosiderosis include:

i) excessive intestinal absorption of iron (e.g. in haemochromatosis);

ii) increased erythropoietic activity (e.g. in haemolytic

anaemia);

iii) excessive intake of dietary iron (e.g. in *Bentley disease*).

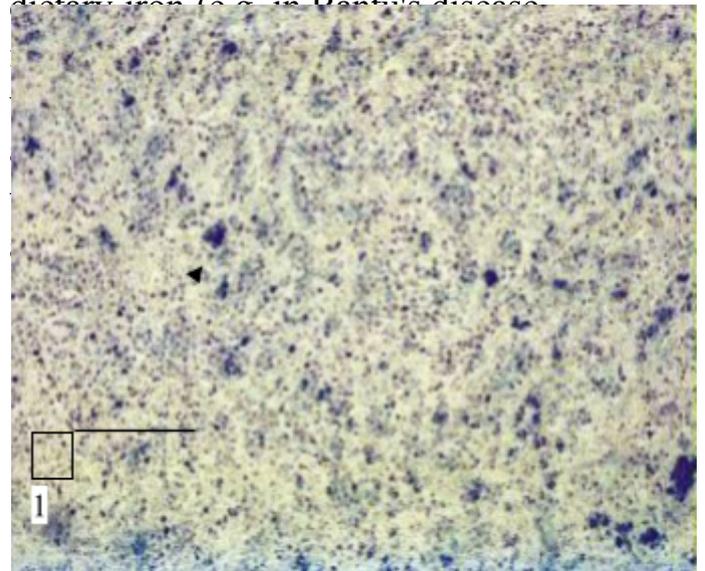


Fig.7 . Haemosiderosis of the lungs. Prussian blue reaction.

1 – storage of haemosiderin.

chromatosis. This is an autosomal dominant disease characterised by excessive absorption

of iron. It is associated with triad of pigmentary liver cirrhosis, pancreatic damage resulting in diabetes mellitus, and skin pigmentation. On the basis of the last two features the disease has come to be termed as 'bronze diabetes'.

- **Bilirubin** is a normal haemoglobin-derived non-iron containing pigment which presents in the bile. It is derived from porphyrin ring of the haem moiety of haemoglobin.

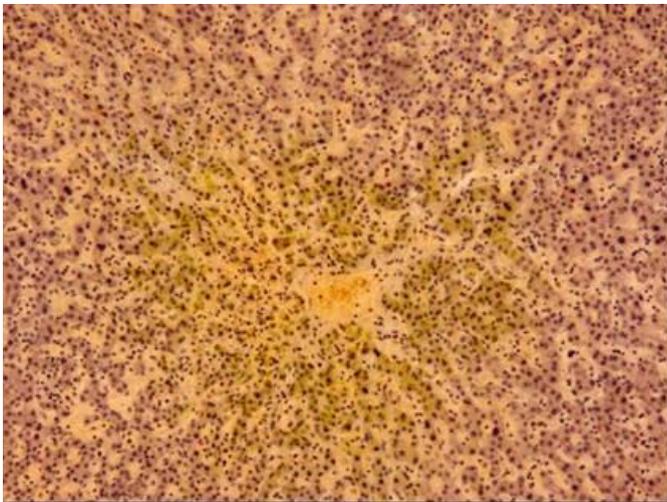
Bilirubin is formed in the cells of reticular-endothelial system in erythrocytes destruction and enters the blood. This is free or indirect bilirubin. It enters the hepatocytes where it is associated with glucuronic acid (direct bilirubin). Then it enters the small intestine, where some of it enter the blood, and last portion color the stool and urine (stercobilin and urobilin).

Normal level of bilirubin in blood is less than 1mg/dl. Excess of bilirubin causes an important clinical condition called jaundice.

Briefly, jaundice appears in one of the following 3 ways:

a) *Prehepatic (or haemolytic)*, when there is excessive destruction of red cells.

b) *Posthepatic (or obstructive, or mechanical)*, which results from obstruction to the outflow of conjugated bilirubin.



c) *Hepatocellular (or hepatic)* that results from failure of hepatocytes to conjugate bilirubin and inability of bilirubin to pass from the liver to intestine. Excessive accumulation of bilirubin pigment can be seen in different tissues and

fluids of the body, especially in the hepatocytes, Kupffer cells and bile sinusoids. Skin and sclerae become distinctly yellow.

Fig.8. «Liver in mechanical jaundice». (hematoxylin and eosin staining).

In infants, rise in unconjugated bilirubin may produce toxic brain injury called kernicterus.

- **Haematin** is a haemoprotein-derived brown-black pigment containing haem iron in ferric form. But it differs from haemosiderin because it cannot be stained by Prussian blue reaction, probably because of formation of complex with a protein so that it is unable to react in the stain. Haematin pigment is seen most commonly in chronic malaria and in mismatched blood transfusions.

There are three types of haematin:

– *Muritic haematin* is formed in gastric erosions and ulcers as the result of interaction between haemoglobin and enzymes with chloric acid of gastric juice.

– *Hemomelanin* is a brown pigment produced by malarial parasites from haemoglobin; it is taken up by monocytes in the blood and subsequently deposited in the liver and spleen.

– *Formalin pigment* looks like dark brown grains, it can be found in the tissues fixed with acid formalin (with pH below 6.0).

● **Porphyrins** are tetrapyrroles which exist in 3 forms in nature combined with different metals:

i) haem contains iron;

ii) chlorophyll contains magnesium; and

iii) cobalamin contains cobalt.

Porphyria results from genetic deficiency of one of the enzymes required for the synthesis of haem so that there is excessive production of porphyrins. Often, the genetic deficiency is precipitated by intake of some drugs. Porphyrias are broadly of 2 types: erythropoietic and hepatic.

(a) Erythropoietic porphyrias. These have defective synthesis of haem in the erythrocytes. These may be further of 2 subtypes:

– *Congenital type*, in which the urine is red due to presence of uroporphyrin I and coproporphyrin 1. The skin of these infants is highly photosensitive. Bones and skin show red brown discoloration.

– *Erythropoietic protoporphyria*, in which there is excess of proto-porphyrin but no excess of porphyrin in the urine.

(b) Hepatic porphyrias. These are more common and have a defect in synthesis of haem in the liver. Its further subtypes include the following:

– *Acute intermittent porphyria* is characterised by acute episodes of 3 patterns: abdominal, neurological, and psychotic. These patients do not have photosensitivity. There is excessive delta aminolaevulinic acid and porphobilinogen in the urine.

– *Variagate porphyria* is common in the whites of South Africa. Photosensitivity occurs and there are acute attacks of colicky abdominal pain and neurological manifestations.

– *Hereditary coproporphyrinuria* is quite rare.

– *Porphyria cutanea tarda* is the most common of all porphyrias.

Porphyryns collect in the liver and small quantity is excreted in the urine. Skin lesions are similar to those in variegate porphyria. Most of the patients have associated haemosiderosis with cirrhosis which may eventually develop into hepatocellular carcinoma.

LIPIDOGENIC PIGMENTS

Lipidogenic pigments include lipofuscin, pigment of vitamin E insufficiency, ceroid, and lipochrome.

Lipofuscin (wear and tear pigment)

Lipofuscin is yellowish-brown intracellular lipid pigment (*lipo* = fat, *fuscus* = dark). The pigment is often found in atrophied cells of old age and hence has the name 'wear and tear pigment'. It is seen in the myocardial fibres, hepatocytes, cells of the testes and neurons. The pigment often accumulates in the central part of the cells around the nuclei. In the heart muscle, the change is associated with wasting of the muscle and is commonly referred to as "brown atrophy". Deposition of lipofuscin in neurons is seen in senile dementia. However, the pigment may, at times, accumulate rapidly in different cells in wasting diseases unrelated to aging.

Lipofuscin represents the collection of indigestible material in the lysosomes after autophagy and is therefore an example of residual bodies. The pigment can be stained by fat stains but differs from other lipids in being fluorescent and acid-fast.

By electron microscopy, lipofuscin appears as intra-lysosomal electron-dense granules in perinuclear location. These granules are composed of lipid-protein complexes.

B. EXOGENOUS

PIGMENTS INHALED

PIGMENTS

The lungs of most individuals, especially of those living in urban areas due to atmospheric pollutants and of smokers, show a large number of inhaled pigmented materials. The most commonly inhaled substances are carbon or coal dust; others are silica or stone dust, iron or iron oxide, asbestos and various other organic substances. These substances may produce occupational lung diseases called pneumoconiosis. The pigment particles after inhalation are taken up by alveolar macrophages. Some of the pigment-laden macrophages are coughed out via bronchi, while some

settle in the interstitial tissue

of the lung and in the respiratory bronchioles and pass into lymphatics to be deposited in the hilar lymph nodes. Anthracosis (i.e. deposition of carbon particles) is seen in almost every adult lung and generally provokes no reaction of tissue injury. However, extensive deposition of particulate material over many years in coal miners' pneumoconiosis, silicosis, asbestosis, etc. provoke low grade inflammation, fibrosis and impaired respiratory function.

INGESTED PIGMENTS

Chronic ingestion of certain metals may produce pigmentation.

The examples are as under:

- i) Argyria is chronic ingestion of silver compounds and results in brownish pigmentation in the skin, bowel, and kidney.
- ii) Chronic lead poisoning may produce the characteristic blue lines on teeth at the gumline.
- iii) Melanosis coli results from prolonged ingestion of certain cathartics.
- iv) Carotenaemia is yellowish-red coloration of the skin caused by excessive ingestion of carrots which contain carotene.

INJECTED PIGMENTS (TATTOOING)

Pigments like India ink, cinnabar and carbon are introduced into the dermis in the process of tattooing where the pigment is taken up by macrophages and lies permanently in the connective tissue. The examples of injected pigments are prolonged use of ointments containing mercury, dirt left accidentally in a wound, and tattooing by pricking the skin with dyes.

DISTURBANCES OF MINERAL METABOLISM.

PATHOLOGIC CALCIFICATION.

Deposition of calcium salts in tissues other than osteoid or enamel is called pathologic or heterotopic calcification. Three distinct types of pathologic calcification are recognised:

- *Dystrophic calcification*, which is characterised by deposition of calcium salts in dead or degenerated tissues with normal calcium metabolism and normal serum calcium levels.
- *Metastatic calcification*, on the other hand, occurs in apparently normal tissues and is associated with deranged calcium metabolism and hypercalcaemia.
- *Metabolic calcification*, main cause of its development is an instability of buffer systems of the blood and tissue liquid.

Etiology and pathogenesis of the three are different but morphologically the deposits in all resemble normal minerals of the bone.

Histologically, in routine H and E stained sections, calcium salts appear as deeply basophilic, irregular and granular clumps. The deposits may be intra-cellular, extracellular, or at both locations. Occasionally, heterotopic bone formation (ossification) may occur. Calcium deposits can be confirmed by special stains like silver impregnation method of von-Kossa producing black colour, and alizarin red S that produces red staining. Pathologic calcification is often accompanied by diffuse or granular deposits of iron giving positive Prussian blue reaction.

DYSTROPHIC CALCIFICATION. As apparent from definition, dystrophic calcification may occur due to 2 types of causes:

- Calcification in dead tissue
- Calcification of degenerated tissue.

Calcification in dead tissue:

1. Caseous necrosis in tuberculosis is the most common site for dystrophic calcification. Living bacilli may be present even in calcified tuberculous lesions, lymph nodes, lungs, etc.

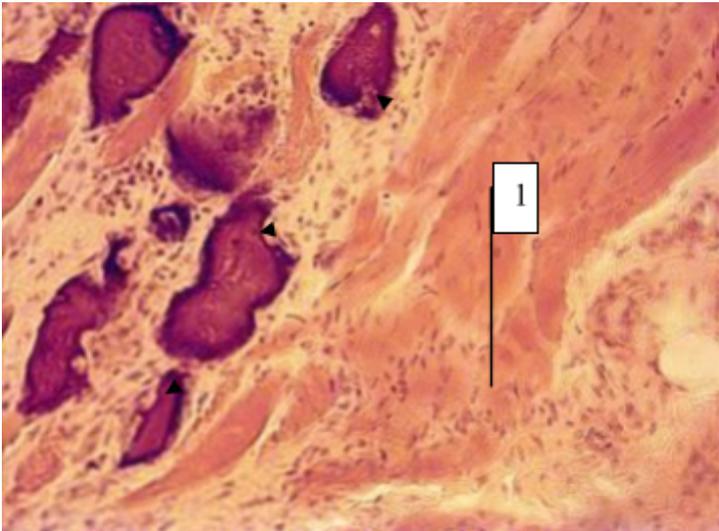


Fig. 9. dystrophic calcification of skeletal muscle in Cencer`s necrosis. H and E stained section, x 200.

1 - deposition of calcium soaps.

Living bacilli may be present even in calcified tuberculous lesions, lymph nodes, lungs, etc.

2. Liquefaction necrosis in chronic abscesses may get calcified.

3. Fat necrosis following acute pancreatitis or traumatic fat necrosis

in the breast results in deposition of calcium soaps.

4. Infarcts may sometimes undergo dystrophic calcification.
5. Thrombi, especially in the veins, may produce phleboliths.
6. Haematomas in the vicinity of bones may undergo dystrophic calcification.
7. Dead parasites like in hydatid cyst, Schistosoma eggs, and cysticercosis are some of the examples showing dystrophic calcification.

8. Calcification in breast cancer detected by mammography.
9. Congenital toxoplasmosis involving the central nervous system visualised by calcification in the infant brain.

Calcification in degenerated tissues:

1. Dense old scars may undergo hyaline degeneration and subsequent calcification.
2. Atheromas in the aorta and coronaries frequently undergo calcification.
3. Monckeberg's sclerosis shows calcification in the tunica media of muscular arteries in elderly people.
4. Stroma of tumours such as uterine fibroids, breast cancer, thyroid adenoma, goitre etc show calcification. Some tumours show characteristic spherules of calcification called psammoma bodies or calcospherites such as in meningioma, papillary serous cystadenocarcinoma of the ovary and papillary carcinoma of the thyroid.
5. Cysts which have been present for a long time may show calcification of their walls e.g. epidermal and pilar cysts.
6. Calcinosis cutis is a condition of unknown cause in which there are irregular nodular deposits of calcium salts in the skin and subcutaneous tissue.
7. Senile degenerative changes may be accompanied by dystrophic calcification such as in costal cartilages, tracheal or bronchial cartilages, and pineal gland in the brain etc.

The **pathogenesis** of dystrophic calcification is not quite clear. A few factors like local alteration of pH in the necrotic tissue and release of enzymes (e.g. alkaline phosphatase) from necrotic or degenerated tissue have been implicated which favour deposition of calcium salts. More recently, however, the process of dystrophic calcification has been likened to the formation of normal hydroxyapatite in the bone involving 2 phases: initiation and propagation.

- Initiation is the phase in which calcium and phosphates begin to accumulate intracellularly in the mitochondria, or extracellularly in membrane-bound vesicles.
- Propagation is the phase in which minerals deposited in the initiation phase are propagated to form mineral crystals.

METASTATIC CALCIFICATION. Since metastatic calcification occurs in normal tissues due to hypercalcaemia, its causes would include one of the following two conditions:

- Excessive mobilisation of calcium from the bone.
- Excessive absorption of calcium from the gut.

Excessive mobilisation of calcium from the bone.

These causes are more common and include the following:

1. Hyperparathyroidism which may be primary such as due to para-thyroid adenoma, or secondary such as from parathyroid hyperplasia, chronic renal failure etc.
2. Bony destructive lesions such as multiple myeloma, metastatic carcinoma.
3. Prolonged immobilisation of a patient results in disuse atrophy of the bones and hypercalcaemia.

Excessive absorption of calcium from the gut. Less often, excess calcium may be absorbed from the gut causing hypercalcaemia and metastatic calcification. These causes are as under:

1. Hypervitaminosis D results in increased calcium absorption.
2. Milk-alkali syndrome caused by excessive oral intake of calcium in the form of milk and administration of calcium carbonate in the treatment of peptic ulcer.
3. Hypercalcaemia of infancy is another condition in which metastatic calcification may occur.

Metastatic calcification may occur in any normal tissue of the body but affects the following organs more commonly:

- Kidneys, especially at the basement membrane of tubular epithelium and in the tubular lumina causing nephrocalcinosis.
- Lungs, especially in the alveolar walls.
- Stomach, on the acid-secreting fundal glands.
- Blood vessels, especially on the internal elastic lamina.
- Cornea is another site affected by metastatic calcification.

The **pathogenesis** of metastatic calcification at the above mentioned sites is based on the hypothesis that these sites have relatively high (alkaline) pH which favours the precipitation of the calcium.

METABOLIC CALCIFICATION. The mechanism of metabolic calcification development is not clearly understood. The principal value is significant

to instability of the organism buffer systems. Owing to this instability salts of calcium are not kept in blood and interstitial fluid. Hereditary sensitivity of tissues to calcium has a significant role too.

NUCLEOPROTEINS METABOLISM DISTURBANCES

Disturbances of nucleoproteins metabolism are characterised by excessive formation of uric acid and precipitation of its salts in the tissues. This process is observed at podagra, urolithiasis and uric acid infarction of kidneys.

The podagra (gout) is characterized by periodic precipitation of sodium salt of uric acid in joints accompanied by a painful attack. An increase in uric acid salts content in blood (hyperuricemia) and in urine (hyperuricuria) is found in patients. Salts (are) usually accumulate in synovia and cartilages of feet and small joints of hands, talocrural and knee joints, in tendons and articular bags, in the cartilage of auricles. There is necrotizing of tissues in which salts precipitate in the form of crystals or amorphous substances. An inflammatory reaction with appearance of giant cells develops around salts depositions and necrotic foci. Podagric tophus develops when there is an increase of salt deposition and connective tissue growth in peri-articular region and adjacent tissue. It arises in auricles and other parts of the body leading to the deformation of joints.

Changes in kidneys. There is accumulation of uric acid and sodium salt of uric acid salts in renal tubules and collecting tubes with the obturation of their lumens and development of secondary inflammatory and atrophic changes (gouty kidneys) at podagra.

The development of podagra is mainly caused by congenital disturbances of metabolism (primary gout), thus the role of dietary habits and over-use of animal proteins is great. Less often gout is the complication of other diseases (secondary gout), such, as nephrocirrhosis, leukaemias, etc.

Urolithiasis is caused, first of all, by the disturbances of purine metabolism i.e. it can be the manifestation of so-called urine acid diathesis. Thus urates are formed in kidneys and urinary tracts.

Uric acid infarct occurs in newborns of two days old and upward. It is manifested by depositions of amorphous substances of sodium salt of uric acid and ammonium in renal tubules and collecting tubes. The cause of uric acid infarction is intensive metabolism in the first days of newborn life. It reflects an adaptative reaction of kidneys to the new conditions of existence.

IRREVERSIBLE CELL INJURY (CELL DEATH)

Cell death is a state of irreversible injury. It may occur in the living body as a local or focal change (i.e. necrosis and apoptosis) and the changes that follow it (i.e. gangrene and pathologic calcification), or result in end of the life (somatic death). These pathologic processes involved in cell death are described below.

NECROSIS

Necrosis is defined as focal death along with degradation of tissue by hydrolytic enzymes liberated by cell. It is invariably accompanied by inflammatory reaction.

Necrosis can be caused by various agents such as hypoxia, chemical and physical agents, microbial agents and immunological injury.

According to the cause there are 5 types of necrosis:

1. Traumatic.
2. Toxic.
3. Trophoneurotic.
4. Allergic.
5. Vascular, or ischemic.

Necrosis may occur due to external or internal factors.

External factors may be mechanical trauma (physical damage to the body which causes cellular breakdown), damage to blood vessels (which may disrupt blood supply to associated tissue), and ischemia, thermal effects (extremely high or low temperature).

Internal factors causing necrosis include: trophoneurotic disorders; injury and paralysis of nerve cells, action of pancreatic enzymes (lipases), components of the immune system, such as the complement system; bacterial toxins; activated natural killer cells; and peritoneal macrophages.

So, according to the mechanism of pathological factor action necrosis is classified into *direct* (produced by mechanical, physical, chemical, and toxic factors) and *indirect* (caused by vascular and neurogenous disturbances).

Two essential changes bring about irreversible cell injury in necrosis – cell digestion by lytic enzymes and denaturation of proteins. These processes are morphologically identified by characteristic cytoplasmic and nuclear changes in necrotic cell. The cytoplasm appears homogeneous and in-

tensely eosinophilic. Occasionally, it may show vacuolation or dystrophic calcification. The nuclear changes include condensation of nuclear chromatin (pyknosis) which may either undergo dissolution (karyolysis) or fragmentation into many granular clumps (karyorrhexis).

There are 4 stages of necrosis: paranecrosis, necrobiosis, necrosis, autolysis.

Autolysis (self-digestion) is disintegration of the cell by its own hydrolytic enzymes liberated from lysosomes. Autolysis can occur in the living body when it is surrounded by inflammatory reaction (vital reaction), or may occur as postmortem change in which there is complete absence of surrounding inflammatory response. Autolysis is rapid in some tissues rich in hydrolytic enzymes such as in the pancreas, and gastric mucosa; intermediate in tissues like the heart, liver and kidney; and slow in fibrous tissue. Morphologically, autolysis is identified by homogeneous and eosinophilic cytoplasm with loss of cellular details and remains of cell as debris.

Morphologically, 6 distinct **types of necrosis** are identified: coagulative (includes fibrinoid, Cencer's, caseous subtypes), liquefaction (or colliquative), fat, gangrene, ischemic necrosis and sequestration.

1. Coagulative necrosis

This is the most common type of necrosis caused by irreversible focal injury, mostly from sudden cessation of blood flow (ischaemia), and less often from bacterial and chemical agents. It develops in tissues rich with the protein. The organs commonly affected are the heart, kidney, and spleen.

Grossly, foci of coagulative necrosis in the early stage are pale, firm, and slightly swollen. With progression, they become more yellowish, softer, and shrunken.

Microscopically, the hallmark of coagulative necrosis is the conversion of normal cells into their 'tombstones' i.e. outlines of the cells are retained so that the cell type can still be recognised but their cytoplasmic and nuclear details are lost. The necrosed cells are swollen and appear more eosinophilic than the normal, along with nuclear changes described above. This pattern of microscopic change results from 2 processes: denaturation of proteins and enzymatic digestion of the cell. But cell digestion and liquefaction fail to occur. Eventually, the necrosed focus is infiltrated by inflammatory cells and the dead cells are phagocytosed leaving granular debris and fragments of cells.

Caseous necrosis is found in the centre of foci of tuberculous infections. It combines features of both coagulative and liquefactive necrosis.

Grossly, foci of caseous necrosis, as the name implies, resemble dry curd and are soft, granular and yellowish. This appearance is partly attributed to the histotoxic effects of lipopolysaccharides present in the capsule of the tubercle bacilli, *Mycobacterium tuberculosis*.

Microscopically, the necrosed foci are structureless, eosinophilic, and contain granular debris. The surrounding tissue shows characteristic granulomatous inflammatory reaction consisting of epithelioid cells with interspersed giant cells of Langhans or foreign body type and peripheral mantle of lymphocytes.

Fibrinoid necrosis or fibrinoid degeneration is characterised by deposition of fibrin-like material which has the staining properties of fibrin. It is encountered in various examples of immunologic tissue injury (e.g. in immune complex vasculitis, autoimmune disease, Arthus reaction etc), arterioles in hypertension, peptic ulcer etc.

Microscopically, fibrinoid necrosis is identified by brightly eosinophilic, hyaline-like deposition in the vessel wall or on the luminal surface of a peptic ulcer. Local haemorrhages may occur due to rupture of these blood vessels

Cancer's necrosis occurs in striated muscles of abdomen cavity in Enteric fever.

2. Liquefaction (colliquative) necrosis

Liquefaction or colliquative necrosis occurs commonly due to ischaemic injury and bacterial or fungal infections. It occurs due to degradation of tissue by the action of powerful hydrolytic enzymes and develops in tissues rich with the liquid. The common examples are brain infarct and abscess cavity.

Grossly, the affected area is soft with liquefied centre containing necrotic debris. Later, a cyst wall is formed.

Microscopically, the cystic space contains necrotic cell debris and macrophages filled with phagocytosed material. The cyst wall is

formed by proliferating capillaries, inflammatory cells, and gliosis (proliferating glial cells) in the case of brain and proliferating fibroblasts in the case of abscess cavity.

3. Fat necrosis

Fat necrosis is a special form of cell death occurring at two anatomically different locations but morphologically similar lesions. These are: following acute pancreatic necrosis, and traumatic fat necrosis commonly in breasts.

In the case of pancreas, there is liberation of pancreatic lipases from injured or inflamed tissue that results in necrosis of the pancreas as well as of the fat depots throughout the peritoneal cavity, and sometimes, even affecting the extra-abdominal adipose tissue.

Fat necrosis in either of the two instances results in hydrolysis of neutral fat present in adipose cells into glycerol and free fatty acids. The damaged adipose cells assume cloudy appearance when only free fatty acids remain behind, after glycerol leaks out. The leaked out free fatty acids, on the other hand, complex with calcium to form calcium soaps (saponification) discussed later under dystrophic calcification.

Grossly, fat necrosis appears as yellowish-white and firm deposits. Formation of calcium soaps imparts the necrosed foci firmer and chalky white appearance.

Microscopically, the necrosed fat cells have cloudy appearance and are surrounded by an inflammatory reaction. Formation of calcium soaps is identified in the tissue sections as amorphous, granular and basophilic material.

4. Gangrene

Gangrene is a form of necrosis of tissue with superadded putrefaction. The type of necrosis is usually coagulative due to ischaemia (e.g. in gangrene of the bowel, gangrene of limb). On the other hand, gangrenous or necrotising inflammation is characterised by primarily inflammation provoked by virulent bacteria resulting in massive tissue necrosis. Thus the end-result of necrotising inflammation and gangrene is the same but the way two are produced is different. The examples of necrotising inflammation are: gangrene lung, gangrenous appendicitis, and noma (cancrum oris).

There are 3 main forms of gangrene – dry, wet and gas gangrene. In

either type of gangrene, coagulative necrosis undergoes liquefaction by the action of putrefactive bacteria.

Dry Gangrene. This form of gangrene begins in the distal part of a limb due to ischaemia. The typical example is the dry gangrene in the toes and feet of an old patient due to arteriosclerosis. Other causes include thromboangiitis obliterans (Buerger's disease), Raynaud's disease, trauma, ergot poisoning. It is usually initiated in one of the toes which is farthest from the blood supply, containing so little blood that even the invading bacteria find it hard to grow in the necrosed tissue. The gangrene spreads slowly upwards until it reaches a point where the blood supply is adequate to keep the tissue viable. A line of separation is formed at this point between the gangrenous part and the viable part.

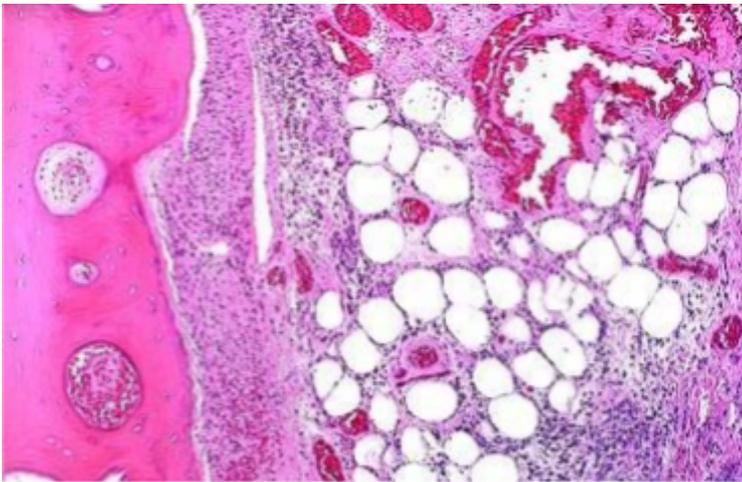


Fig. 10. Dry Gangrene. H and E stained section, x 200.

Macroscopically, the affected part is dry, shrunken and dark black, resembling the foot of a mummy. It is black due to liberation of haemoglobin from haemolysed red blood cells which is acted upon by hydrogen disulfide (H_2S) produced by bacteria resulting in for-

mation of black iron sulfide. The line of separation usually brings about complete separation with eventual falling off of the gangrenous tissue if it is not removed surgically.

Histologically, there is necrosis with smudging of the tissue (see Fig. 10). The line of separation consists of inflammatory granulation tissue.

Wet Gangrene. This occurs in naturally moist tissues and organs such as the mouth, bowel, lung, cervix, vulva etc. Diabetic foot is another example of wet gangrene due to high sugar content in the necrosed tissue which favours growth of bacteria. Bed sores occurring in a bed-ridden patient due to pressure on sites like the sacrum, buttocks and heels are the other important clinical conditions included in wet gangrene. Wet gangrene

usually develops rapidly due to blockage of venous and less commonly arterial blood flow from thrombosis or embolism. The affected part is stuffed with blood which favours the rapid growth of putrefactive bacteria. The toxic products formed by bacteria are absorbed causing systemic manifestations of septicaemia, and finally death. The spreading wet gangrene lacks clear cut line of demarcation and may spread to peritoneal cavity causing peritonitis.

Macroscopically, the affected part is soft, swollen, putrid, rotten and dark. The classic example is gangrene of bowel, commonly due to strangulated hernia, volvulus or intussusception. The part is stained dark due to the same mechanism as in dry gangrene.

Histologically, there is coagulative necrosis with stuffing of affected part with blood. There is ulceration of the mucosa and intense inflammatory infiltration. Lumen of the bowel contains mucus and blood. The line of demarcation between gangrenous segment and viable bowel is generally not clear cut.

Gas Gangrene. Gas gangrene is a special form of wet gangrene caused by gas-forming clostridia (gram-positive anaerobic bacteria) which gain entry into the tissues through open contaminated wounds, especially in the muscles, or as a complication of operation on colon which normally contains clostridia. Clostridia produce various toxins which produce necrosis and oedema locally and are also absorbed producing profound systemic manifestations.

Grossly, the affected area is swollen, oedematous, painful and crepitant due to accumulation of gas bubbles within the tissues. Subsequently, the affected tissue becomes dark black and foul smelling.

Microscopically, the muscle fibres undergo coagulative necrosis with liquefaction. Large number of gram-positive bacilli can be identified. At the periphery, a zone of leucocytic infiltration, oedema and congestion are found. Capillary and venous thrombi are common.

5. Infarction, or vascular (ischemic) necrosis.

Infarcts are classified depending upon different features:

– *according to the shape of infarction zone:*

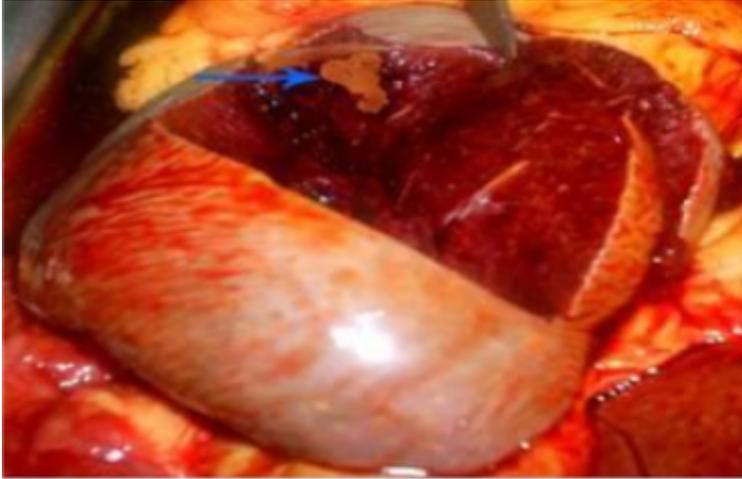


Fig. 11. Wedge-shaped pale infarction of the spleen.

1. Wedge-shaped infarction (in organs with the main-line type of vascularization e.g. in spleen, kidney, lung);



Fig. 12. Irregular-shape red infarction of the intestine.

2. Irregular-shape infarction (in organs with disseminated or mixed type of blood supply e.g. myocardium, brain, intestine).

– *according to the color of infarction zone:*

1. Pale or anaemic, due to arterial occlusion and are seen in compact organs (e.g. spleen, brain).

2. Pale with haemorrhagic crown (e.g. heart, kidneys).

3. Red or haemorrhagic, seen in soft loose tissues and are caused either by pulmonary arterial obstruction (e.g. in the lungs) or by arterial or venous occlusion (e.g. in the intestines).

– *according to their age:*

- recent, or fresh,

- old, or healed.

– *according to presence or absence of infection:*

- bland, when free of bacterial contamination,
- septic, when infected.

6. Sequestration develops in bones and is characterized by the absence of boundary between the necrotic zone and surrounded tissue.

FATE OF NECROSIS

- Regeneration of tissues – the replacement of the dead tissue with a new one.
- Incapsulation – the formation of the connective tissue capsule around necrotic area.
- Organization – the replacement of the dead tissue with connective tissue.
- Petrification – replacement of the dead tissue and the deposition of calcium salts.
- Ossification – the formation of the bone tissue in the necrotic area.
- Hyaline change – the appearance of the hyaline-like substance in the necrotic area.
- Suppuration, or purulent fusion of necrotic tissues.
- Sequestration – formation of sequester.
- Mutilation – spontaneous tearing away of the dead tissue.
- Cyst formation.

APOPTOSIS

Apoptosis is a form of “coordinated and internally programmed cell death” which is of significance in a variety of physiologic and pathologic conditions (apoptosis is a Greek word meaning “failing off” or “dropping off”). The term was first coined in 1972 as distinct from necrosis.

Morphologic changes: The characteristic morphologic changes in apoptosis as seen in histologic and electron microscopic examination are as under:

1. Shrinkage of cell with dense cytoplasm and almost normal organelles.
2. Convulsions of the cell membrane with formation of membrane-bound near-spherical bodies called apoptotic bodies containing com-

pacted organelles.

3. Chromatin condensation around the periphery of nucleus.
4. Characteristically, there is no acute inflammatory reaction.
5. Phagocytosis of apoptotic bodies by macrophages takes place at varying speed. There may be swift phagocytosis, or loosely floating apoptotic cells after losing contact, with each other and basement membrane as single cells, or may result in major cell loss in the tissue without significant change in the overall tissue structure.

Histologic examination of tissues stained with H & E shows apoptosis involving single cells or small clusters of cells in the background of viable cells. The apoptotic cells are round to oval shrunken masses of intensely eosinophilic cytoplasm containing condensed or fragmented nuclear chromatin. Unlike necrosis, inflammatory response around apoptosis is absent.

Biochemical changes: Biochemical processes underlying the morphologic changes are as under:

1. Proteolysis of cytoskeletal proteins.
2. Protein-protein cross linking.
3. Fragmentation of nuclear chromatin by activation of nuclease.
4. Appearance of phosphatidylserine on the outer surface of cell membrane.
5. In some forms of apoptosis, appearance of an adhesive glycoprotein thrombospondin on the outer surface of apoptotic bodies.
6. Appearance of phosphatidylserine and thrombospondin on the outer surface of apoptotic cell facilitates early recognition by macrophages for phagocytosis prior to appearance of inflammatory cells.
7. The biochemical mechanism underlying rapid cell shrinkage, however, remains unexplained.

Identifying apoptotic cells. Identifying and counting of apoptotic cells is possible by following methods:

1. Staining of chromatin condensation (by haematoxylin, Feulgen, or acridine orange).
2. Flow cytometry to visualise rapid cell shrinkage.
3. DNA changes detected by in situ techniques or by gel electrophoresis.
4. Annexin V as marker for apoptotic cell membrane having phosphatidylserine on the cell exterior.

Molecular mechanisms of apoptosis.

Several physiologic and pathologic processes activate apoptosis in a variety of ways. However, in general the following events sum up the sequence involved in apoptosis:

1. *Initiators of apoptosis.* Stimuli for signalling programmed cell death act either at the cell membrane or intracellularly. These include:

i) Absence of stimuli required for normal cell survival (e.g. absence of certain hormones, growth factors, cytokines).

ii) Activators of programmed cell death (e.g. receptors for TNF).

iii) Intracellular stimuli include heat, radiation, hypoxia etc.

2. *Regulators of apoptosis.* After a cell has been initiated into apoptosis by above-mentioned signals, next comes the phase in which certain proteins convert death signals to the final programmed cell death and thus determine the *outcome*. These regulator proteins include the following:

i) bcl-2. bcl-2 protein is a human counterpart of ced-9 (cell death) gene found in programmed cell death of nematode worm *C. elegans*. bcl-2 is located in the outer mitochondrial membrane and may regulate the apoptotic process by binding to some other related proteins e.g. to bax and bad for promoting apoptosis, and bcl-XL for inhibiting apoptosis. Another important bcl-2 binding protein in the cytosol is the pro-apoptotic protease activating factor (apaf-1) which is a mammalian counterpart of gene ced-4 of nematode. The net effect on the mitochondrial membrane is thus based on the pro-apoptotic and anti-apoptotic members of bcl-2 protein family.

ii) Other apoptotic regulator proteins. Besides bcl-2, other important regulator proteins of apoptosis are p53 protein, caspases, bax and certain viruses (adenovirus, papillomavirus, hepatitis B virus).

3. *Programmed cell death.* The final outcome of apoptotic regulators in the programmed cell death involves the following pathways:

i) Fas receptor activation. Cell surface receptor Fas (CD 95) presents on cytotoxic (CD 8 +) T cells. On coming in contact with the target cell, the Fas receptor is activated. This leads to activation of caspases and subsequent proteolysis.

ii) Ceramide generation. Due to hydrolysis of phospholipid sphingo- myelin of the plasma membrane, ceramide is generated. Ceramide is implicated in further mitochondrial injury.

iii) DNA damage. Damage to DNA produced by various agents such as ionising radiation, chemotherapeutic agents, activated oxygen species lead to apoptosis. DNA damage affects nuclear protein *p53* which induces the synthesis of cell death promoting protein Bax.

Phagocytosis. The dead apoptotic cells and their fragments possess cell surface receptors which facilitate their identification by adjacent phagocytes. The phagocytosis is unaccompanied by any other inflammatory cells.

Apoptosis in biologic processes.

Apoptosis is responsible for mediating cell death in a wide variety of physiologic and pathologic processes as under:

Physiologic Processes:

1. Organized cell destruction in sculpting of tissues during development of embryo.
2. Physiologic involution of cells in hormone-dependent tissues e.g. endometrial shedding, regression of lactating breast after withdrawal of breast feeding.
3. Normal cell destruction followed by replacement proliferation such as in intestinal epithelium.
4. Involution of the thymus in early age.

Pathologic Processes:

1. Cell death in tumours exposed to chemotherapeutic agents.
2. Cell death by cytotoxic T cells in immune mechanisms such as in graft-versus-host disease and rejection reactions.
3. Cell death in viral infections e.g. formation of Councilman bodies in viral hepatitis
4. Pathologic atrophy of organs and tissues on withdrawal of stimuli e.g. prostatic atrophy after orchiectomy, atrophy of kidney or salivary gland on obstruction of ureter or ducts, respectively.
5. Progressive depletion of CD4⁺ T cells in the pathogenesis of AIDS.
6. Cell death in response to injurious agents involved in causation of necrosis e.g. radiation, hypoxia and mild thermal injury.
7. In degenerative diseases of CNS e.g. in Alzheimer's disease, Parkinson's disease, and chronic infective dementias.

GENERAL DEATH. DEATH SIGNS. POSTMORTAL CHANGES.

Death is a biological process of irreversible organism vital activity cessation. Death results in the man transformation into the corpse (cadaver).

Depending on the causes of death there are nature (physiological), violent death and the death due to diseases.

Physiological death comes in elder persons and long-livers due to nature (physiological) wear of the organism. A man lifetime is not limited. According to the world data the age of long-livers may be 150 years and more.

Violent death happens from such acts (deliberate in deliberate) as murder, suicide, death due to traumas (e.g. street, industrial, home), accidents (e.g. transport catastrophe). Being social-legal categories it is studied by forensic medicine and justice institutions.

The death due to diseases results from incompatibility of the life and changes in the organism, caused by pathological (ill) processes. Usually death comes slowly and is accompanied by the disappearance of vital functions. But sometimes the death arises suddenly – sudden death. It occurs in latent or enough compensated disease, when suddenly mortal complication develops (profuse bleeding in aorta rupture, acute myocardium infarction due to coronary artery thrombosis, hemorrhagic stroke).

Depending on development of vital activity reversible and irreversible changes there are clinical and biological death. Clinical death is characterized by the breath and blood circulation stop. But these changes of organism activity are reversible during some minutes (the time of brain cortex surviving). The base of clinical death is hypoxia state (mainly CNS) due to blood circulation stop) and absence of its central regulation.

Clinical death is predisposed by agonies, which is characterized by the uncoordinated activity of homeostatic system in terminal period (arrhythmia, paralyze of sphincters, lung oedema). Due to this agony, continuing from some minutes to some hours, is considered as terminal state resulting in clinical death.

Terminal state (agony, shock, haemorrhage and other) and clinical death need the reanimation methods.

Biological death means irreversible changes of organism vital activity, onset of autolytic processes. However, in biological death the death of cells and tissues don't occur simultaneously. First the central nervous sys-

tem dies. In 5-6 min after breath and blood circulation stop the ultra structural elements of brain and spinal cord parenchymatous cells have been destroyed. In the other organs and tissues (spleen, kidney, heart, lungs and other) this process continues during hours and sometime days. Common structure of many organs and tissues, observed microscopically after the death, is kept during long time. And only electron microscopy helps to determine destruction of the cells ultra structure. Due to this pathologist can assume the pathological changes of cadaver organs and tissues using the microscopic methods.

Biological death is accompanied with the appearing of some signs of death and post mortal changes:

- cadaveric cooling,
- cadaveric rigidity,
- cadaveric desiccation,
- blood redistribution,
- livores mortis (cadaveric spots),
- putrefaction (cadaveric decay).

Cadaver cooling (algor mortis) results from the heat producing cessation and equalization of the body and environment temperature. The cooling of cadaver comes slowly in patients with fever before death or with spasms in long agonic period. Sometimes (tetanus, poisoning with strychnine) during first hours after death the cadaver temperature may increase.

Cadaver rigidity (rigor mortis) is characterized by the hardening of the voluntary and involuntary muscles. It results from disappearance of the ATF from the muscles after death and accumulation of the lactase acid there. Cadaver rigidity starts usually in 2-5 hours after death and to the end of 1st day involves whole musculature. First the muscles of facial expression and mystification undergo the rigidity and then the muscles of neck, trunk and extremities. Cadaver rigidity continues during 2-3 days and then disappears in the same sequence. Cadaver rigidity is more expressed and early in persons with developed musculature and in cases when the death is accompanied by the spasms (e.g. tetanus, poisoning with strychnine). Poor cadaver rigidity occurs in elder persons and children, in patients died due to sepsis, in cachectic patients. In prematurely born fetus cadaver rigidity is absent. Low temperature of environment makes difficult the cadaver rigidity development and prolongs its continuation. High temperature accelerates the cadaver rigidity disappearance.

Cadaver desiccation arises due to evaporation of moisture from the body surface. This process may be local, but may spread and involve whole corpse (cadaver mummification). First desiccation involves the skin, eyes-balls, and mucous coats. It results in turbidity of cornea, dry brown triangle spots appearance on it. The base of these spots is facing to the cornea, and the apex – to eye angle. The mucous coats become dry, hard, of brown colour. First of all, dry, yellow-brown, parchment spots appear in the places of epidermis damage and maceration of the skin. These spots should be diagnosed with the vital burns and grazes.

Blood redistribution means the fullness of the veins, whereas the arteries become almost empty. In the veins and right chambers of the heart the post mortal blood coagulation occurs. The post mortal blood clots have yellow or red colour, smooth surface, elastic consistence, they don't attach to the wall. In prolonged death there are a lot of blood clots, in sudden death – less number.

Death resulting from asphyxia doesn't accompanied by the blood coagulation. With time haemolysis comes.

Cadaver spots (livores mortis) arise due to blood redistribution in the corpse. Their location depends on corpse position. In 3-6 hours after death the cadaveric (post-mortem) hypostases appear on the skin of underlying body parts due to accumulation of the blood in the veins of these regions. They are dark violet and become pale when pressing. The cadaver hypostases are absent in the regions of body undergoing to compression (sacrum region, scapula in the back lying position). They are more expressed in death due to diseases resulting in general venous hyperaemia and worse expressed in cachexia, hypovolemia, and anaemia.

When postmortal haemolysis of red blood cells comes, the regions of cadaver hypostases are impregnated with the blood plasma, diffusing from blood vessels and colouring with haemoglobin. The late cadaver spots appear – cadaver imbibitions. These spots are red or pink colour and don't disappear when pressing.

Cadaver decay is accompanied with autolysis and putrefaction. Postmortal autolysis arises early and more expressed in the glandular organs (liver, pancreas) and stomach, the cells of which are rich with hydrolytic enzymes. Self-digestion of pancreas arises very early. Due to activity of gastric juices increase the post mortal self-digestion of stomach develops

(*gastromalacia*). *Esophagomalacia* due to regurgitation and *pneumomalacia acida* due to aspiration of stomach contents are possible.

Putrefactive bacteria undergo the multiplication in the intestine, spread in the organism and cause the general putrefactive processes. Putrefaction increases postmortal autolysis, resulting in tissues melting and change of the colour into green due to destruction of haemoglobin and FeS formation.

Forming due to cadaver decay gases blow out the intestine, enter the tissues and organs and give them the foamy shape (palpation reveals crepitating cadaver emphysema). To stop or to prevent the cadaver putrefaction the cold freezing chamber and embalming are used.

CELLULAR ADAPTATIONS

The cell responds to injuries that fail to destroy it with either increased or decreased function. This response results from changes in its metabolism of structural and functional substances and may include cell proliferation. This also applies to the term dystrophy, which harkens back to the time before biochemistry and refers to the development of weakened structures originally attributed to nutritional deficiencies, and to the term degeneration, referring to transformation of a tissue into one of lesser quality.

Adaptive response with increased function (hypertrophy and hyperplasia): Hypertrophy results when functional internal cellular structures increase, causing the volume of the organ to increase. In hyperplasia, the expansion of the organ is also due to an increase in the number of cells. Cellular proliferation in these cases continues to follow the normal pattern of cell division and maturation. However, it is not always possible to draw a clear histological distinction between this type of proliferation and the autonomous cell growth of a tumour.

Adaptive response with decreased function (atrophy): This response initially manifests itself as atrophy in which a reduction in only the structural components of the cells occurs (simple atrophy). Later the total number of cells in the organ also decreases (numeric atrophy). The reduction in the quantity of cells is effected by the programmed death of individual cells.

Particular form of physiologic and pathologic adaptations is the changing of cells phenotypic differentiation pathway (i.e. metaplasia and dysplasia).

In general, the adaptive responses are reversible on withdrawal of stimulus. However, if the irritant stimulus persists for long time, the cell may not be able to survive and may either die or progress further (e.g. cell death in sustained atrophy, progression of dysplasia into carcinoma-in-situ). Thus, the concept of evolution “survival of the fittest” holds true for adaptation as “survival of the adaptable”.

Various mechanisms which may be involved in adaptive cellular responses include:

- Altered cell surface receptor binding
- Alterations in signal for protein synthesis

- Synthesis of new proteins by the target cell such as heat-shock proteins (HSPs).

Common forms of cellular adaptive responses along with examples of physiologic and pathologic adaptations are briefly discussed below.

ATROPHY

Reduction of the number and size of parenchymal cells of an organ or its parts which was once normal is called atrophy (c.f. hypoplasia which is the term used for developmentally small size, and aplasia for extreme failure of development so that only rudimentary tissue is present).

Atrophy may occur from **physiologic or pathologic** causes.

A. **Physiologic atrophy.** Atrophy is a normal process of aging in some tissues which could be due to loss of endocrine stimulation or arteriosclerosis. For example:

- i) Atrophy of lymphoid tissue in lymph nodes, appendix and thymus,
- ii) Atrophy of gonads after menopause,
- iii) Atrophy of brain.

B. **Pathologic atrophy.** It may be general and local.

General atrophy is observed in **cachexy (starvation)** due to:

- Oncologic diseases,
- Chronic infectious diseases,
- Injury of hypophysis (endocrine cachexia),
- Injury of hypothalamus (cerebral cachexia).

In starvation, there is first depletion of carbohydrate and fat stores followed by protein catabolism. There is general weakness, emaciation and anaemia referred to as cachexia seen in cancer and severely-ill patients.

Local atrophy may develop due to different causes:

1. **Ischaemic atrophy.** Gradual diminution of blood supply due to atherosclerosis may result in shrinkage of the affected organ e.g.

- i) Small atrophic kidney in atherosclerosis of renal artery.
- ii) Atrophy of brain in cerebral atherosclerosis.

2. **Disuse atrophy.** Prolonged diminished functional activity is associated with disuse atrophy of the organ e.g.

- i) Wasting of muscles of limb immobilised in cast,
- ii) Atrophy of the pancreas in obstruction of pancreatic duct.

3. **Neuropathic atrophy.** Interruption in nerve supply leads to wasting of muscles e.g.

- i) Poliomyelitis
- ii) Motor neuron disease
- iii) Nerve section.

4. **Endocrine atrophy.** Loss of endocrine regulatory mechanism results in reduced metabolic activity of tissues and hence atrophy e.g.

- i) Hypopituitarism may lead to atrophy of thyroid, adrenal and gonads.
- ii) Hypothyroidism may cause atrophy of the skin and its adnexal structures.

5. **Pressure atrophy.** Prolonged pressure from benign tumours or cyst or aneurysm may cause compression and atrophy of the tissues e.g.

- i) Atrophy of kidney parenchyma by tumor or stone in urolithiasis.
- ii) Atrophy of sternum by aneurysm of aorta arch.

6. **Idiopathic atrophy.** There are some examples of atrophy where no obvious cause is present e.g.

- i) Myopathies
- ii) Testicular atrophy.

7. **Atrophy due to chemical and physical factors.** For example: action of the radiation lead to atrophy of bone marrow and genital organs.

Pathologic changes. Irrespective of the underlying cause for atrophy, the pathologic changes are similar. The organ is small, often shrunken. The cells become smaller in size but they are not dead. Shrinkage in cell size is due to reduction in cell organelles, chiefly mitochondria, myofilaments and endoplasmic reticulum. There is often increase in the number of autophagic vacuoles containing cell debris. These autophagic vacuoles may persist to form 'residual bodies' in the cell cytoplasm e.g. lipofuscin pigment granules in brown atrophy.

HYPERTROPHY

Hypertrophy is an increase in the size of parenchymal cells resulting in enlargement of the organ or tissue without any change in the number of cells.

Pathogenesis: A stimulus inducing increased function activates proto-oncogenes. The resulting expression of transcription factors increases the

synthesis of RNA and proteins and later DNA as well (causing nuclear polyploidy). This accelerates intracellular growth processes and proliferation of organelles while growth inhibitors simultaneously prevent mitosis. The limiting factor for organ function is density of capillaries in the tissue, which does not increase proportionally.

Causes: Hypertrophy may be physiologic or pathologic. In both cases, it is caused either by increased functional demand or by hormonal stimulation. Hypertrophy without accompanying hyperplasia affects mainly muscles. In non-dividing cells too, only hypertrophy occurs.

A. ***Physiologic hypertrophy.*** It results from the increased workload on an organ. Examples include weight training, which produces hypertrophy of skeletal muscle (e.g. hypertrophied muscles in athletes and manual labourers), pregnancy with enlarged size of the uterus. All physiologic hypertrophies are reversible once the triggering stimulus is removed.

B. ***Pathologic hypertrophy.***

Pathologic hypertrophy can be classified as:

1. *Neurohumoral hypertrophy* develops due to impairment of endocrine functions.

Examples are endometrial glandular hyperplasia following estrogen excess production which results in metrorrhagia; atrophy of testis leads to increase of breast (gynecomastia); hyperfunction of anterior hypophysis lobe (adenoma) leads to increased skeleton (acromegaly).

2. *Compensatory working hypertrophy* develops in tissues consisting of stable undivided cells due to increase of their size. It may be often in cardiac muscle at some cardiac diseases (systemic hypertension, aortic valve disease (stenosis and insufficiency), mitral insufficiency), in smooth muscle (e.g. cardiac achalasia (in esophagus), pyloric stenosis (in stomach), and intestinal stricture; hypertrophy of urine bladder in adenoma of prostatic glands).

3. *Compensatory vicarious (substitutional) hypertrophy* may occur in an organ when the contralateral organ is removed. Examples are:

i) Following nephrectomy on one side in a young patient, there is compensatory hypertrophy as well as hyperplasia of the nephrons of the other kidney.

ii) Adrenal hyperplasia following removal of one adrenal gland.

4. *Compensatory reparative hyperplasia.* E.g. regeneration of the liver following partial hepatectomy, regeneration of epidermis after skin abrasion; bone marrow after blood loss.

5. *Hypertrophic vegetations* develop due to chronic inflammation in mucous membranes (polyps and condylomas); lymphostasis leads to ingrowth of connective tissue, examples of false hypertrophy. In wound healing, there is formation of granulation tissue.

Pathologic changes. The affected organ is enlarged and heavy. For example a hypertrophied heart of a patient with systemic hypertension may weigh 700-800 g as compared to average normal adult weight of 350 g. There is enlargement of muscle fibres as well as of nuclei. At ultrastructural level, there is increased synthesis of DNA and RNA, increased protein synthesis and increased number of organelles like mitochondria, endoplasmic reticulum and myofibrils.

HYPERPLASIA

Hyperplasia is an increase in the number of parenchymal cells resulting in enlargement of the organ or tissue. Quite often, both hyperplasia and hypertrophy occur together. Hyperplasia occurs due to increased recruitment of cells from G₀ (resting) phase of the cell cycle to undergo mitosis, when stimulated. Labile cells (e.g. epithelial cells of the skin and mucous membranes, cells of the bone marrow and lymph nodes) and stable cells (e.g. parenchymal cells of the liver, pancreas, kidney, adrenal, and thyroid) can undergo hyperplasia, while permanent cells (e.g. neurons, cardiac and skeletal muscle) have little or no capacity for regenerative hyperplastic growth. Neoplasia differs from hyperplasia in having hyperplastic growth with loss of growth-regulatory mechanism due to change in genetic composition of the cell. Hyperplasia, on the other hand, persists so long as stimulus presents.

Pathogenesis: Increased load on the tissue causes cellular hypertrophy. When the critical cell mass is exceeded, proto-oncogenes are activated and factors triggering mitosis (mitogenes) are expressed. This results in cell proliferation. The limiting factor for organ function is density of capillaries in the tissue, which does not increase proportionally.

Causes. As with other non-neoplastic disorders of growth, hyperplasia has also been divided into physiologic and pathologic.

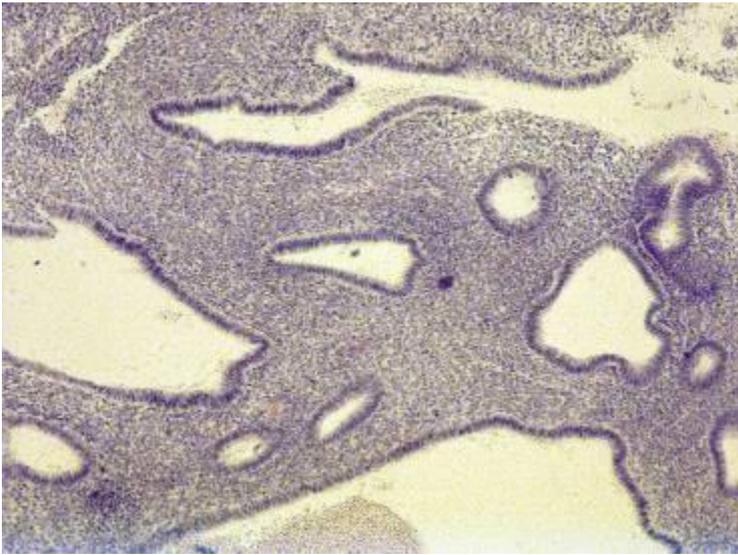


Fig. 13. Hyperplasia of uterus endometrium. H and E stained section, x 200.

A. Physiologic hyperplasia. The two most common types are as follows:

1. **Hormonal hyperplasia** i.e. hyperplasia occurring under the influence of hormonal stimulation e.g.

i) Hyperplasia of female breast at puberty, during pregnancy and lactation.

ii) Hyperplasia of

pregnant uterus.

iii) Proliferative activity of normal endometrium after a normal men- strual cycle.

iv) Prostatic hyperplasia in old age.

2. **Compensatory hyperplasia** i.e. hyperplasia occurring following removal of part of an organ or a contralateral organ in paired organ e.g.

i) Regeneration of the liver following partial hepatectomy

ii) Regeneration of epidermis after skin abrasion iii) Following nephrec- tomy on one side, there is hyperplasia of nephrons of the other kidney.

B. Pathologic hyperplasia. Most examples of pathologic hyperplasia are due to excessive stimulation of hormones or growth factors e.g.

i) Endometrial hyperplasia following oestrogen excess.

ii) In wound healing, there is formation of granulation tissue due to proliferation of fibroblasts and endothelial cells.

iii) Formation of skin warts from hyperplasia of epidermis due to human papilloma virus.

iv) Pseudocarcinomatous hyperplasia of the skin.

Pathologic changes. There is enlargement of the affected organ or tissue and increase in the number of cells. This is due to increased rate of DNA synthesis and hence increased mitoses of the cells.

METAPLASIA

Metaplasia (*meta* = transformation, *plasia* = growth) is defined as a reversible change of one type of epithelial or mesenchymal adult cells to another type of adult epithelial or mesenchymal cells, usually in response to abnormal stimuli, and often reverts back to normal on removal of stimulus. However, if the stimulus persists for a long time, epithelial metaplasia may transform into cancer.

Metaplasia is broadly divided into 2 types: **epithelial and mesenchymal**.

A. ***Epithelial metaplasia***. This is more common type. The metaplastic change may be patchy or diffuse and usually results in replacement by stronger but less well-specialised epithelium. However, the metaplastic epithelium being less well-specialised such as squamous type, results in deprivation of protective mucus secretion and hence more prone to infection.

Some common types of epithelial metaplasia are as under:

1. ***Squamous metaplasia***. Various types of epithelium are capable of undergoing squamous metaplastic change due to chronic irritation that may be mechanical, chemical or infective in origin. Some common examples of squamous metaplasia are seen at following sites:

i) In bronchus (normally lined by pseudostratified columnar ciliated epithelium) in chronic smokers.

ii) In uterine endocervix (normally lined by simple columnar epithelium) in prolapse of the uterus and in old age.

iii) In gall bladder (normally lined by simple columnar epithelium) in chronic cholecystitis with cholelithiasis.

iv) In prostate (ducts normally lined by simple columnar epithelium) in chronic prostatitis and oestrogen therapy.

v) In renal pelvis and urinary bladder (normally lined by transitional epithelium) in chronic infection and stones.

vi) In vitamin A deficiency, apart from xerophthalmia, there is squamous metaplasia in the nose, bronchi, urinary tract, lacrimal and salivary glands.

2. ***Columnar metaplasia***. There are some conditions in which there is transformation to columnar epithelium. For example:

i) Intestinal metaplasia in healed chronic gastric ulcer.

ii) Conversion of pseudostratified columnar epithelium in chronic bronchitis and bronchiectasis to columnar type.

iii) In cervical erosion (congenital and adult type), there is variable area of endocervical glandular mucosa everted into the vagina.

B. ***Mesenchymal metaplasia.*** Less often, there is transformation of one adult type of mesenchymal tissue to another. The examples are as under:

1. ***Osseous metaplasia.*** Osseous metaplasia is formation of bone in fibrous tissue, cartilage and myxoid tissue. Examples of osseous metaplasia are as under:

i) In arterial wall in old age (Monckeberg's medial calcific sclerosis),

ii) In soft tissues in myositis ossificans,

iii) In cartilage of larynx and bronchi in elderly people,

iv) In scar of chronic inflammation of prolonged duration,

v) In the fibrous stroma of tumour.

2. ***Cartilaginous metaplasia.*** In healing of fractures, cartilaginous metaplasia may occur where there is undue mobility.

DYSPLASIA

Dysplasia means “disordered cellular development”, often accompanied with metaplasia and hyperplasia; it is therefore also referred to as atypical hyperplasia. Dysplasia occurs most often in epithelial cells. Epithelial dysplasia is characterized by cellular proliferation and cytologic changes. These changes include:

1. Hyperplasia of epithelial layers,

2. Disorderly arrangement of cells from basal layer to the surface layer,

3. Cellular and nuclear pleomorphism,

4. Increased nucleocytoplasmic ratio,

5. Nuclear hyperchromatism,

6. Increased mitotic activity.

The most common two examples of dysplastic changes are the uterine cervix and respiratory tract. Dysplastic changes often occur due to chronic irritation or prolonged inflammation. On removal of the inciting stimulus, the changes may disappear. In a proportion of cases, however, dysplasia progresses into carcinoma in situ (cancer confined to layers superficial to basement membrane) or invasive cancer.

REGENERATION OF SOME TISSUES AND ORGANS

Regeneration of blood. Blood plasma is compensated by tissue liquid, blood cells - due to formation of new cells from hemopoietic tissue. Extramedullar haemopoiesis (the appearance of hemopoietic centers in internal organs as in embryonal period) and myeloid transformation of yellow bone marrow (replacement of fatty bone marrow by hemopoietic tissue) take place at reparative regeneration of blood. At some diseases haemopoiesis can be heavily depressed (radiation sickness, agranulocytosis) or perverted (leukemia). In these cases pathological regeneration of blood is observed.

Bone marrow regenerates well and quickly; it can be restored even at great damages.

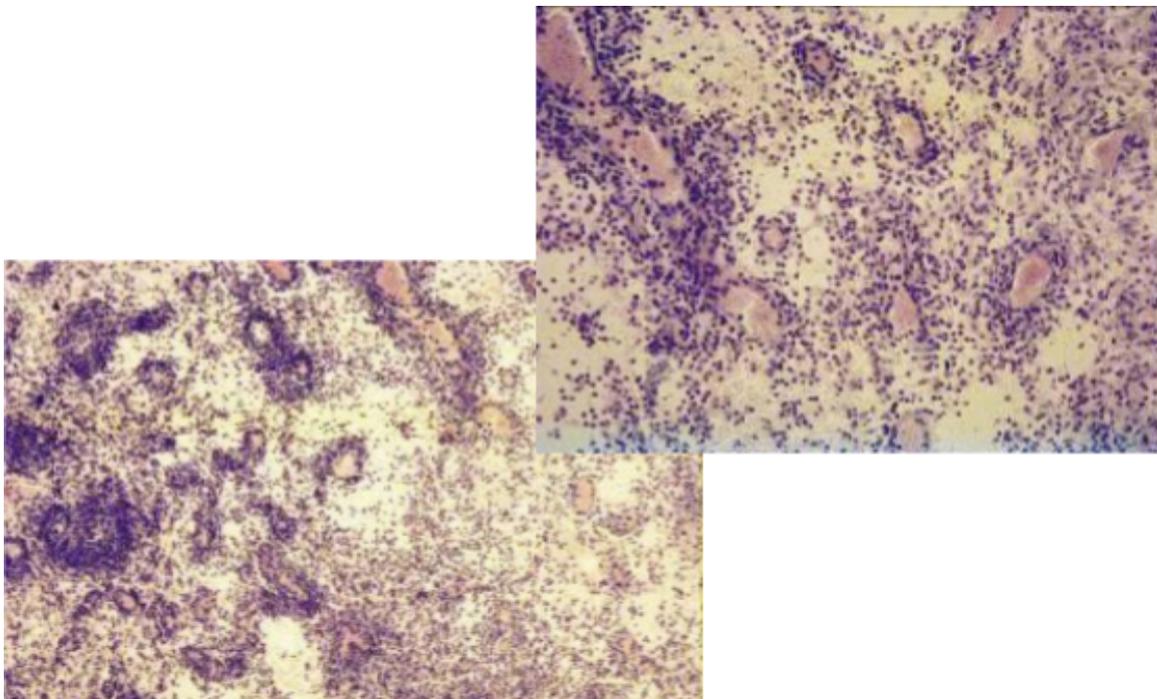


Fig. 14. Granulation tissue. **H and E stained section,**
x 200.

Regeneration of vessels. Small vessels (capillaries, arterioles) regenerate by gemmation (there is a lateral outpouching of endothelium cells in the vessels wall with the formation of cellular bar). Then there is a formation of lumen and a development of vessel in it). Another way of regeneration is autogenic (due to reproduction of connective tissue cells, which afterwards differentiate in endothelium). Large vessels regenerate worse: the injured media and external membrane are replaced by connective tissue.

Regeneration of connective tissue. The process begins with formation of granulation tissue (a young tissue with a lot of thin-walled vessels). At maturing of granulation tissue the quantity of vessels decreases, a number of fibroblasts increases, there are a synthesis of fibrous structures and formation of fibrous connective tissue scar on the place of granulation tissue. At retardation of granulation tissue maturing (due to inflammatory process) there is a formation of keloid - a cicatricial tissue of bright red color elevated above a surface of the skin.

Regeneration of bone tissue. At simple fractures of bones there is primary bone fusion. Thus the young connective tissue grows into the area of fracture (preliminary connective tissue clavus) which then transforms to a bone clavus. Then under action of physical activity the new formed bone tissue is reconstructed with the formation of mature bone. At the complicated fractures the secondary bone fusion is observed: thus at first there is a formation of new cartilage tissue from connective tissue clavus, and only then a bone tissue. At suppuration the disturbance of bone tissue formation in a place of fracture and formation of a false joint are possible. Thus bone fragments are connected by fibrous tissue and are mobile.

Regeneration of nervous tissue

Central nervous system. The nerve cells of brain, spinal cord and ganglia once destroyed are not replaced. Axons of CNS also do not show any significant regeneration. The damaged neuroglial cells, however, may show proliferation of astrocytes called gliosis.

Peripheral nervous system. In contrast to the cells of CNS, the peripheral nerves show regeneration, mainly from proliferation of Schwann cells and fibrils from distal end. Briefly, it consists of the following:

- myelin sheath and axon of the intact distal nerve undergo Wallerian degeneration up to the next node of Ranvier towards the proximal end;
- the degenerated debris are cleared away by macrophages;
- regeneration in the form of sprouting of fibrils takes place from the viable end of axon. These fibrils grow along the track of degenerated nerves so that in about 6-7 weeks, the peripheral stump consists of tube filled with elongated Schwann cells;

- one of the fibrils from the proximal stump enters the old neural tube and develops into new functional axon.

Regeneration of muscle

All three types of muscle fibers have limited capacity to regenerate.

Skeletal muscles. The regeneration of striated muscle is similar to peripheral nerves. On injury, the cut ends of muscle fibers retract but are held together by stromal connective tissue. The injured site is filled with fibrinous material, polymorphs and macrophages. After clearance of damaged fibres by macrophages, one of the following two types of regeneration of muscle fibres can occur. If the muscle sheath is intact, sarcolemmal tubes containing histiocytes appear along the endomysial tube which, in about 3 months time, restores properly oriented muscle fibres e.g. in Zenker's degeneration of muscle in typhoid fever. If the muscle sheath is damaged, it forms a disorganized multinucleate mass and scar composed of fibrovascular tissue, e.g. in Volkman's ischaemic contracture.

Smooth muscles. Non-striated muscle has limited regenerative capacity, e.g. appearance of smooth muscle in the arterioles in granulation tissue. However, in large destructive lesions, the smooth muscle is replaced by permanent scar tissue.

Regeneration of mucous membrans

The cells of mucosa have very good regeneration and are normally being lost and replaced continuously, e.g. mucosa of alimentary tract, respiratory tract, urinary tract, uterine endometrium, etc. This occurs by proliferation from margins, migration, multilayering and differentiation of epithelial cells in the same way as in the epidermal cells in healing of skin wounds.

Regeneration of solid epithelial organs

Following gross tissue damage to organs like kidney, liver and thyroid, the replacement is by fibrous scar, e.g. in chronic pyelonephritis and cirrhosis of liver. However, in parenchymal cell damage with intact basement membrane or intact supporting stromal tissue, regeneration may occur. For example, in tubular necrosis of kidney with intact basement membrane, proliferation and slow migration of tubular epithelial cells may occur to form renal tubules; in viral hepatitis if part of the liver lobule is damaged with in-

tact stromal network, proliferation of hepatocytes may result in restoration of liver lobule.

ORGANIZATION

Organization is the replacement of sites with pathological changes by mature connective tissue that leads to the sclerosis. Sclerosis is a pathological process which leads to the diffusive or focal induration of internal organs, connective tissue, blood vessels due to excessive growth of mature dense connective tissue.

Classification of sclerosis.

– *according to etiology and pathogenesis.*

1. Sclerosis is the outcome of chronic proliferative inflammation (at infectious, infectious-allergic, immune diseases, encapsulation of foreign substances).

2. Sclerosis is the outcome of connective tissue disorganization (at rheumatic diseases).

3. The substitutive sclerosis. It is the outcome of necrosis and atrophy.

4. Scars as the result of wounds and ulcers healing.

5. Organization of blood clots, hematomas, formation of adhesions.

– *according to morphogenesis three mechanisms are revealed.*

1. Neoformation of young connective tissue due to proliferation of fibroblasts (at proliferative inflammation, necrosis).

2. Increased collagen synthesis by fibroblasts without significant hyperplasia of cells; transformation of loose connective tissue into fibrous tissue (at chronic venous congestion).

HAEMODYNAMIC DISTURBANCES

There are three main basic requirements for normal circulatory function: normal anatomic features, normal physiologic controls, and normal biochemical composition of the blood. These are essential to maintain normal blood flow and perfusion of tissues.

Derangements of blood flow or haemodynamic disturbances are considered under 2 broad headings:

I. Disturbances in the volume of the circulating blood. These include: hyperaemia and congestion, haemorrhage and shock.

II. Circulatory disturbances of obstructive nature. These are: thrombosis, embolism, ischaemia and infarction.

I. DISTURBANCES IN THE VOLUME OF CIRCULATING BLOOD. HYPERAEMIA AND CONGESTION

Hyperaemia and congestion are the terms used for increased volume of blood within dilated vessels of an organ or tissue; the increased volume from arterial and arteriolar dilatation being referred to as hyperaemia or active hyperaemia, whereas the impaired venous drainage is called venous congestion or passive hyperaemia. If the condition develops rapidly it is called acute, while more prolonged and gradual response is known as chronic.

ACTIVE (OR ARTERIAL) HYPERAEMIA

The dilatation of arteries, arterioles and capillaries is effected either through sympathetic neurogenic mechanism or via the release of vasoactive substances. The affected tissue or organ is pink or red in appearance (erythema).

Active hyperaemia is classified into physiological and pathological, local and general, acute and chronic.

The examples of *active physiological hyperaemia* are seen in the following conditions:

- blushing i.e. flushing of the skin of face in response to emotions,
- muscular exercise,
- menopausal flush.

The *active pathological hyperaemia* is classified into next subtypes:

- inflammatory (dilation of the vessels due to mediators discharge),
- collateral,
- angioneurotic (or neuroparalytic),
- hyperaemia after ischemia,
- vacatous (vacuus – empty),
- due to arteio-venous fistula,

The general active pathological hyperaemia may be as the result of polycythemia, erythrocythemia.

PASSIVE HYPERAEMIA (VENOUS CONGESTION)

The dilatation of veins and capillaries due to impaired venous drainage results in passive hyperaemia or venous congestion, commonly referred to as congestion. Congestion may be acute or chronic, the latter being more common and called chronic venous congestion (CVC). The affected tissue or organ is bluish in colour due to accumulation of venous blood (cyanosis). Obstruction to the venous outflow may be local or systemic. Accordingly, venous congestion is of 2 types:

- *Local venous congestion* results from obstruction to the venous outflow from an organ or part of the body e.g. portal venous obstruction in cirrhosis of the liver, outside pressure on the vessel wall as occurs in tight bandage, plasters, tumours, pregnancy, hernia etc, or intraluminal occlusion by thrombosis.

- *Systemic (General) venous congestion* is engorgement of systemic veins e.g. in left-sided and right-sided heart failure and diseases of the lungs which interfere with pulmonary blood flow like pulmonary fibrosis, emphysema etc. Usually the fluid accumulates upstream to the specific chamber of the heart which is initially affected. For example, in left-sided heart failure (such as due to mechanical overload in aortic stenosis, or due to weakened left ventricular wall as in myocardial infarction) pulmonary congestion results, whereas in right-sided heart failure (such as due to pulmonary stenosis or pulmonary hypertension) systemic venous congestion results.

Morphology of chronic venous congestion of organs

Morphologic changes seen in CVC of the lungs, liver, spleen and kidney are discussed below.

Chronic venous congestion of lung occurs in left heart failure, especially in rheumatic mitral stenosis so that there is consequent rise in pulmonary venous pressure.

Grossly, the lungs are heavy and firm in consistency. The sectioned surface is dark brown in colour referred to as brown induration of the lungs. Histologically, the alveolar septa are widened due to the presence of interstitial oedema as well as due to dilated and congested capillaries. The septa are mildly thickened due to slight increase in fibrous connective tissue. Rupture of dilated and congested capillaries may result in minute intra-alveolar haemorrhages. The breakdown of erythrocytes liberates haemosiderin pigment which is taken up by alveolar macrophages, so called heart failure cells, present in the alveolar lumina. The brown induration of the cut surface of the lungs is due to the pigmentation and fibrosis.

Chronic venous congestion of the liver occurs in right heart failure and sometimes due to occlusion of inferior vena cava and hepatic vein.

Grossly, the liver is enlarged and tender and the capsule is tense. Cut surface shows characteristic nutmeg liver due to red and yellow mottled appearance, corresponding to congested centre of lobules and fatty peripheral zone respectively. Microscopically, the changes of congestion are more marked in the centrilobular zone due to severe hypoxia than in the peripheral zone. The central veins as well as the adjacent sinusoids are distended and filled with blood. The centrilobular hepatocytes undergo degenerative changes, and eventually centrilobular haemorrhagic necrosis may be seen. Long-standing cases may show fine centrilobular fibrosis and regeneration of hepatocytes, resulting in cardiac cirrhosis. The peripheral zone of the lobule is less severely affected by chronic hypoxia and shows some fatty change in the hepatocytes.

Chronic venous congestion of spleen occurs in right heart failure and in portal hypertension from cirrhosis of liver.

Grossly, the spleen in early stage is slightly to moderately enlarged (up- to 250 g as compared to normal 150 g), while in long-standing cases there is progressive enlargement and may weigh upto 500 g to 1000 g. The organ is deeply congested, tense and cyanotic. Sectioned surface is gray tan.

Microscopically, the red pulp shows congestion and marked sinusoidal dilatation with areas of recent and old haemorrhages. These haemorrhages may get organised and form Gamna-Gandy bodies or sidero-fibrotic nodules which are deposits of haemosiderin pigment and calcium salts on

fibrous connective tissue and elastic fibres. In the late stage, there is hyperplasia of macrophages and fibroblasts resulting in increase in fibrous tissue of the capsule and hyperplasia of red pulp all of which account for firmness of the spleen. This advanced stage seen more commonly in hepatic cirrhosis is called congestive splenomegaly and is the commonest cause of hypersplenism.

Chronic venous congestion of kidney. Grossly, the kidneys are slightly enlarged and the medulla is congested. Microscopically, the changes are rather mild. The tubules may show degenerative changes like cloudy swelling and fatty change. The glomeruli may show mesangial proliferation.

Edema and ascites: venous congestion impedes the flow of blood in the capillaries, thereby increasing hydrostatic pressure and promoting edema formation. The accumulation of edema fluid in heart failure is particularly noticeable in dependent tissues: the legs and feet in ambulatory patients and the back in bedridden persons. Ascites, the accumulation of fluid in the peritoneal space, reflects (among other factors) the lack of tissue rigor, a condition in which there is no countervailing external pressure to oppose hydrostatic pressure.

Stasis (stasis – stop) is the arrest of blood flow in the vessels of microcirculatory system (capillaries).

Causes of stasis: physical factors (temperature elevation, cold), chemical factors, infection, infectious-allergic factors, autoimmune factors.

Plasmorrhagia means outflow of plasma from vessels. The cause of plasmorrhagia is increased vascular permeability.

Lymph circulation disturbance manifests itself as lymphatic system insufficiency (mechanical, dynamic and resorption).

HAEMORRHAGE

Hemorrhage (i.e., bleeding) is a discharge of blood from the vascular compartment to the exterior of the body or into nonvascular body spaces.

Causes of haemorrhage: the blood loss may be large and sudden (acute), or small repeated bleeds may occur over a period of time (chronic). The various causes of haemorrhage are listed below:

1. Trauma to the vessel wall, e.g. penetrating wound in the heart or great vessels, during labour etc.
2. Spontaneous haemorrhage e.g. rupture of an aneurysm, septicaemia, bleeding diathesis (such as purpura), acute leukaemias, pernicious anaemia, scurvy.
3. Inflammatory lesions of the vessel wall, e.g. bleeding from chronic peptic ulcer, typhoid ulcers, blood vessels traversing a tuberculous cavity in the lung, syphilitic involvement of the aorta, polyarteritis nodosa.
4. Neoplastic invasion, e.g. haemorrhage following vascular invasion in carcinoma of the tongue.
5. Vascular diseases, e.g. atherosclerosis.
6. Elevated pressure within the vessels, e.g. cerebral and retinal haemorrhage in systemic hypertension, severe haemorrhage from varicose veins due to high pressure in the veins of legs or oesophagus.

The most common and obvious cause is trauma. An artery may be ruptured in ways other than laceration. For instance, severe atherosclerosis may so weaken the wall of the abdominal aorta that it balloons to form an aneurysm, which then ruptures and bleeds in to the retroperitoneal space. By the same token, an aneurysm may complicate a congenitally weak cerebral artery (berry aneurysm) and lead to subarachnoid hemorrhage. Certain infections (*e.g.*, pulmonary tuberculosis) erode blood vessels; a similar vascular injury is caused by invasive tumors.

Hemorrhage also results from damage at the level of the capillaries. For instance, the rupture of capillaries by blunt trauma is evidenced by the appearance of a bruise. Increased venous pressure also causes extravasation of blood from capillaries in the lung.

Vitamin C deficiency is associated with capillary fragility and bleeding, owing to a defect in the supporting structures. It is important to recognize that the capillary barrier by itself is not sufficient to contain the blood within the intravascular space. The minor trauma imposed on small vessels and capillaries by normal movement requires an intact coagulation system to prevent hemorrhage. Thus, a severe decrease in the number of platelets (thrombocytopenia) or a deficiency of a coagulation factor (*e.g.*, factor VIII in hemophilia) is associated with spontaneous hemorrhages unrelated to any apparent trauma.

A person may exsanguinate into an internal cavity, as in the case of gastrointestinal hemorrhage from a peptic ulcer (arterial hemorrhage) or

esophageal varices (venous hemorrhage). In such cases large amounts of fresh blood fill the entire gastrointestinal tract. Bleeding into a serous cavity can result in the accumulation of a large amount of blood, even to the point of exsanguination. A few definitions are in order:

- Hemothorax: Hemorrhage into the pleural cavity.
- Hemopericardium: Hemorrhage into the pericardial space.
- Hemoperitoneum: Bleeding into the peritoneal cavity.
- Hemarthrosis: Bleeding into a joint space.
- Hematoma: Hemorrhage into the soft tissues. Such collections of blood can be merely painful, as in a muscle bruise, or fatal, if located in the brain.
- Purpura: Diffuse superficial hemorrhages in the skin, up to 1 cm in diameter.
- Ecchymosis: A larger superficial hemorrhage. Following a bruise or in association with a coagulation defect, an initially purple discoloration of the skin turns green and then yellow before resolving. This sequence reflects the progressive oxidation of bilirubin released from the hemoglobin of degraded erythrocytes. A good example of an ecchymosis is a "black eye."
- Petechia: A pinpoint hemorrhage, usually in the skin or conjunctiva. This lesion represents the rupture of a capillary or arteriole and occurs in conjunction with coagulopathies or vasculitis, the latter classically associated with infections of the heart valves (bacterial endocarditis).

Effects of haemorrhage: The effects of blood loss depend upon 3 main factors:

- The amount of blood loss;
- The speed of blood loss; and
- The site of the haemorrhage.

The loss up to 20% of blood volume suddenly or slowly generally has little clinical effects because of compensatory mechanisms. A sudden loss of 33% of blood volume may cause death, while loss of up to 50% of blood volume over a period of 24 hours may not be necessarily fatal. However, chronic blood loss generally produces iron deficiency anaemia, whereas acute haemorrhage may lead to serious immediate consequences such as hypovolaemic shock.

SHOCK

Shock is defined as a clinical state of cardiovascular collapse characterised by:

- An acute reduction of effective circulating blood volume; and
- An inadequate perfusion of cells and tissues.

The end result is hypotension and cellular hypoxia and, if uncompensated, may lead to impaired cellular metabolism and death. Shock may be of 2 main types: primary (initial) and secondary (true) shock.

Primary or initial shock: It is transient and usually a benign vasovagal attack resulting from sudden reduction of venous return to the heart caused by neurogenic vasodilatation and consequent peripheral pooling of blood. It can occur immediately following trauma, severe pain or emotional over-reaction such as due to fear, sorrow or surprise. Clinically, the patient generally develops unconsciousness, weakness, sinking sensation, pale and clammy limbs, weak and rapid pulse, and low blood pressure. The attack usually lasts for a few seconds or minutes.

Secondary or true shock: this is the form of shock which occurs due to haemodynamic derangements with hypoperfusion of the cells. This type of shock is the true shock which is commonly referred to as 'shock' if not specified and is the type described below.

Etiology and Classification. Many types of injuries and diseases can cause shock. These causes are broadly grouped under 3 major headings, and accordingly shock is classified into 3 main etiologic forms: hypovolaemic, cardiogenic, and septic.

1. *Hypovolaemic shock.* Reduction in blood volume induces hypovolaemic shock. The causes of hypovolaemia include the following:

- i) Severe haemorrhage (external or internal) e.g. in trauma, surgery.
- ii) Fluid loss e.g. in severe burns, crush injury to a limb, persistent vomitings and severe diarrhoea causing dehydration.

2. *Septic shock.* Severe bacterial infections or septicaemia induce septic shock. The predominant causes are as under:

- i) Gram-negative septicaemia (endotoxic shock) e.g. infection with *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas* and *bacteroides*. Endotoxins of gram-negative bacilli have been implicated as the most important mediator of septic shock.

- ii) Gram-positive septicaemia (exotoxic shock) is less common e.g. infection with streptococci, pneumococci. Lysis of gram-negative bacteria

releases endotoxin, a lipopolysaccharide (LPS), into circulation where it binds to lipopolysaccharide-binding protein (LBP). The complex of LPS-LBP binds to CD14 molecule on the surface of the monocyte/macrophage which in turn is stimulated to elaborate tumour necrosis factor- α (TNF- α). TNF- α induces septic shock by endothelial cell injury by many mechanisms which include:

- a) Direct cytotoxicity
- b) Promotes the adherence of polymorphs to endothelium
- c) Stimulates the release of interleukin-1 (IL-1)
- d) Promotes the release of procoagulant tissue factor that causes thrombosis and local ischaemia.

3. *Cardiogenic shock*. Acute circulatory failure with sudden fall in cardiac output from acute diseases of the heart without actual reduction of blood volume (normovolaemia) results in cardiogenic shock. The causes include the following:

- i) Deficient emptying, e.g.
 - Myocardial infarction
 - Rupture of the heart
 - Cardiac arrhythmias
- ii) Deficient filling e.g.
 - Cardiac tamponade from haemopericardium
- iii) Obstruction to the outflow, e.g.
 - Pulmonary embolism
 - Ball valve thrombus.

Besides the three major forms of shock described above, traumatic shock (following severe trauma with the tissues compression), neurogenic shock (following anaesthesia or spinal cord injury) and anaphylactic shock are other types of shock. The last 2 ones result from peripheral vasodilation with pooling of blood.

Pathogenesis. There are 2 basic features in the pathogenesis of shock:

- reduced effective circulating blood volume; and
- reduced supply of oxygen to the cells and tissues with resultant anoxia.

1. *Reduced effective circulating volume*. It may result by either of the following mechanisms:

- i) by actual loss of blood volume as occurs in hypovolaemic shock; or
- ii) by decreased cardiac output without actual loss of blood (normovolaemia) as occurs in cardiogenic shock and septic shock.

2. *Tissue anoxia.* Following reduction in the effective circulating blood volume from either of the above two mechanisms and from any of the etiologic agents, there is decreased venous return to the heart resulting in decreased cardiac output. This consequently causes reduced supply of oxygen to the organs and tissues and hence tissue anoxia, and shock ensues.

In contrast to hypovolaemic and cardiogenic shock, patients in septic shock have hyperdynamic circulation due to peripheral vasodilatation and pooling of blood, as well as there is increased vascular permeability. This result in reduction of effective circulating blood volume, lowered cardiac output, reduced blood flow (hypotension) and inadequate perfusion of cells and tissues. Disseminated intravascular coagulation (DIC) is prone to develop in septic shock due to endothelial cell injury by toxins.

Stages of Shock. Deterioration of the circulation in shock is a progressive phenomenon and can be divided arbitrarily into 3 stages:

1. Non-progressive (initial, compensated reversible) shock.
2. Progressive decompensated shock.
3. Decompensated (irreversible) shock.

1. *Non-progressive (initial, compensated reversible) shock.* In the early stage of shock, an attempt is made to maintain adequate cerebral and coronary blood supply by redistribution of blood so that the vital organs (brain and heart) are adequately perfused and oxygenated. This is achieved by activation of various neurohormonal mechanisms causing widespread vasoconstriction and by fluid conservation by the kidney. If the condition that caused the shock is adequately treated, the compensatory mechanism may be able to bring about recovery and re-establish the normal circulation; this is called compensated or reversible shock. These compensatory mechanisms are as under:

i) Widespread vasoconstriction. In response to reduced blood flow (hypotension) and tissue anoxia, the neural and humoral factors (e.g. baroreceptors, chemoreceptors, catecholamines, renin, and VEM or vasoexcitor material from hypoxic kidney) are activated. All these bring about vasoconstriction, particularly in the vessels of the skin and abdominal viscera. Widespread vasoconstriction is a protective mechanism as it causes increased peripheral resistance, increased heart rate and increased blood pressure. However, in septic shock, there is initial vasodilatation followed by vasoconstriction. Besides, in severe septic shock there is elevated level of

thromboxane A₂ which is a potent vasoconstrictor and may augment the cardiac output along with other sympathetic mechanisms.

ii) Fluid conservation by the kidney. In order to compensate the actual loss of blood volume in hypovolaemic shock, the following factors may assist in restoring the blood volume and improve venous return to the heart:

- Release of aldosterone from hypoxic kidney.
- Release of ADH due to decreased effective circulating blood volume.
- Reduced glomerular filtration rate (GFR) due to arteriolar constriction.
- Shifting of tissue fluids into the plasma due to lowered capillary hydrostatic pressure (hypotension).

iii) Vascular autoregulation. In response to hypoxia and acidosis, regional blood flow to the heart and brain is preserved by vasodilatation of the coronary and cerebral circulation.

2. Progressive decompensated shock.

This is a stage when the patient suffers from some other stress or risk factors (e.g. pre-existing cardiovascular and lung disease) besides persistence of the shock so that there is progressive deterioration. The effects of progressive decompensated shock include the following:

- a) Pulmonary hypoperfusion with resultant tachypnoea and adult respiratory distress syndrome.
- b) Tissue anoxia causing anaerobic glycolysis resulting in metabolic lactic acidosis.
- c) Anoxia of liver causing reduced clearance of lactate from it leading to acidosis.

3. Decompensated (irreversible) shock.

When the shock is so severe that in spite of compensatory mechanisms and despite therapy and control of etiologic agent which caused the shock, no recovery takes place, it is called decompensated or irreversible shock. Its effects include the following:

- a) Progressive fall in the blood pressure due to deterioration in cardiac output attributed to release of myocardial depressant factor (MDF).
- b) Severe metabolic acidosis due to anaerobic glycolysis.
- c) Respiratory distress due to pulmonary oedema, tachypnoea and adult respiratory distress syndrome (ARDS).

d) Ischaemic cell death of brain, heart and kidneys due to reduced blood supply to these organs resulting in coma, worsened heart function and progressive renal failure.

A number of hypotheses or factors have been described in irreversibility of shock, with tissue anoxia occupying the central role. These are as under:

i) Persistence of widespread vasoconstriction.

Although widespread vasoconstriction is a compensatory mechanism, its persistence for prolonged duration can cause anoxia of tissues and organs like liver, spleen, kidney and intestine. Particularly significant is the postcapillary venular constriction contributing to tissue anoxia.

ii) Vasodilatation and increased vascular permeability. Anoxia damages the capillary and venular wall so that there is vasodilatation and increased vascular permeability. Vasodilatation results in peripheral pooling of blood while increased vascular permeability causes escape of fluid from circulation into the interstitial tissues, both of which further deteriorate the effective circulating blood volume.

iii) Myocardial ischaemia. Persistently reduced blood flow to myocardium causes coronary insufficiency and myocardial ischaemia due to release of myocardial depressant factor (MDF). This results in depression of cardiac function, reduced cardiac output and decreased blood flow.

iv) Cerebral ischaemia. Cerebral ischaemia resulting from persistent reduction of blood flow causes depression of the vasomotor centre. This results in vasodilatation and peripheral pooling of blood, thus reducing the venous return to the heart and consequent lowered cardiac output.

v) Vasodepressor material (VDM). VDM is a substance produced by the spleen and skeletal muscle and is normally inactivated in the liver. In severe hypoxia of the liver as occurs in irreversible shock, the mechanism of inactivation of VDM in liver is damaged so that its blood levels rise. VDM causes peripheral vasodilatation (reverse of VEM) and thus diverts blood from the systemic circulation, resulting in deterioration of the circulation.

vi) Tumour necrosis factor (TNF). In septic shock, monocyte-macrophage cell system gets activated by bacterial products causing release of substances like prostaglandins, leukotrienes, platelet activating factor and

interleukins-1, 6 and 18. These substances have been implicated in producing irreversibility of endotoxic shock.

vii) Intestinal factor. Due to prolonged vasoconstriction, haemorrhagic necrosis of the intestinal tract occurs. This results in loss of blood and plasma into the intestine from haemorrhagic lesions, causing further reduction in effective circulating blood volume.

viii) Bacterial factor. Prolonged anoxic injury to the reticuloendothelial organs like the liver and spleen impairs the normal antibacterial defense mechanism of these organs. This results in release of undetoxified endotoxins derived from intestinal bacteria into the circulation, which cause further vasoconstriction and its harmful effects.

ix) Hypercoagulability of blood. Excessive accumulation of lactic acid in the blood in prolonged shock enhances the release of catecholamines (e.g. epinephrine) into the circulation. The effects of catecholamines include the release of clot promoting factor, release of thromboplastin and release of platelet aggregator, ADP. Excess lactic acid in the blood can also cause endothelial injury and thus initiate thrombosis. In this way, hypercoagulability of blood with consequent microthrombi impair the blood flow and may even cause tissue necrosis.

Morphologic Complications in Shock. Eventually, shock is characterised by multisystem failure. The morphologic changes in shock develop due to hypoxia resulting in degeneration and necrosis in various organs. The major organs affected are the brain, heart, lungs and kidneys. Morphologic changes are also noted in the adrenals, gastrointestinal tract, liver and other organs.

1. *Hypoxic encephalopathy.* Cerebral ischaemia in compensated shock may produce altered state of consciousness. However, if the blood pressure falls below 50 mmHg as occurs in systemic hypotension in prolonged shock and cardiac arrest, brain suffers from serious ischaemic damage with loss of cortical functions, coma, and a vegetative state.

Grossly, the area supplied by the most distal branches of the cerebral arteries suffers from severe ischaemic necrosis which is usually the border zone between the anterior and middle cerebral arteries.

Microscopically, the changes are noticeable if ischaemia is prolonged for 12 to 24 hours. Neurons, particularly Purkinje cells, are more prone to develop the effects of ischaemia. The cytoplasm of the affected neurons is

intensely eosinophilic and the nucleus is small pyknotic. Dead and dying nerve cells are replaced by gliosis.

2. *Heart in shock.* Heart is more vulnerable to the effects of hypoxia than any other organ. Heart is affected in cardiogenic as well as in other forms of shock. There are 2 types of morphologic changes in heart in all types of shock:

i) Haemorrhages and necrosis. There may be small or large ischaemic areas or infarcts, particularly located in the subepicardial and subendocardial region.

ii) Zonal lesions. These are opaque transverse contraction bands in the myocytes near the intercalated disc.

3. *Shock lung.* Lungs due to dual blood supply are generally not affected by hypovolaemic shock but in septic shock the morphologic changes in lungs are quite prominent termed 'shock lung'.

Grossly, the lungs are heavy and wet. Microscopically, changes of adult respiratory distress syndrome (ARDS) are seen. Briefly, the changes include congestion, interstitial and alveolar oedema, interstitial lymphocytic infiltrate, alveolar hyaline membranes, thickening and fibrosis of alveolar septa, and fibrin and platelet thrombi in the pulmonary microvasculature.

4. *Shock kidney.* One of the important complications of shock is irreversible renal injury, first noted in persons who sustained crush injuries in building collapses in air raids in World War II. The renal ischaemia following systemic hypotension is considered responsible for renal changes in shock. The end-result is generally anuria and death.

Grossly, the kidneys are soft and swollen. Sectioned surface shows blurred architectural markings. Microscopically, the tubular lesions are seen at all levels of nephron and are referred to as acute tubular necrosis (ATN) which can occur following other causes besides shock. If extensive muscle injury or intravascular haemolysis are also associated, peculiar brown tubular casts are seen.

5. *Adrenals in shock.* The adrenals show stress response in shock. This includes release of aldosterone in response to hypoxic kidney, release of glucocorticoids from adrenal cortex and catecholamines like adrenaline from adrenal medulla. In severe shock, adrenal haemorrhages may occur.

6. *Haemorrhagic gastroenteropathy.*

The hypoperfusion of the alimentary tract in conditions such as shock and cardiac failure may result in mucosal and mural infarction called haem-

orrhagic gastroentero-pathy. This type of non-occlusive ischaemic injury of bowel must be distinguished from full-fledged infarction in which case the deeper layers of gut (muscularis and serosa) are also damaged. In shock due to burns, acute stress ulcers of the stomach or duodenum may occur and are known as Curling's ulcers.

Grossly, the lesions are multifocal and widely distributed throughout the bowel. The lesions are superficial ulcers, reddish purple in colour. The adjoining bowel mucosa is oedematous and haemorrhagic. Microscopically, the involved areas show dilated and congested vessels and haemorrhagic necrosis of the mucosa and sometimes submucosa. Secondary infection may supervene and condition may progress into pseudomembranous enterocolitis.

7. *Liver in shock.* Due to effects of hypoxia on liver, VDM is released from the liver which causes vasodilatation. Besides, focal necrosis may be seen, fatty change may occur and the liver function may be impaired.

8. *Other organs.* Other organs such as lymph nodes, spleen and pancreas may also show foci of necrosis in shock. In addition, the patients who survive acute phase of shock succumb to overwhelming infection due to altered immune status and host defense mechanism.

Clinical Features. The classical features of decompensated shock are characterised by depression of 4 vital processes:

- Very low blood pressure
- Subnormal temperature
- Feeble and irregular pulse
- Shallow and sighing respiration

In addition, the patients in shock have pale face, sunken eyes, weakness, cold and clammy skin. Renal dysfunction in shock is clinically characterised by a phase of oliguria due to ATM and a later phase of diuresis due to regeneration of tubular epithelium. Haemo-concentration is present in early oliguric phase while marked electrolyte imbalance occurs in diuretic phase. With progression of the condition, the patient may develop stupor, coma and death.

TURE

II. CIRCULATORY DISTURBANCES OF OBSTRUCTIVE NA-

THROMBOSIS

Thrombosis is the process of formation of solid mass in circulation

from the constituents of flowing blood; the mass itself is called a thrombus. A blood clot is the mass of coagulated blood formed in vitro e.g. in a test tube. Haematoma is the extravascular accumulation of blood clot e.g. into the tissues. Haemostatic plugs are the blood clots formed in healthy individuals at the site of bleeding e.g. in injury to the blood vessel. In other words, haemostatic plug at the cut end of a blood vessel may be considered the simplest form of thrombosis. Haemostatic plugs are useful as they stop the escape of blood and plasma, whereas thrombi developing in the unruptured cardiovascular system may be life-threatening by causing one of the following harmful effects:

1. *Ischaemic injury.* Thrombi may decrease or stop the blood supply to part of an organ or tissue and cause ischaemia which may subsequently result in infarction.
2. *Thromboembolism.* The thrombus or its part may get dislodged and be carried along in the blood stream as embolus to lodge in a distant vessel.

Pathophysiology. Since the protective haemostatic plug formed as a result of normal haemostasis is an example of thrombosis, it is essential to describe thrombogenesis in relation to the normal haemostatic mechanism.

Human beings possess inbuilt system by which the blood remains in fluid state normally and guards against the hazards of thrombosis and haemorrhage. However, injury to the blood vessel initiates haemostatic repair mechanism or thrombogenesis. Virchow described three primary events which predispose to thrombus formation (Virchow's triad): endothelial injury, alteration in flow of blood, and hypercoagulability of blood. These events are discussed below:

1. *Role of blood vessel wall.* The integrity of blood vessel wall is important for maintaining normal blood flow. An intact endothelium has the following functions:

- i) It protects the flowing blood from the thrombogenic influence of subendothelium.

ii) It elaborates a few anti-thrombotic factors (thrombosis inhibitory factors) e.g.

a) Heparin-like substance which accelerates the action of antithrombin III and inactivates some other clotting factors.

b) Thrombomodulin which converts thrombin into activator of protein C, an anticoagulant.

c) Inhibitors of platelet aggregation such as ADPase, PGI₂ or prostacyclin.

d) Tissue plasminogen activator which accelerates the fibrinolytic activity.

iii) It can release a few prothrombotic factors which have procoagulant properties (thrombosis favouring factors)

a) Thromboplastin or tissue factor released from endothelial cells.

b) von Willebrand factor that causes adherence of platelets to the subendothelium.

c) Platelet activating factor which is activator and aggregator of platelets.

d) Inhibitor of plasminogen activator that suppresses fibrinolysis.

Vascular injury exposes the subendothelial connective tissue (e.g. collagen, elastin, fibronectin, laminin and glycosaminoglycans) which are thrombogenic and thus plays important role in initiating haemostasis as well as thrombosis. Injury to vessel wall also causes vasoconstriction of small blood vessels briefly so as to reduce the blood loss. Endothelial injury is of major significance in the formation of arterial thrombi and thrombi of the heart, especially of the left ventricle. A number of factors and conditions may cause vascular injury and predispose to the formation of thrombi. These are as under:

i) Endocardial injury in myocardial infarction, myocarditis, cardiac surgery, prosthetic valves.

ii) Ulcerated plaques in advanced atherosclerosis.

iii) Haemodynamic stress in hypertension.

iv) Arterial diseases.

v) Diabetes mellitus.

vi) Endogenous chemical agents such as hypercholesterolaemia, endotoxins.

vii) Exogenous chemical agents such as cigarette smoke.

2. Role of platelets. Following endothelial cell injury, platelets come to play a central role in normal haemostasis as well as in thrombosis. The sequence of events is as under:

i) Platelet adhesion. The platelets in circulation recognise the site of endothelial injury and adhere to exposed subendothelial collagen (primary aggregation), von Willebrand's factor is required for such adhesion between platelets and collagen. Normal non-activated platelets have open canalicular system with cytoplasmic organelles (granules, mitochondria, endoplasmic reticulum) dispersed throughout the cytoplasm. During the early adhesion process, there is dilatation of canalicular system with formation of pseudopods and the cytoplasmic organelles shift to the centre of the cell.

ii) Platelet release reaction. The activated platelets then undergo re-lease reaction by which the platelet granules are released to the exterior. Two main types of platelet granules are released:

a) Alpha granules containing fibrinogen, fibronectin, platelet-derived growth factor, platelet factor 4 (an anti-heparin) and cationic proteins.

b) Dense bodies containing ADP (adenosine diphosphate), ionic calcium, 5-HT (serotonin), histamine and epinephrine.

As a sequel to platelet activation and release reaction, the phospholipid complex-platelet factor 3 gets activated which plays important role in the intrinsic pathway of coagulation.

iii) Platelet aggregation. Following release of ADP, a potent platelet aggregating agent, aggregation of additional platelets takes place (secondary aggregation). This results in formation of temporary haemostatic plug. However, stable haemostatic plug is formed by the action of fibrin, thrombin and thromboxane A₂.

3. Role of coagulation system. Coagulation mechanism is the conversion of the plasma fibrinogen into solid mass of fibrin. The coagulation system is involved in both haemostatic process and thrombus formation.

i) In the intrinsic pathway, contact with abnormal surface leads to activation of factor XII and the sequential interactions of factors XI, IX, VIII and finally factor X, alongwith calcium ions (factor IV) and platelet factor 3.

ii) In the extrinsic pathway, tissue damage results in the release of tissue factor or thromboplastin. Tissue factor on interaction with factor VII activates factor X.

iii) The common pathway begins where both intrinsic and extrinsic

pathways converge to activate factor X which forms a complex with factor

Va and platelet factor 3, in the presence of calcium ions. This complex activates prothrombin (factor II) to thrombin (factor IIa) which, in turn, converts fibrinogen to fibrin. Initial monomeric fibrin is polymerised to form insoluble fibrin by activation of factor XIII.

iv) Regulation of coagulation system. The blood is kept in fluid state normally and coagulation system kept in check by controlling mechanisms. These are as under:

a) Protease inhibitors. These act on coagulation factors so as to oppose the formation of thrombin e.g. antithrombin III, protein C, C1 inactivator, α 1-antitrypsin, α 2-macroglobulin.

b) Fibrinolytic system. Plasmin, a potent fibrinolytic enzyme, is formed by the action of plasminogen activator on plasminogen present in the normal plasma. Two types of plasminogen activators (PA) are identified:

- Tissue-type PA derived from endothelial cells and leucocytes.
- Urokinase-like PA present in the plasma. Plasmin so formed acts on fibrin to destroy the clot and produces fibrin split products (FSP).

4. Hypercoagulability of blood. The occurrence of thrombosis in some conditions such as in nephrotic syndrome, advanced cancers, extensive trauma, burns and during puerperium is explained on the basis of hypercoagulability of blood. The effect of hypercoagulability on thrombosis is favoured by advancing age, smoking, use of oral contraceptives and obesity. Hypercoagulability may occur by the following changes in the composition of blood:

i) Increase in coagulation factors e.g. fibrinogen, prothrombin, factor VIII, VIII and Xa.

ii) Increase in platelet count and their adhesiveness.

iii) Decreased levels of coagulation inhibitors e.g. antithrombin III, fibrin split products.

5. Alteration of blood flow. Formation of arterial and cardiac thrombi is facilitated by turbulence in the blood flow, while stasis initiates the venous thrombi even without evidence of endothelial injury.

i) Normally, there is axial flow of blood in which the most rapidly-moving central stream consists of leucocytes and red cells. The platelets are present in the slow-moving laminar stream adjacent to the central stream while the peripheral stream consists of most slow-moving cell-free plasma close to endothelial layer.

ii) In turbulence and stasis, the normal axial flow of blood is disturbed so that the platelets come into contact with the endothelium.

Besides, the inhibitors of coagulation fail to reach the site of thrombus resulting in enlargement of thrombus size. Turbulence may actually injure the endothelium resulting in deposition of platelets and fibrin.

Morphology of various types. Thrombosis may occur in the heart, arteries, veins and the capillaries. Beside the differences in mechanisms of thrombus formation at these sites, the clinical effects of these are even more different. Arterial thrombi produce ischaemia and infarction, whereas cardiac and venous thrombi cause embolism.

The general morphologic features of thrombi are as under:

Grossly, thrombi may be of various shapes, sizes and composition depending upon the site of origin. Arterial (or white) thrombi tend to be white and mural while the venous thrombi are red and occlusive. Mixed or laminated thrombi are also common and consist of alternate white and red layers called lines of Zahn. Red (or venous) thrombi are soft, red and gelatinous whereas white thrombi are firm and pale.

Microscopically, the composition of thrombus is determined by the rate of flow of blood i.e. whether it is formed in the rapid arterial and cardiac circulation, or in the slow moving flow in veins. The lines of Zahn are formed by alternate layers of light-staining aggregated platelets admixed with fibrin meshwork and dark-staining layer of red cells. Red (venous) thrombi have more abundant red cells, leucocytes and platelets entrapped in fibrin meshwork. Thus, red thrombi closely resemble blood clots in vitro.

Red thrombi (ante-mortem) have to be distinguished from postmortem clots.

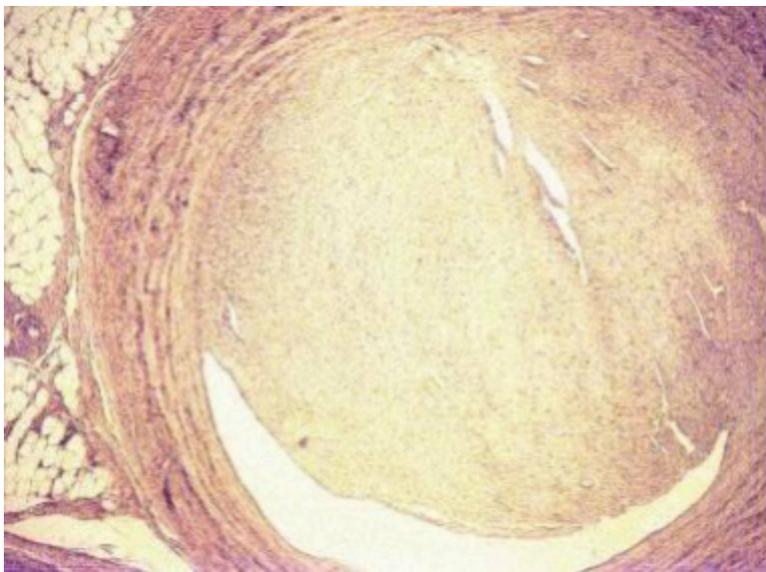
Cardiac thrombi. Thrombi may form in any of the chambers of heart and on the valve cusps. They are more common in the atrial appendages, especially of the right atrium, and on mitral and aortic valves called vegetations which may be seen in infective endocarditis and non-bacterial thrombotic endocarditis. Cardiac thrombi are mural (non-occlusive) as are the mural thrombi encountered in the aorta in atherosclerosis and in aneurysmal dilatations. Rarely, large round thrombus may form and obstruct the mitral valve and is called ball-valve thrombus. Agonal thrombi are formed shortly before death and may occur in either or both the ventricles. They are composed mainly of fibrin.

Arterial and venous thrombi. The distinguishing features between thrombi formed in rapidly-flowing arterial circulation and slow-moving venous blood.

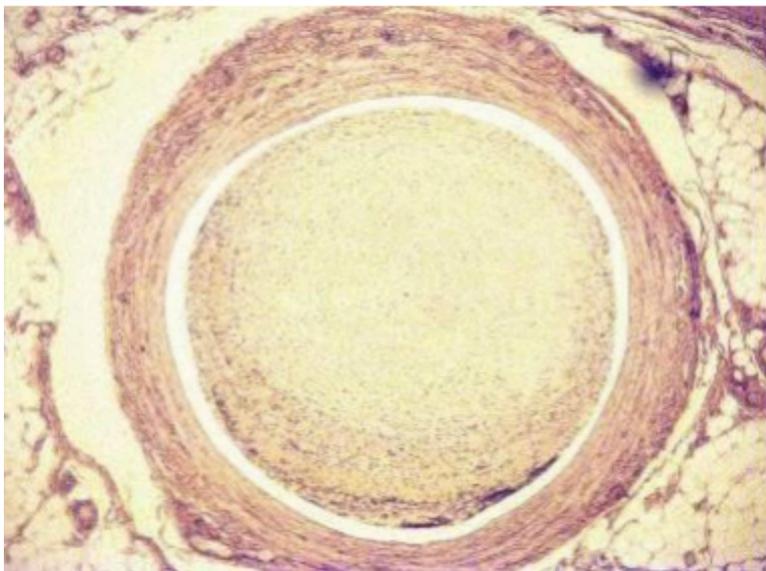
Capillary thrombi. Minute thrombi composed mainly of packed red cells are formed in the capillaries in acute inflammatory lesions, vasculitis and in disseminated intravascular coagulation (DIC).

Fate of thrombus. The possible fate of thrombi:

1. *Resolution.* Thrombus activates the fibrinolytic system with consequent release of plasmin which may dissolve the thrombus completely resulting in resolution. Usually, lysis is completed in small venous thrombi while



large thrombi may not be dissolved. Fibrinolytic activity can be accentuated by administration of thrombolytic substances (e.g. urokinase, streptokinase), especially in the early stage when fibrin is in monomeric form.



2. *Organization.* If the thrombus is not removed, it starts getting organized. Phagocytic cells (neutrophils and macrophages) appear and begin to phagocytose fibrin and cell debris. The proteolytic enzymes liberated by leucocytes and endothelial cells start digesting coagulum. Capillaries grow into the thrombus from the site of its at-

Fig. 16. Thromboembolism. H and E stained section, x 200.

tachment and fibroblasts start invading the thrombus. Thus, fibro-

vascular granulation tissue is formed which subsequently becomes dense and less vascular and is covered over by endothelial cells. The thrombus in this way is excluded from the vascular lumen and becomes part of vessel wall. The new vascular channels in it may be able to re-establish the blood flow, called recanalization. The fibrosed thrombus may undergo hyalinisation and calcification e.g. phleboliths in the pelvic veins.

3. *Propagation.* The thrombus may enlarge in size due to more and more deposition from the constituents of flowing blood. In this way, it may ultimately cause obstruction of some important vessel.

4. *Thromboembolism.* The thrombi in early stage and infected thrombi are quite friable and may get detached from the vessel wall. These are released in part or completely in blood stream as emboli which produce ill-effects at the site of their lodgement.

Factors predisposing to thrombosis. A number of primary (genetic) and secondary (acquired) factors favour thrombosis.

Primary (Genetic) factors:

- i) Deficiency of antithrombin
- ii) Deficiency of protein C or S.
- iii) Defects in fibrinolysis
- iv) Mutation in factor V

Secondary (acquired) factors:

- a) Risk factors are:
 - i) Advanced age
 - ii) Prolonged bed-rest
 - iii) Immobilisation
 - iv) Use of oral contraceptives
 - v) Cigarette smoking
 - vi) Tissue damage e.g. trauma, fractures, burns
- b) Clinical conditions predisposing to thrombosis:
 - i) Heart diseases e.g. myocardial infarction, CHF, rheumatic mitral stenosis, cardiomyopathy.
 - ii) Atherosclerosis
 - iii) Aneurysms of the aorta and other vessels
 - iv) Varicosities of leg veins
 - v) Nephrotic syndrome
 - vi) Disseminated cancers
 - vii) Late pregnancy and puerperium.

Clinical effects of thrombosis. These depend upon the site of thrombi, rapidity of formation, and nature of thrombi.

1. Cardiac thrombi. Large thrombi in the heart may cause sudden death by mechanical obstruction of blood flow or through thromboembolism to vital organs.

2. Arterial thrombi. These cause ischaemic necrosis of the deprived part (infarct) which may lead to gangrene. Sudden death may occur following thrombosis of coronary artery.

3. Venous thrombi (Phlebothrombosis). These may cause various effects such as:

i) Thromboembolism

ii) Oedema of area drained

iii) Poor wound healing

iv) Skin ulcer

v) Painful thrombosed veins (thrombophlebitis)

vi) Painful white leg (phlegmasia alba dolens) due to iliofemoral venous thrombosis in post-partum cases

vii) Thrombophlebitis migrans in cancer.

4. Capillary thrombi. Microthrombi in microcirculation may give rise to disseminated intravascular coagulation (DIC).

EMBOLISM

Embolism is the process of partial or complete obstruction of some parts of the cardiovascular system by any mass carried in the circulation; the transported intravascular mass detached from its site of origin is called an embolus. Most usual forms of emboli (90%) are thromboemboli i.e. originating from thrombi or their parts detached from the vessel wall. Emboli may be of various types:

A. Depending upon the matter in the emboli, they can be:

i) Solid e.g. detached thrombi (thromboemboli), atheromatous material, tumour cell clumps, tissue fragments, parasites, bacterial clumps, foreign bodies.

ii) Liquid e.g. fat globules, amniotic fluid, bone marrow.

iii) Gaseous e.g. air, other gases.

B. Depending upon whether infected or not, they are called:

i) Septic, when sterile.

ii) Septic, when infected.

C. Depending upon the source of the emboli, they are classified as:

i) Cardiac emboli from left side of heart e.g. emboli originating from atrium and atrial appendages, infarct in the left ventricle, vegetations of endocarditis.

ii) Arterial emboli e.g. in systemic arteries in the brain, spleen, kidney, intestine.

iii) Venous emboli e.g. in pulmonary arteries.

iv) Lymphatic emboli can also occur.

D. Depending upon the flow of blood, two special types of emboli are mentioned:

i) Paradoxical embolus. An embolus which is carried from the venous side of circulation to the arterial side or vice versa is called paradoxical or crossed embolus e.g. through arteriovenous communication such as in patent foramen ovale, septal defect of the heart, and arteriovenous shunts in the lungs.

ii) Retrograde embolus. An embolus which travels against the flow of blood is called retrograde embolus e.g. metastatic deposits in the spine from carcinoma prostate. The spread occurs by retrograde embolism through intraspinal veins which carry tumour emboli from large thoracic and abdominal veins due to increased pressure in body cavities e.g. during coughing or straining.

Thromboembolism.

It is the most frequent kind of embolism. There are three main ways of blood clot moving:

1) From venous part of the systemic circle (*venous thromboembolism*) to the right chambers of heart and then to the vessels of a pulmonary circle;

2) From the left chambers of the heart to the aorta and large arteries (*arterial (systemic) thromboembolism*) and then to the small arteries of systemic circle (thromboembolic syndrome);

3) From branches of portal system to the portal vein of liver.

A detached thrombus or part of thrombus constitutes the most common type of embolism. These may arise in the arterial or venous circulation:

Arterial (systemic) thromboembolism. Arterial emboli may be derived from the following sources:

A. Causes within the heart (80-85%): These are mural thrombi in the left atrium or left ventricle, vegetations on the mitral or aortic valves, prosthetic heart valves and cardiomyopathy.

B. Causes within the arteries: these include emboli developing in relation to atherosclerotic plaques, aortic aneurysms, pulmonary veins and paradoxical arterial emboli from the systemic venous circulation.

The effects of arterial emboli depend upon their size, site of lodgement, and adequacy of collateral circulation. If the vascular occlusion occurs, the following ill-effects may happen:

i) Infarction of the organ or its affected part e.g. ischaemic necrosis in the lower limbs (70-75%), spleen, kidneys, brain, intestine.

ii) Gangrene following infarction in the lower limbs if the collateral circulation is inadequate.

iii) Arteritis and mycotic aneurysm formation from bacterial endocarditis.

iv) Myocardial infarction may occur following coronary embolism.

v) Sudden death may result from coronary embolism or embolism in the middle cerebral artery.

Venous thromboembolism. Venous emboli may arise from the following sources:

i) Thrombi in the veins of the lower legs are the most common cause of venous emboli.

ii) Thrombi in the pelvic veins.

iii) Thrombi in the veins of the upper limbs.

iv) Thrombosis in cavernous sinus of the brain.

v) Thrombi in the right side of heart.

The most significant effect of venous embolism is obstruction of pulmonary arterial circulation leading to pulmonary embolism described below.

Pulmonary embolism

Pulmonary embolism is the most common and fatal form of venous thromboembolism in which there is occlusion of pulmonary arterial tree by thromboemboli. Pulmonary thrombosis as such is uncommon and may occur in pulmonary atherosclerosis and pulmonary hypertension.

Etiology. Pulmonary emboli are more common in hospitalised or bed-ridden patients, though they can occur in ambulatory patients as well. The causes are as follows:

i) Thrombi originating from large veins of lower legs (such as popliteal, femoral and iliac) are the cause in 95% of pulmonary emboli.

ii) Less common sources include thrombi in varicosities of superficial veins of the legs, and pelvic veins such as periprostatic, periovarian, uterine and broad ligament veins.

Pathogenesis. Detachment of thrombi from any of the above-mentioned sites produces a thromboembolus that flows through venous drainage into the larger veins draining into right side of the heart.

- If the thrombus is large, it is impacted at the bifurcation of the main pulmonary artery (saddle embolus), or may be found in the right ventricle or its outflow tract.

- More commonly, there are multiple emboli, or a large embolus may be fragmented into many smaller emboli which are then impacted in a number of vessels, particularly affecting the lower lobes of lungs.

- Rarely, paradoxical embolism may occur by passage of an embolus from right heart into the left heart through atrial or ventricular septal defect. In this way, pulmonary emboli may reach systemic circulation.

Consequences of pulmonary embolism.

Pulmonary embolism occurs more commonly as a complication in patients of acute or chronic debilitating diseases who are immobilised for a long duration. Women in their reproductive period are at higher risk such as in late pregnancy, following delivery and with use of contraceptive pills. The effects of pulmonary embolism depend mainly on the size of the occluded vessel, the number of emboli, and on the cardiovascular status of the patient. The following consequences can result:

i) *Sudden death.* Massive pulmonary embolism results in instantaneous death, without occurrence of chest pain or dyspnoea. However, if the death is somewhat delayed, the clinical features resemble myocardial infarction i.e. severe chest pain, dyspnoea and shock.

ii) *Acute cor pulmonale.* Numerous small emboli may obstruct most of the pulmonary circulation resulting in acute right heart failure. Another mechanism is by release of vasoconstrictor substances from platelets or by reflex vasoconstriction of pulmonary vessels.

iii) *Pulmonary infarction.* Obstruction of relatively small-sized pulmonary arterial branches may result in pulmonary infarction. The clinical

features include chest pain due to fibrinous pleuritis, haemoptysis and dyspnoea due to reduced functioning pulmonary parenchyma.

iv) *Pulmonary haemorrhage.* Obstruction of terminal branches (endarteries) leads to central pulmonary haemorrhage. The clinical features are haemoptysis, dyspnoea, and less commonly, chest pain due to central location of pulmonary haemorrhage. Sometimes, there may be concomitant pulmonary infarction.

v) *Resolution.* Vast majority of small pulmonary emboli (60-80%) are resolved by fibrinolytic activity. These patients are clinically silent owing to bronchial circulation so that lung parenchyma is adequately perfused.

vi) *Pulmonary hypertension, chronic cor pulmonale and pulmonary arteriosclerosis.* These are the sequelae of multiple small thromboemboli undergoing healing rather than resolution.

Systemic embolism.

This is the type of arterial embolism that originates commonly from thrombi in the diseased heart, especially in the left ventricle. These diseases of heart include myocardial infarction, cardiomyopathy, RHD, congenital heart disease, infective endocarditis, and prosthetic cardiac valves. These arterial emboli invariably cause infarction at the sites of lodgement which include, in descending order of frequency, lower extremity, brain, and internal visceral organs (spleen, kidneys, intestines). Thus, the effects and sites of arterial emboli are in striking contrast to venous emboli which are often lodged in the lungs.

Fat embolism.

Obstruction of arterioles and capillaries by fat globules constitutes fat embolism. If the obstruction in the circulation is by fragments of adipose tissue, it is called fat-tissue embolism.

Etiology. Following are the important causes of fat embolism:

i) Traumatic causes:

- Trauma to bones is the most common cause of fat embolism e.g. in fractures of long bones leading to passage of fatty marrow in circulation, concussions of bones, after orthopaedic surgical procedures etc.

- Trauma to soft tissue e.g. laceration of adipose tissue and in puerperium due to injury to pelvic fatty tissue.

ii) Non-traumatic causes:

- Extensive burns
- Diabetes mellitus
- Fatty liver
- Pancreatitis
- Sickle cell anaemia
- Decompression sickness
- Inflammation of bones and soft tissues
- Extrinsic fat or oils introduced into the body.

Pathogenesis. The following mechanisms are hypothesised to explain the pathogenesis of fat embolism. These may be acting singly or in combination.

i) *Mechanical theory.* Mobilisation of fluid fat may occur following trauma to the bone or soft tissues. The fat globules released from the injured area may enter venous circulation and finally most of the fat is arrested in the small vessels in the lungs. Some of the fat globules may further pass through into the systemic circulation to lodge in other organs.

ii) *Emulsion instability theory.* This theory explains the pathogenesis of fat embolism in non-traumatic cases. According to this theory, fat emboli are formed by aggregation of plasma lipids (chylomicrons and fatty acids) due to disturbance in natural emulsification of fat.

iii) *Intravascular coagulation theory.* In stress, release of some factor activates disseminated intravascular coagulation (DIC) and aggregation of fat emboli.

iv) *Toxic injury theory.* According to this theory, the small blood vessels of lungs are chemically injured by high plasma levels of free fatty acid, resulting in increased vascular permeability and consequent pulmonary oedema.

Consequences of fat embolism. The effects of fat embolism depend upon the size and quantity of fat globules, and whether or not the emboli pass through the lungs into the systemic circulation.

i) *Pulmonary fat embolism.* In patients dying after fractures of bones, presence of numerous fat emboli in the capillaries of the lung is a frequent autopsy finding because the small fat globules are not likely to appreciably obstruct the vast pulmonary vascular bed. However, widespread obstruction of pulmonary circulation due to extensive pulmonary embolism can occur and result in sudden death.

Microscopically, the lungs show hyperaemia, oedema, patchial haemorrhages and changes of adult respiratory distress syndrome (ARDS). Pulmonary infarction is usually not a feature of fat embolism because of the small size of globules. In routine stains, the fat globules in the pulmonary arteries, capillaries and alveolar spaces appear as vacuoles. Frozen section is essential for confirmation of globules by fat stains such as Sudan dyes (Sudan black, Sudan III and IV), oil red O and osmic acid.

ii) *Systemic fat embolism.* Some of the fat globules may pass through the pulmonary circulation such as via patent foramen ovale, arteriovenous shunts in the lungs and vertebral venous plexuses, and get lodged in the capillaries of organs like the brain, kidney, skin etc.

- *Brain.* The pathologic findings in the brain are patchial haemorrhages on the leptomeninges and minute haemorrhages in the parenchyma.

Microscopically, microinfarct of brain, oedema and haemorrhages are seen. The CNS manifestations include delirium, convulsions, stupor, coma and sudden death.

- *Kidney.* Renal fat embolism present in the glomerular capillaries, may cause decreased glomerular filtration. Other effects include tubular damage and renal insufficiency.

- *Other organs.* Besides the brain and kidneys, other findings in systemic fat embolism are patchiae in the skin, conjunctivae, serosal surfaces, fat globules in the urine and sputum.

Gas embolism

Air, nitrogen and other gases can produce bubbles within the circulation and obstruct the blood vessels causing damage to tissue. Two main forms of gas embolism – air embolism and decompression sickness are described below.

Air embolism occurs when air is introduced into venous or arterial circulation.

Venous air embolism. Air may be sucked into systemic veins under the following circumstances:

- i) *Operations on head and neck, and trauma.* The accidental opening of a major vein of the neck like jugular, or neck wounds involving the major neck veins, may allow air to be drawn into venous circulation.

ii) *Obstetrical operations and trauma.* During childbirth by normal vaginal delivery, caesarean section, abortions and other procedures, fatal air embolism may result from the entrance of air into the opened-up uterine venous sinuses and endometrial veins.

iii) *Intravenous infusion of blood and fluid.* Air embolism may occur during intravenous blood or fluid infusions if only positive pressure is employed.

iv) *Angiography.* During angiographic procedures, air may be entrapped into a large vein causing air embolism.

The effects of venous air embolism depend upon the following factors:

i) Amount of air introduced into the circulation. The volume of air necessary to cause death is variable but usually 100-150 ml of air entry is considered fatal.

ii) Rapidity of entry of a smaller volume of air is important determinant of a fatal outcome.

iii) Position of the patient during or soon after entry of air is another factor. The air bubbles may ascend into the superior vena cava if the position of head is higher than the trunk (e.g. in upright position) and reach the brain.

iv) General condition of the patient e.g. in severely ill patients, as little as 40 ml of air may have serious results.

The mechanism of death is caused by entrapment of air emboli in the pulmonary arterial trunk in the right heart. If bubbles of air in the form of froth pass further out into pulmonary arterioles, they cause widespread vascular occlusions. If death from pulmonary air embolism is suspected, the heart and pulmonary artery should be opened in situ under water so that escaping froth or foam formed by mixture of air and blood can be detected.

Arterial air embolism. Entry of air into pulmonary vein or its tributaries may occur in the following conditions:

i) Cardiothoracic surgery and trauma. Arterial air embolism may occur following thoracic operations, thoracocentesis, rupture of the lung, penetrating wounds of the lung, artificial pneumothorax etc.

ii) Paradoxical air embolism. This may occur due to passage of venous air emboli to the arterial side of circulation through a patent foramen ovale or via pulmonary arteriovenous shunts.

iii) Arteriography. During arteriographic procedures, air embolism may occur.

The effects of arterial air embolism are in the form of certain characteristic features:

- i) Marble skin due to blockage of cutaneous vessels.
- ii) Air bubbles in the retinal vessels seen ophthalmoscopically.
- iii) Pallor of the tongue due to occlusion of a branch of lingual artery.
- iv) Coronary or cerebral arterial air embolism may cause sudden death by much smaller amounts of air than in the venous air embolism.

Decompression Sickness. This is a specialised form of gas embolism known by various names such as Caisson's disease, divers' palsy or aeroembolism.

Pathogenesis. Decompression sickness is produced when the individual decompresses suddenly, either from high atmospheric pressure to normal level, or from normal pressure to low atmospheric pressure.

- In divers, workers in caissons (diving-bells), offshore drilling and runnels, who descend to high atmospheric pressure, increased amount of atmospheric gases (mainly nitrogen; others are O₂, CO₂) are dissolved in blood and tissue fluids. When such an individual ascends too rapidly i.e. comes to normal level suddenly from high atmospheric pressure, the gases come out of the solution as minute bubbles, particularly in fatty tissues which have affinity for nitrogen. These bubbles may coalesce together to form large emboli.

- In aeroembolism, seen in those who ascend to high altitudes or air flight in unpressurised cabins, the individuals are exposed to sudden decompression from low atmospheric pressure to normal levels. This results in similar effects as in divers and workers in caissons.

Effects. The effects of decompression sickness depend upon the following:

- Depth or altitude reached
- Duration of exposure to altered pressure
- Rate of ascent or descent
- General condition of the individual

The pathologic changes are more pronounced in sudden decompression from high pressure to normal levels than in those who decompress from low pressure to normal levels. The changes are more serious in obese persons as nitrogen gas is more soluble in fat than in body fluids.

Clinical effects of decompression sickness are of 2 types – acute and chronic.

- *Acute form* occurs due to acute obstruction of small blood vessels in the vicinity of joints and skeletal muscles. The condition is clinically characterised by the following:

- i) The bends, as the patient doubles up in bed due to acute pain in joints, ligaments and tendons,

- ii) The chokes occur due to accumulation of bubbles in the lungs, resulting in acute respiratory distress,

- iii) Cerebral effects may manifest in the form of vertigo, coma, and sometimes death.

- *Chronic form* is due to foci of ischaemic necrosis throughout body, especially the skeletal system. Ischaemic necrosis may be due to embolism per se, but other factors such as platelet activation, intravascular coagulation and hypoxia might contribute. The features of chronic form are as under:

- i) Avascular necrosis of bones e.g. head of femur, tibia, humerus.

- ii) Neurological symptoms may occur due to ischaemic necrosis in the central nervous system. These include paraesthesias and paraplegia.

- iii) Lung involvement in the form of haemorrhage, oedema, emphysema and atelectasis may be seen. These result in dyspnoea, nonproductive cough and chest pain.

- iv) Skin manifestations include itching, patchy erythema, cyanosis and oedema.

- v) Other organs like parenchymal cells of the liver and pancreas may show lipid vacuoles.

Amniotic fluid embolism

This is the most serious, unpredictable and unpreventable cause of maternal mortality. During labour and in the immediate post-partum period, the contents of amniotic fluid may enter the uterine veins and reach right side of the heart resulting in fatal complications. The amniotic fluid components which may be found in uterine veins, pulmonary artery and vessels of other organs are: epithelial squames, vernix caseosa, lanugo hair, bile from meconium, and mucus. The mechanism by which these amniotic fluid contents enter the maternal circulation is not clear. Possibly, they gain entry either through tears in the myometrium and endocervix, or the amniotic fluid is forced into uterine sinusoids by vigorous uterine contractions.

Pathologic changes. Notable changes are seen in the lungs such as haemorrhages, congestion, oedema and changes of ARDS, and dilatation of right side of the heart.

These changes are associated with identifiable amniotic fluid contents within the pulmonary microcirculation.

The clinical syndrome is characterised by the following features:

- Sudden respiratory distress and dyspnoea,
- Deep cyanosis,
- Cardiovascular shock,
- Convulsions,
- Coma,
- Unexpected death.

The cause of death may not be obvious but can occur as a result of the following mechanisms:

i) Mechanical blockage of the pulmonary circulation in extensive embolism.

ii) Anaphylactoid reaction to amniotic fluid components.

iii) Disseminated intravascular coagulation (DIC) due to liberation of thromboplastin by amniotic fluid.

iv) Haemorrhagic manifestations due to thrombocytopenia and afibrinogenaemia.

Atheroembolism

Atheromatous plaques, especially from aorta, may get eroded to form atherosclerotic emboli which are then lodged in medium-sized and small arteries. These emboli consist of cholesterol crystals, hyaline debris and calcified material, and may evoke foreign body reaction at the site of lodgement.

The pathologic changes and their effects in atheroembolism are as under:

i) Ischaemia, atrophy and necrosis of tissue distal to the occluded vessel.

ii) Infarcts in the organs affected such as the kidneys, spleen, brain and heart.

iii) Gangrene in the lower limbs.

iv) Hypertension, if widespread renal vascular lesions are present.

Tumour embolism

Malignant tumour cells invade the local blood vessels and may form tumour emboli to be lodged elsewhere, producing metastatic tumour deposits. Notable examples are clear cell carcinoma of kidney, carcinoma of the lung, malignant melanoma etc.

Miscellaneous emboli.

Various other endogenous and exogenous substances may act as emboli. These are:

- i) Fragments of tissue,
- ii) Placental fragments,
- iii) Red cell aggregates (sludging),
- iv) Bacteria,
- v) Parasites,
- vi) Barium emboli following enema,
- vii) Foreign bodies e.g. needles, talc, sutures, bullets, catheters etc.

Microbial embolism takes place when microbes or fungi occlude capillaries, thus abscesses are formed in different organs.

ISCHAEMIA

Definition. Ischaemia is defined as deficient blood supply to part of a tissue. The cessation of blood supply may be complete (complete ischaemia) or partial (partial ischaemia). The harmful effects of ischaemia may result from 3 ways:

1. Hypoxia due to deprivation of oxygen to tissues.
2. Inadequate supply of nutrients to the tissue such as glucose and aminoacids.
3. Inadequate clearance of metabolites resulting in accumulation of metabolic waste-products in the affected tissue.

Etiology. A number of causes may produce ischaemia. These are as under:

1. Causes in the heart. Inadequate cardiac output resulting from heart block, ventricular arrest and fibrillation may cause hypoxic injury to brain.
 - If the arrest continues for 15 seconds, consciousness is lost.
 - If the condition lasts for more than 4 minutes, irreversible ischaemic damage to brain occurs.
 - If it is prolonged for more than 8 minutes, death is inevitable.

2. Causes in the arteries. The most common and most important causes of ischaemia are due to obstruction in arterial blood supply. These are:

i) Luminal occlusion such as due to:

- Thrombosis,
- Embolism.

ii) Causes in the arterial wall such as:

- Vasospasm (e.g. in Raynaud's disease),
- Hypothermia, ergotism,
- Arteriosclerosis,
- Polyarteritis nodosa,
- Thromboangiitis obliterans (Buerger's disease),
- Severed vessel wall.

iii) Outside pressure on an artery such as:

- Ligature,
- Tourniquet,
- Tight plaster, bandages,
- Torsion.

3. Causes in the veins. Blockage of venous drainage may lead to engorgement and obstruction to arterial blood supply resulting in ischaemia. The examples include the following:

i) Luminal occlusion such as in:

- Thrombosis of mesenteric veins,
- Cavernous sinus thrombosis.

ii) Causes in the vessel wall such as in:

- Varicose veins of the legs.

iii) Outside pressure on a vein as in:

- Strangulated hernia,
- Intussusception,
- Volvulus.

4. Causes in the microcirculation. Ischaemia may result from occlusion of arterioles, capillaries and venules. The causes are as under:

i) Luminal occlusion such as:

- By red cells e.g. in sickle cell anaemia, red cells parasitised by malaria, acquired haemolytic anaemia, sludging of the blood.
- By white cells e.g. in chronic myeloid leukaemia,
- By fibrin e.g. defibrination syndrome,
- By precipitated cryoglobulins

- By fat embolism,
 - In decompression sickness.
- ii) Causes in the microvasculature wall such as:
- Vasculitis e.g. in polyarteritis nodosa, Henoch-Schonlein purpura, Arthus reaction, septicaemia.
 - Frost-bite injuring of the wall of small blood vessels.
- iii) Outside pressure on microvasculature as in:
- Bedsores.

Factors determining the severity of ischaemic injury. The extent of damage produced by ischaemia due to occlusion of arterial or venous blood vessels depends upon a number of factors. These are as under:

1. *Anatomic pattern.* The extent of injury by ischaemia depends upon the anatomic pattern of arterial blood supply of the organ or tissue affected. There are 4 different patterns of arterial blood supply:

i) *Single arterial supply without anastomosis.* Some organs receive blood supply from arteries which do not have significant anastomosis and are thus functional endarteries. Occlusion of such vessels invariably results in ischaemic necrosis. The examples are:

- Central artery of the retina,
- Interlobular arteries of the kidneys.

ii) *Single arterial supply with rich anastomosis.* Arterial supply to some organs has rich interarterial anastomoses so that blockage of one vessel can re-establish blood supply bypassing the blocked arterial branch, and hence the infarction is less common in such circumstances. For example:

- Superior mesenteric artery supplying blood to the small intestine.
- Inferior mesenteric artery supplying blood to distal colon.
- Arterial supply to the stomach by 3 separate vessels derived from coeliac axis.
- Interarterial anastomoses in the 3 main trunks of the coronary arterial system.

iii) *Parallel arterial supply.* Blood supply to some organs and tissues is such that the vitality of the tissue is maintained by alternative blood supply in case of occlusion of one. The examples are:

- Blood supply to brain in the region of circle of Willis.
- Arterial supply to forearm by radial and ulnar arteries.

iv) *Double blood supply.* The effect of occlusion of one set of vessels is modified if an organ has dual blood supply. For example:

- Lungs are perfused by bronchial circulation as well as by pulmonary arterial branches.

- Liver is supplied by both portal circulation and hepatic arterial flow. However, collateral circulation is of little value if the vessels are severely affected with spasm, atheroma or any other condition.

2. *General and cardiovascular status.* The general status of an individual as regards cardiovascular function is an important determinant to assess the effect of ischaemia. Some of the factors which render the tissues more vulnerable to the effects of ischaemia are:

- i) Anaemias (sickle cell anaemia, in particular)
- ii) Lowered oxygenation of blood (hypoxaemia)
- iii) Senility with marked coronary atherosclerosis
- iv) Cardiac failure
- v) Blood loss
- vi) Shock.

3. *Type of tissue affected.* The vulnerability of tissue of the body to the effect of ischaemia is variable. The mesenchymal tissues are quite resistant to the effect of ischaemia as compared to parenchymal cells of the organs. The following tissues are more vulnerable to ischaemia:

- i) Brain (cerebral cortical neurons, in particular).
- ii) Heart (myocardial cells).
- iii) Kidney (especially epithelial cells of proximal convoluted tubules).

4. *Rapidity of development.* Sudden vascular obstruction results in more severe effects of ischaemia than if it is gradual since there is less time for collaterals to develop.

5. *Degree of vascular occlusion.* Complete vascular obstruction results in more severe ischaemic injury than the partial occlusion.

Effects. The effects of ischaemia are variable and range from 'no change' to 'sudden death'.

1. *No effects on the tissues,* if the collateral channels develop adequately so that the effect of ischaemia fails to occur.

2. *Functional disturbances.* These result when collateral channels are able to supply blood during normal activity but the supply is not adequate to

withstand the effect of exertion. The examples are angina pectoris and intermittent claudication.

3. *Cellular changes.* Partial ischaemia may produce cellular changes such as cloudy swelling, fatty change, atrophy and replacement fibrosis. Infarction results when the deprivation of blood supply is complete so as to cause necrosis of tissue affected.

4. *Sudden death.* The cause of sudden death from ischaemia is usually myocardial and cerebral infarction.

INFARCTION

Definition. Infarction is the process of tissue necrosis resulting from some form of circulatory insufficiency; the localised area of necrosis so developed is called an infarct.

Etiology. All the causes of ischaemia discussed above can cause infarction. There are a few other noteworthy features in infarcts:

- Most commonly, infarcts are caused by interrupted arterial blood supply, called ischaemic necrosis.

- Less commonly, venous obstruction can produce infarcts termed stagnant hypoxia.

- Generally, sudden, complete, and continuous occlusion by thrombosis or embolism produces infarcts.

- Infarcts may be produced by nonexclusive circulatory insufficiency as well e.g. incomplete atherosclerotic narrowing of coronary arteries may produce myocardial infarction due to acute coronary insufficiency.

Types of infarcts see above.

Pathogenesis. The process of infarction takes place as follows:

i) Localised hyperaemia due to local anoxaemia occurs immediately after obstruction of the blood supply.

ii) Within a few hours, the affected part becomes swollen due to oedema and haemorrhage. The amount of haemorrhage is variable, being more marked in the lungs and spleen, and less extensive in the kidneys and heart.

iii) Cellular changes such as cloudy swelling and degeneration appear early, while death of the cells or necrosis occurs in 12-48 hours.

iv) There is progressive autolysis of the necrotic tissue and haemolysis of the red cells.

v) An acute inflammatory reaction and hyperaemia appear at the same time in the surrounding tissues in response to products of autolysis.

vi) Blood pigments, haematoidin and haemosiderin, liberated by haemolysis are deposited in the infarct. At this stage, most infarcts become pale due to loss of red cells.

vii) Following this, there is progressive in growth of granulation tissue from the margin of the infarct so that eventually the infarct is replaced by a fibrous scar. Dystrophic calcification may occur sometimes. However, in the case of infarct brain, there is liquefactive necrosis which heals by gliosis.

Pathologic changes. Some general morphological features of infarcts are described below, followed by pathologic changes in infarcts of different organs.

Grossly, infarcts of solid organs are usually wedge-shaped, the apex pointing towards the occluded artery and the wide base on the surface of the organ. Infarcts due to arterial occlusion are generally pale while those due to venous obstruction are haemorrhagic. Most infarcts become pale later as the red cells are lysed but pulmonary infarcts never become pale due to extensive amount of blood.

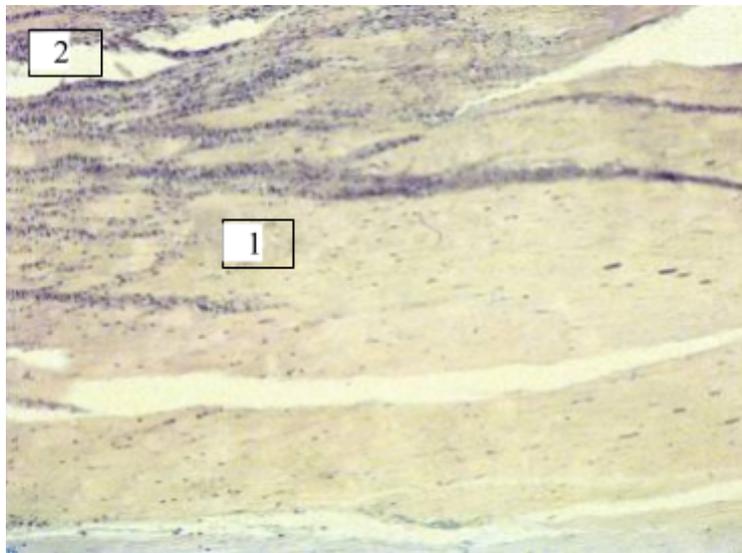


Fig. 17. Myocardium infarction. H and E stained section, x 200.
 inflammatory reaction at the periphery of an infarct.

Cerebral infarcts are poorly defined with central softening (encephalomalacia). Recent infarcts are generally slightly elevated over the surface while the old infarcts are shrunken and depressed under the surface of the organ.

Microscopically,

the pathognomonic cyto-

logic change in all infarcts is coagulative necrosis of the affected area of tissue

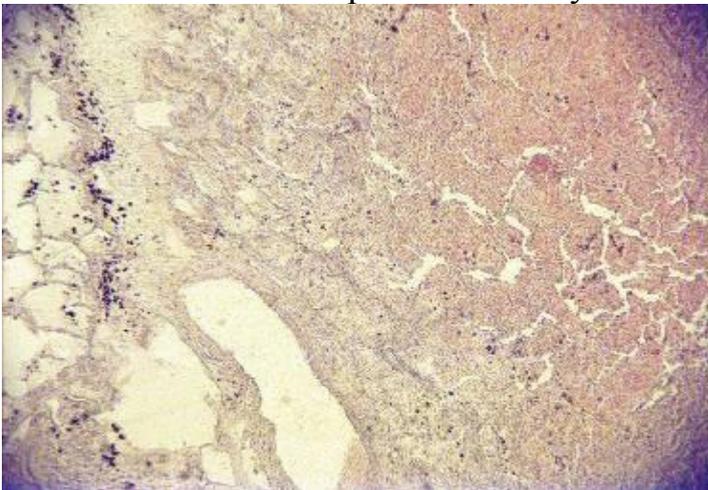
or organ. In cerebral infarcts, however, there is characteristic liquefactive necrosis. Some amount of haemorrhage is generally present in any infarct. At the periphery of an infarct, inflammatory reaction is noted. Initially, neutrophils predominate but subsequently macrophages and fibroblasts appear.

Eventually, the necrotic area is replaced by fibrous scar tissue, which at times may show dystrophic calcification. In cerebral infarcts, the liquefactive necrosis is followed by gliosis i.e. replacement by microglial cells distended by fatty material (gitter cells).

Infarcts of different organs. A few representative examples of infarction of some organs (lungs, kidney, liver and spleen) are discussed below. Myocardial infarction, cerebral infarction and infarction of small intestines are covered in detail later in respective chapters of Systemic Pathology.

Infarct of the lung. Embolism of the pulmonary arteries may produce pulmonary infarction, though not always. This is because lungs receive blood supply from bronchial arteries as well, and thus occlusion of pulmonary artery ordinarily does not produce infarcts. However, it may occur in patients who have inadequate circulation such as in chronic lung diseases and congestive heart failure.

Grossly, the pulmonary infarcts are classically wedge-shaped with base on the pleura, haemorrhagic, variable in size, and most often in the lower lobes. Fibrinous pleuritis usually covers the area of infarct. Cut sur-



face is dark purple and may show the blocked vessel near the apex of the infarcted area. Old organised and healed pulmonary infarcts appear as retracted fibrous scars.

Microscopically, the distinctive histologic feature is the necrosis of the alveolar

Fig. 18 . pulmonary infarct. H and E stained section, x 200.

walls (see Fig. 18). Initially, there is infiltration by neutrophils

and intense alveolar capillary congestion, but later their place is taken by haemosiderin, phagocytes and granulation tissue.

Infarct of the kidney. Renal infarction is common, found in upto 5% of autopsies. Majority of them are caused by thromboemboli, most commonly originating from the heart such as in mural thrombi in the left atrium, myocardial infarction, vegetative endocarditis and aortic aneurysm. Less commonly, renal infarcts may occur due to advanced renal artery atherosclerosis, arteritis and sickle cell anaemia.

Grossly, renal infarcts are often multiple and may be bilateral. Characteristically, they are pale or anaemic and wedge-shaped with base resting under the capsule and apex pointing towards the medulla. Generally, a narrow rim of preserved renal tissue under the capsule is spared because it draws its blood supply from the capsular vessels. Cut surface of renal infarct in the first 2 to 3 days is red and congested but by 4th day the centre becomes pale yellow. At the end of one week, the infarct is typically anaemic and depressed below the surface of the kidney.

Microscopically, the affected area shows characteristic coagulative necrosis of renal parenchyma i.e. there are ghosts of renal tubules and glomeruli without intact nuclei and cytoplasmic content. The margin of the infarct shows inflammatory reaction – initially acute but later macrophages and fibrous tissue predominate.

Infarct of the spleen. Spleen is one of the common sites for infarction. Splenic infarction results from occlusion of the splenic artery or its branches. Occlusion is caused most commonly by thromboemboli arising in the heart (e.g. in mural thrombi in the left atrium, vegetative endocarditis, myocardial infarction), and less frequently by obstruction of microcirculation (e.g. in myeloproliferative diseases, sickle cell anaemia, arteritis, Hodgkin's disease, bacterial infections).

Grossly, splenic infarcts are often multiple. They are characteristically pale or anaemic and wedge-shaped with their base at the periphery and apex pointing towards hilum.

Microscopically, the features are similar to those found in anaemic infarcts in kidney. Coagulative necrosis and inflammatory reaction are seen. Later, the necrotic tissue is replaced by shrunken fibrous scar.

Infarct of the liver. Just as in lungs, infarcts in the liver are uncommon due to dual blood supply – from portal vein and from hepatic artery. Obstruction of the portal vein is usually secondary to other diseases such as hepatic cirrhosis, intravenous invasion of primary carcinoma of the liver,

carcinoma of the pancreas and pylephlebitis. Occlusion of portal vein or its branches generally does not produce ischaemic infarction but instead reduced blood supply to hepatic parenchyma causes non-ischaemic infarct called infarct of Zahn. Obstruction of the hepatic artery or its branches, on the other hand, caused by arteritis, arteriosclerosis, bland or septic emboli, results in ischaemic infarcts of the liver.

Grossly, ischaemic infarcts of the liver are usually anaemic but sometimes may be haemorrhagic due to stuffing of the site by blood from the portal vein. Infarcts of Zahn (non-ischaemic infarcts) produce sharply defined red-blue area in liver parenchyma.

Microscopically, ischaemic infarcts show characteristics of pale or anaemic infarcts as in kidney or spleen. Infarcts of Zahn occurring due to reduced portal blood flow result in atrophy of hepatocytes and dilatation of sinusoids.

DISSEMINATED INTRAVASCULAR COAGULATION (DIVC)

Disseminated intravascular coagulation (DIVC), also termed defibrination syndrome or consumption coagulopathy, is a complex thrombohaemorrhagic disorder (intravascular coagulation and haemorrhage) occurring as a secondary complication in some systemic diseases.

Etiology: although there are numerous conditions associated with DIC, most frequent causes are listed below:

1. *Massive tissue injury:* in obstetrical syndromes (e.g., abruption placentae, amniotic fluid embolism, retained dead foetus), massive trauma, metastatic malignancies, surgery.
2. *Infections:* especially endotoxaemia, gram-negative and meningococcal septicaemia, certain viral infections, malaria, aspergillosis.
3. *Widespread endothelial damage:* in aortic aneurysm, haemolytic-uraemic syndrome, severe burns, acute glomerulonephritis.
4. *Miscellaneous:* snake bite, shock, acute intravascular haemolysis, heat stroke.

Pathogenesis: Although in each case, a distinct triggering mechanism has been identified, the sequence of events, in general, can be summarised:

1. *Activation of coagulation.* The etiologic factors listed above initiate widespread activation of coagulation pathway by release of tissue factor.



Fig. 19. Multiple hemorrhages in the heart and pericardium.

2. *Thrombotic phase.* Endothelial damage from the various thrombogenic stimuli causes generalised platelet aggregation and adhesion with resultant deposition of small thrombi and emboli throughout the micro vasculature.

3. *Consumption phase.* The early thrombotic phase is followed by a phase of consumption of coagulation factors and platelets.

4. *Secondary fibrinolysis.* As a protective mechanism, fibrinolytic system is secondarily activated at the site of intravascular coagulation. Secondary fibrinolysis causes breakdown of fibrin resulting in formation of FDPs in the circulation.

Clinical features: There are 2 main features of DIC – *bleeding* as the most common manifestation, and *organ damage* due to ischaemia caused

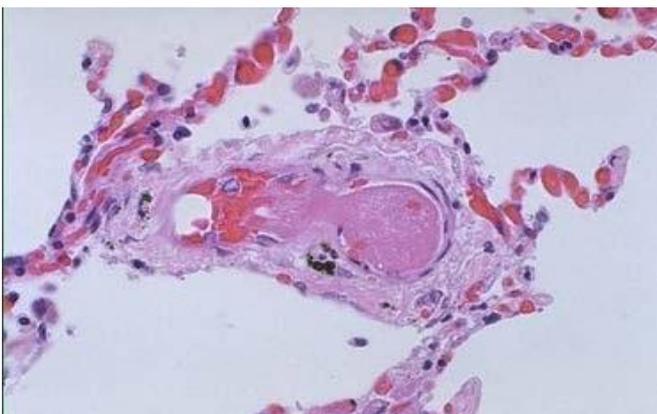


Fig. 20. Intravascular thrombosis of pulmonary vessels. H and E stained section, x 200.

by the effect of widespread intravascular thrombosis such as in the kidney and brain. Less common manifestations include: microangiopathic haemolytic anaemia and thrombosis in larger arteries and veins.

INFLAMMATION

DEFINITION AND CAUSES

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. Inflammation is a complex vascular-mesenchymal reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrotized cells and tissues.

The agents causing inflammation may be as under:

1. Physical agents like heat, cold, radiation, mechanical trauma.
2. Chemical agents like organic and inorganic poisons.
3. Infective agents like bacteria, viruses and their toxins.
4. Immunological agents like cell-mediated and antigen-antibody re- actions.

Thus inflammation is distinct from infection – the former being a protective response by the body while the latter is invasion into the body by harmful microbes and their resultant ill-effects by toxins. Inflammation involves 2 basic processes with some overlapping, viz. early inflammatory response and later followed by healing. Though both these processes generally have protective role against injurious agents, inflammation and healing may cause considerable harm to the body as well e.g. anaphylaxis to bites by insects or reptiles, drugs, toxins, atherosclerosis, chronic rheumatoid arthritis, fibrous bands and adhesions in intestinal obstruction.

SIGNS OF INFLAMMATION

The Roman writer Celsus in 1st century A.D. named the famous 4 cardinal signs of inflammation as:

- rubor (redness);
- tumor (swelling);
- calor (heat); and
- dolor (pain).

To these, fifth sign *functio laesa* (loss of function) was later added by Virchow. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only one of the signs of inflammation.

Types of inflammation: Depending upon the defense capacity of the host and duration of response, inflammation can be classified as acute and chronic.

I. **Acute** inflammation is of short duration and represents the early body reaction and is usually followed by repair.

The main features of acute inflammation are:

1. Accumulation of fluid and plasma at the affected site.
2. Intravascular activation of platelets.
3. Polymorphonuclear neutrophils as inflammatory cells.

II. **Chronic** inflammation is of longer duration and occurs either after the causative agent of acute inflammation persists for a long time, or the stimulus is such that it induces chronic inflammation from the beginning.

The characteristic feature of chronic inflammation is presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages.

ACUTE INFLAMMATION

The changes in acute inflammation can be conveniently described under the following 2 headings:

- I. Vascular events; and
- II. Cellular events.

I. Vascular events

Alteration in the microvasculature (arterioles, capillaries and venules) is the earliest response to tissue injury. These alterations include: haemodynamic changes and changes in vascular permeability.

Haemodynamic changes. The earliest features of inflammatory response result from changes in the vascular flow and calibre of small blood vessels in the injured tissue. The sequence of these changes is as under:

1. Irrespective of the type of injury, immediate vascular response is of transient vasoconstriction of arterioles. With mild form of injury, the blood flow may be re-established in 3-5 seconds while with more severe injury the vasoconstriction may last for about 5 minutes.

2. Next follows persistent progressive vasodilatation which involves mainly the arterioles, but to a lesser extent, affects other components of the microcirculation like venules and capillaries. This change is obvious within half an hour of injury. Vasodilatation results in increased blood volume in

microvascular bed of the area, which is responsible for redness and warmth at the site of acute inflammation.

3. Progressive vasodilatation, in turn, may elevate the local hydrostatic pressure resulting in transudation of fluid into the extracellular space. This is responsible for swelling at the local site of acute inflammation.

4. Slowing or stasis of microcirculation occurs next. Slowing is attributed to increased permeability of microvasculature that results in increased concentration of red cells, and thus, raised blood viscosity.

5. Stasis or slowing is followed by leucocytic margination or peripheral orientation of leucocytes (mainly neutrophils) along the vascular endothelium. The leucocytes stick to the vascular endothelium briefly, and then move and migrate through the gaps between the endothelial cells into the extravascular space. This process is known as emigration (discussed later in detail).

The features of haemodynamic changes in inflammation are best demonstrated by the Lewis experiment. Lewis induced the changes in the skin of inner aspect of forearm by firm stroking with a blunt point. The reaction so elicited is known as triple response or red line response consisting of the following:

i) Red line appears within a few seconds following stroking and results from local vasodilatation of capillaries and venules.

ii) Flare is the bright reddish appearance or flush surrounding the red-line and results from vasodilatation of the adjacent arterioles.

iii) Wheal is the swelling or oedema of the surrounding skin occurring due to transudation of fluid into the extravascular space.

These features, thus, elicit the classical signs of inflammation – redness, heat, swelling and pain.

Altered vascular permeability. In and around the inflamed tissue, there is accumulation of oedema fluid in the interstitial compartment which comes from blood plasma by its escape through the endothelial wall of peripheral vascular bed. In the initial stage, the escape of fluid is due to vasodilatation and consequent elevation in hydrostatic pressure. This is transudate in nature. But subsequently, the characteristic inflammatory oedema, exudate, appears by increased vascular permeability of microcirculation.

The appearance of inflammatory oedema due to increased vascular permeability of microvascular bed is explained on the basis of Starling's hypothesis. In normal circumstances, the fluid balance is maintained by two opposing sets of forces:

i) Forces that cause outward movement of fluid from microcirculation are intravascular hydrostatic pressure and osmotic pressure of interstitial fluid,

ii) Forces that cause inward movement of interstitial fluid into circulation are intravascular osmotic pressure and hydrostatic pressure of interstitial fluid. Whatever little fluid is left in the interstitial compartment is drained away by lymphatics and, thus, no oedema results normally. However, in inflamed tissues, the endothelial lining of microvasculature becomes leakier. Consequently, intravascular osmotic pressure decreases and osmotic pressure of the interstitial fluid increases resulting in excessive outward flow of fluid into the interstitial compartment which is exudative inflammatory oedema.

Mechanisms of increased vascular permeability: In acute inflammation, normally non-permeable endothelial layer of microvasculature becomes leaky. This is explained by one or more of the following mechanisms:

i) *Contraction of endothelial cells.* This is the most common mechanism of increased leakiness that affects venules exclusively while capillaries and arterioles remain unaffected. The endothelial cells develop temporary gaps between them due to their contraction resulting in vascular leakiness. It is mediated by the release of histamine, bradykinin and other chemical mediators. The response begins immediately after injury, is usually reversible, and is for short duration (15-30 minutes). Example of such immediate transient leakage is mild thermal injury of skin of forearm.

ii) *Retraction of endothelial cells.* In this mechanism, there is structural re-organisation of the cytoskeleton of endothelial cells that causes reversible retraction at the intercellular junctions. This change too affects venules and is mediated by cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF). The onset of response takes 4-6 hours after injury and lasts for 2-4 hours or more (somewhat delayed and prolonged leakage). The example of this type of response exists in vitro experimental work only.

iii) *Direct injury to endothelial cells.* Direct injury to the endothelium causes cell necrosis and appearance of physical gaps at the sites of detached endothelial cells. Process of thrombosis is initiated at the site of damaged endothelial cells. The change affects all levels of microvasculature (venules, capillaries and arterioles). The increased permeability may either appear immediately after injury and last for several hours or days (immediate sus-

tained leakage), or may occur after a delay of 2-12 hours and last for hours or days (delayed prolonged leakage). The examples of immediate sustained leakage are severe bacterial infections while delayed prolonged leakage may occur following moderate thermal injury and radiation injury.

iv) *Endothelial injury mediated by leucocytes.* Adherence of leucocytes to the endothelium at the site of inflammation may result in activation of leucocytes. The activated leucocytes release proteolytic enzymes and toxic oxygen species which may cause endothelial injury and increased vascular leakiness. This form of increased vascular leakiness is a late response. The examples are seen in sites where leucocytes adhere to the vascular endothelium e.g. in pulmonary venules and capillaries.

v) *Other mechanisms.* In addition, the newly formed capillaries during the process of repair are excessively leaky. Vesicles and vacuoles within the cytoplasm of endothelial cells of blood vessels in tumours may account for leakage of fluid across the cytoplasm.

II. Cellular events

The cellular phase of inflammation consists of 2 processes:

1. Exudation of leucocytes; and
2. Phagocytosis.

Exudation of leucocytes. The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important feature of inflammatory response. In acute inflammation, polymorphonuclear neutrophils (PMNs) comprise the first line of body defense, followed later by monocytes and macrophages.

The changes leading to migration of leucocytes are as follows:

1. *Changes in the formed elements of blood.* In the early stage of inflammation, the rate of flow of blood is increased due to vasodilatation. But subsequently, there is slowing or stasis of blood stream. With stasis, changes in the normal axial flow of blood in the microcirculation take place. The normal axial flow consists of central stream of cells comprised by leucocytes and RBCs and peripheral cell-free layer of plasma close to vessel wall. Due to slowing and stasis, the central stream of cells widens and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as margination. As a result of this redis-

tribution, the neutrophils of the central column come close to the vessel wall; this is known as pavementing.

2. *Adhesion or rolling.* Peripherally margined and paved neutrophils stick briefly to the endothelial cells lining the vessel wall or roll over it. Injury leads to neutralisation of the normal negative charge on leucocytes and endothelial cells so as to cause adhesion. The phenomenon of loose and transient adhesions between endothelial cells and leucocytes and later tight adherence of the leucocytes to the vascular endothelium is brought about by 4 types of distinct adhesion molecules.

i) Selectins partake in rolling of PMNs over endothelial surface. These consist of P-selectin (preformed and stored in endothelial cells and platelets), E-selectin (synthesised by cytokine-activated endothelial cells) and L-selectin (also termed Leu-8 antigenase expressed on the surface of lymphocytes and neutrophils).

ii) Addressins expressed on the surface of leucocytes and endothelium and regulate the localisation of subpopulation of leucocytes.

iii) Integrins consisting of ($\beta 1$ and $\beta 2$ molecules bring about firm adhesion between leukocyte and endothelium.

iv) Immunoglobulin superfamily adhesion molecule such as intercellular adhesion molecule (ICAM-1, 2) helps in localising leucocytes to the site of tissue injury and thus helps in transmigration of PMNs.

3. *Emigration.* After sticking of neutrophils to endothelium, the former move along the endothelial surface till a suitable site between the endothelial cells is found where the neutrophils throw out cytoplasmic pseudopods. Subsequently, the neutrophils lodged between the endothelial cells and basement membrane cross the basement membrane by damaging it locally with secreted collagenases and escape out into the extravascular space; this is known as emigration. The damaged basement membrane is repaired almost immediately. As already mentioned, neutrophils are the dominant cells in acute inflammatory exudate in the first 24 hours, and monocyte-macrophages appear in the next 24-48 hours. However, neutrophils are short-lived (24-48 hours) while monocyte-macrophages survive much longer.

Simultaneous to emigration of leucocytes, escape of red cells through gaps between the endothelial cells, diapedesis, takes place. It is a passive phenomenon – RBCs being forced out either by raised hydrostatic pressure or may

escape through the endothelial defects left after emigration of leucocytes. Diapedesis gives haemorrhagic appearance to the inflammatory exudate.

4. *Chemotaxis*. The chemotactic factor-mediated transmigration of leucocytes after crossing several barriers (endothelium, basement membrane, perivascular myofibroblasts and matrix) to reach the interstitial-tissues is called chemotaxis. The concept of chemotaxis is well illustrated by Boyden's chamber experiment. In this, a millipore filter (3 μ m pore size) separates the suspension of leucocytes from the test solution in tissue culture chamber. If the test solution contains chemotactic agent, the leucocytes migrate through the pores of filter towards the chemotactic agent.

The agents acting as potent chemotactic substances for different leucocytes called chemokines are as follows:

- i) Leukotriene B₄ (LTB₄)
- ii) Platelet factor 4 (PF₄)
- iii) Components of complement system (C_{5a} in particular)
- iv) Cytokines (particularly IL-8)
- v) Soluble bacterial products (such as formylated peptides)
- vi) Monocyte chemoattractant protein (MCP-1)
- vii) Chemotactic factor for CD₄ + T cells
- viii) Exotoxin chemotactic for eosinophils.

There are specific receptors for each of the chemoattractants listed above. In addition, chemotactic agents also induce leukocyte activation that includes: the production of arachidonic acid metabolites, degranulation and secretion of lysosomal enzymes, generation of oxygen metabolites, increased intracellular calcium, and increase in leukocyte surface adhesion molecules.

Phagocytosis. Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

- i) Polymorphonuclear neutrophils (PMNs) which appear early in acute inflammatory response, also called as microphages.
- ii) Circulating monocytes and fixed tissue mononuclear phagocytes called as macrophages.

The process of phagocytosis is similar for both polymorphs and macrophages and involves the following 4 steps:

1. Attachment stage (opsonisation).
2. Engulfment stage.

3. Secretion (degranulation) stage.

4. Killing or degradation stage.

1. *Attachment stage (opsonisation)*. The phagocytic cells as well as microorganisms to be ingested have usually negatively-charged surface and, thus, they repel each other. In order to establish a bond between bacteria and the cell membrane of phagocytic cell, the microorganisms get coated with opsonins which are naturally-occurring factors in the serum. The two main opsonins present in the serum and their corresponding receptors on the surface of phagocytic cells are as under:

i) IgG opsonin and its corresponding receptor on the surface of polymorphs and monocytes is Fc fragment of immunoglobulin.

ii) C3b opsonin fragment of complement and corresponding receptor for C3b on the surface of phagocytic cells.

2. *Engulfment stage*. The opsonised particle bound to the surface of phagocyte is ready to be engulfed. This is accomplished by formation of cytoplasmic pseudopods around the particle, enveloping it in a phagocytic vacuole. Eventually, the plasma membrane enclosing the phagocytic vacuole breaks from the cell surface so that membrane lined phagocytic vacuole lies free in the cell cytoplasm. The lysosomes of the cell fuse with the phagocytic vacuole and form phagolysosome or phagosome.

3. *Secretion (degranulation) stage*. During this process, the pre-formed granule-stored products of PMNs are discharged or secreted into the phagosome and the extracellular environment. In particular, the specific or secondary granules of PMNs are discharged (e.g. lysosomes) while the azurophilic granules are fused with phagosomes. Besides the discharge of pre-formed granules, mononuclear phagocytes synthesise and secrete certain enzymes (e.g. interleukin 2 and 6, TNF), arachidonic acid metabolites (e.g. prostaglandins, leukotrienes, platelet activating factor) and oxygen metabolites (e.g. superoxide oxygen, hydrogen peroxide, hypochlorous acid).

4. *Killing or degradation stage*. Next the stage of killing comes and digestion of microorganism completing the role of phagocytes as scavenger cells. The microorganisms after being killed by antibacterial substances are degraded by hydrolytic enzymes. However, this mechanism fails to kill and degrade some bacteria like tubercle bacilli.

The antimicrobial agents act by either of the following mechanisms:

i) Oxygen-dependent bactericidal mechanism;

ii) Oxygen-independent bactericidal mechanism; and

iii) Nitric oxide mechanism.

i) Oxygen-dependent bactericidal mechanism. An important mechanism of microbicidal killing is by the production of reactive oxygen metabolites ($O_2^{\cdot -}$, H_2O_2 , OH^{\cdot} , $HOCl$, HOI , $HOBr$).

A phase of increased oxygen consumption ('respiratory burst') by activated phagocytic leucocytes requires the essential presence of NADPH oxidase.

NADPH-oxidase present in the cell membrane of phagosome reduces oxygen to superoxide ion ($O_2^{\cdot -}$).

This type of bactericidal activity is carried out either via enzyme myeloperoxidase (MPO) present in the granules of neutrophils and monocytes, or independent of enzyme MPO, as under:

a) MPO-dependent killing (HA-MPO-halide system). In this mechanism, the enzyme MPO acts on H_2O_2 in the presence of halides (chloride, iodide or bromide) to form hypohalous acid ($HOCl$, HOI , $HOBr$) which is more potent antibacterial agent than H_2O_2 .

b) MPO-independent killing. Mature macrophages lack the enzyme MPO and they carry out bactericidal activity by producing OH^{\cdot} ions and superoxide singlet oxygen (O^{\cdot}) from H_2O_2 in the presence of $O_2^{\cdot -}$ (Haber-Weiss reaction) or in the presence of Fe^{++} (Fenton reaction).

Reactive oxygen metabolites are particularly useful in eliminating microbial organisms that grow within phagocytes e.g. *M. tuberculosis*, *Histoplasma capsulatum*.

ii) Oxygen-independent bactericidal mechanism. Some agents released from the granules of phagocytic cells do not require oxygen for bactericidal activity. These include lysosomal hydrolases, permeability increasing factors, defensins and cationic proteins.

iii) Nitric oxide mechanism. In addition to oxygen-dependent and oxygen-independent mechanisms, recently role of nitric oxide (NO) in inflammatory reaction has been emphasised. NO is produced by endothelial cells as well as by activated macrophages. In experimental animals, NO has been shown to have fungicidal and anti-parasitic action but its role in bactericidal activity in human beings is yet not clear.

Chemical mediators of inflammation. Also called as permeability factors or endogenous mediators of increased vascular permeability, these are a large and increasing number of endogenous compounds which can enhance vascular permeability. However, currently many chemical mediators

have been identified which partake in other processes of acute inflammation as well e.g. vasodilatation, chemotaxis, fever, pain and cause tissue damage.

The substances acting as chemical mediators of inflammation may be released from the cells, the plasma, or damaged tissue itself. They are broadly classified into 2 groups:

- i) mediators released by cells; and
- ii) mediators originating from plasma.

I. Cell-derived mediators

1. *Vasoactive amines*. Two important pharmacologically active amines that have role in the early inflammatory response (first one hour) are histamine and 5-hydroxytryptamine (5-HT) or serotonin.

i) Histamine. It is stored in the granules of mast cells, basophils and platelets. Histamine is released from these cells by various agents like:

- a) Stimuli or substances inducing acute inflammation e.g. heat, cold, irradiation, trauma, irritant chemicals, immunologic reactions etc.
- b) Anaphylatoxins like fragments of complement C3a, and C-a, which increase vascular permeability and cause oedema in tissues.
- c) Histamine-releasing factors from neutrophils, monocytes and platelets.
- d) Neuropeptides such as substance P.
- e) Interleukins.

The main actions of histamine are: vasodilatation, increased vascular (venular) permeability, itching and pain. Stimulation of mast cells and basophils also releases products of arachidonic acid metabolism including the release of slow-reacting substances of anaphylaxis (SRS-As). The SRS-As consist of various leukotrienes (LTC₄, LTD₄ and LTE₄).

ii) 5-Hydroxytryptamine (5-HT or serotonin). It is present in tissues like chromaffin cells of GIT, spleen, nervous tissue, mast cells and platelets. The actions of 5-HT are similar to histamine but it is a less potent mediator of increased vascular permeability and vasodilatation than histamine.

2. *Arachidonic acid metabolites*. Arachidonic acid is a fatty acid, eicosatetraenoic acid, and its 2 main sources are:

- from diet directly; and
- conversion of essential fatty acid, linoleic acid to arachidonic acid.

Arachidonic acid must be first activated by stimuli or other mediators like C5a so as to form arachidonic acid metabolites by one of the following 2 pathways: via cyclo-oxygenase pathway and via lipo-oxygenase pathway.

i) Metabolites via cyclo-oxygenase pathway (prostaglandins, thromboxane A₂, prostacyclin). The name 'prostaglandin' was first given to a substance found in human seminal fluid but now the same substance has been isolated from a number of other body tissues. Prostaglandins and related compounds are also called autocooids.

Cyclo-oxygenase is a fatty acid enzyme which acts on activated arachidonic acid to form prostaglandin endoperoxide (PGG₂). PGG₂ is enzymatically transformed into PGH₂ with generation of free radical of oxygen. PGH₂ is further acted upon by enzymes and results in formation of the following 3 metabolites:

a) Prostaglandins (PGD₂, PGE₂ and PGF₂- α). PGD₂ and PGE₂ act on blood vessels to cause increased venular permeability, vasodilatation and bronchodilatation and inhibit inflammatory cell function. PGF₂- α induces vasodilatation and bronchoconstriction.

b) Thromboxane A₂ (TXA₂). It is a vasoconstrictor and bronchoconstrictor and enhances inflammatory cell function by causing platelet aggregation.

c) Prostacyclin (PGI₂). PGI₂ induces vasodilatation, bronchodilatation and inhibits inflammatory cell function by acting as anti-aggregating agent for platelets.

ii) Metabolites via lipo-oxygenase pathway (5-HETE, leukotrienes). The enzyme, lipo-oxygenase, acts on activated arachidonic acid to form hydroperoxy compound, 5-HPETE (hydroperoxy eicosatetraenoic acid) which on further peroxidation forms the following 2 metabolites:

a) 5-HETE (hydroxy compound) which is a potent chemotactic agent for neutrophils.

b) Leukotrienes (LT) or slow-reacting substances of anaphylaxis (SRS-As) are so named as they were first isolated from leucocytes. Firstly, unstable Leukotriene A₄ (LTA₄) is formed which is acted upon by enzymes to form LTB₄ (chemotactic for phagocyte cells and stimulates phagocyte cell adherence) while LTC₄, LTD₄ and LTE₄ have common actions by causing smooth muscle contraction and thereby induce vasoconstriction, bronchoconstriction and increased vascular permeability.

3. *Lysosomal components.* The inflammatory cells – neutrophils and monocytes, contain lysosomal granules which on release elaborate a variety of mediators of inflammation. These are as under:

i) Granules of neutrophils. These are of two types: specific or secondary, and azurophil or primary. The specific granules contain lactoferrin, lysozyme, alkaline phosphatase and collagenase while the large azurophil granules have myeloperoxidase, acid hydrolases and neutral proteases such as elastase, collagenase and proteinase.

Acid proteases act within the cell to cause destruction of bacteria in phagolysosome while neutral proteases attack on the extracellular constituents such as basement membrane, collagen, elastin, cartilage etc.

However, degradation of extracellular components like collagen, basement membrane, fibrin and cartilage by proteases results in harmful tissue destruction which is kept in check by antiproteases like α 1-antitrypsin and α 2-macroglobulin.

ii) Granules of monocytes and tissue macrophages. These cells on degranulation also release mediators of inflammation like acid proteases, collagenase, elastase and plasminogen activator. However they are more active in chronic inflammation than acting as mediators of acute inflammation.

4. *Platelet activating factor (PAF).* It is released from IgE-sensitised basophils or mast cells, other leucocytes, endothelium and platelets. Apart from its action on platelet aggregation and release reaction, the actions of PAF as mediator of inflammation are:

- increased vascular permeability;
- vasodilatation in low concentration and vasoconstriction otherwise;
- bronchoconstriction;
- adhesion of leucocytes to endothelium; and
- chemotaxis.

5. *Cytokines.* Cytokines are polypeptide substances produced by activated lymphocytes (lymphokines) and activated monocytes (monokines). These agents may act on 'self cells producing them or on other cells. Currently, main cytokines acting as mediators of inflammation are: interleukin- 1 (IL-1), tumour necrosis factor (TNF) α and β interferon (IF)- γ , and chemokines (IL-8, PF-4).

IL-1 and TNF- α are formed by activated macrophages while TNF- β and IF- γ are produced by activated T cells. The chemokines include interleukin 8 (released from activated macrophages) and platelet factor-4 from activated platelets, both of which are potent chemoattractant for inflammatory cells and hence their name.

The actions of various cytokines as mediator of inflammation are as under:

i) IL-1 and TNF- α , TNF- β induce endothelial effects in the form of increased leucocyte adherence, thrombogenicity, elaboration of other cytokines, fibroblastic proliferation and acute phase reactions.

ii) IF- γ causes activation of macrophages and neutrophils and is associated with synthesis of nitric acid synthase.

iii) Chemokines are a family of chemoattractants for inflammatory cells and include

- IL-8 chemotactic for neutrophils;
- platelet factor-4 chemotactic for neutrophils, monocytes and eosinophils;
- MCP-1 chemotactic for monocytes; and
- eotaxin chemotactic for eosinophils.

6. *Nitric oxide and oxygen metabolites.* Nitric oxide (NO) was originally described as vascular relaxation factor produced by endothelial cells. It has recently been included as a mediator of inflammatory responses since activated macrophages also produce NO during the oxidation of arginine by the action of enzyme, NO synthase.

NO plays the following role in inflammation:

- Vasodilatation
- Anti-platelet activating agent
- Possibly microbicidal action. Oxygen-derived metabolites are released from activated neutrophils and macrophages and include superoxide oxygen (O'²), H₂O₂, OH' and toxic NO products. These oxygen-derived free radicals have the following action in inflammation:

- Endothelial cell damage and thereby increased vascular permeability.
- Activation of protease and inactivation of antiprotease causing tissue matrix damage.
- Damage to other cells.

The actions of free radicals are counteracted by antioxidants present

in tissues and serum which play a protective role.

II. Plasma-derived mediators (Plasma proteases)

These include the various products derived from activation and interaction of 4 interlinked systems: kinin, clotting, fibrinolytic and complement. Each of these systems has its inhibitors and accelerators in plasma with negative and positive feedback mechanisms respectively.

Hageman factor (factor XII) of clotting system plays a key role in interactions of the four systems. Activation of factor XII in vivo by contact with basement membrane and bacterial end toxins, and in vitro with glass or kaolin, leads to activation of clotting, fibrinolytic and kinin systems. In inflammation, activation of factor XII is brought about by contact of the factor leaking through the endothelial gaps. The end-products of the activated clotting, fibrinolytic and kinin systems activate the complement system that generate permeability factors. These permeability factors, in turn, further activate clotting system.

The inter-relationship between 4 systems.

1. *The kinin system.* This system on activation by factor XIIa generates bradykinin, so named because of the slow contraction of smooth muscle it induces. First, kallikrein is formed from plasma prekallikrein by the action of prekallikrein activator which is a fragment of factor XIIa. Kallikrein then acts on high molecular weight kininogen to form bradykinin.

Bradykinin acts in the early stage of inflammation and its effects include:

- smooth muscle contraction;
- vasodilatation;
- increased vascular permeability; and
- pain.

2. *The clotting system.* Factor XIIa initiates the cascade of the clotting system resulting in formation of fibrinogen which is acted upon by thrombin to form fibrin and fibrinopeptides.

The actions of fibrinopeptides in inflammation are:

- increased vascular permeability;
- chemotaxis for leucocyte; and
- anticoagulant activity.

3. *The fibrinolytic system.* This system is activated by plasminogen activator, the sources of which include kallikrein of the kinin system, endo-

thelial cells and leucocytes. Plasminogen activator acts on plasminogen present as component of plasma proteins to form plasmin. Further breakdown of fibrin by plasmin forms fibrinopeptides or fibrin split products.

The actions of plasmin in inflammation are:

- activation of factor XII to form prekallikrein activator that stimulates the kinin system to generate bradykinin;
- splits off complement C3 to form C3a which is a permeability factor; and
- degrades fibrin to form fibrin split products which increase vascular permeability and are chemotactic to leucocytes.

4. *The complement system.* The activation of complement system can occur either:

- i) by classic pathway through antigen-antibody complexes; or
- ii) by alternate pathway via non-immunologic agents such as bacterial toxins, cobra venoms and IgA.

Complement system on activation by either of these two pathways yields anaphylatoxins C3a, C4a and C5a, and membrane attack complex (MAC). The relative potencies of anaphylatoxins are in the descending sequence of C3a, C5a and C4a.

The actions of anaphylatoxins in inflammation are:

- release of histamine from mast cells and basophils;
- increased vascular permeability causing oedema in tissues;
- C3b augments phagocytosis; and
- C5a is chemotactic for leucocytes.

The action of MAC is to cause pores in the cell membrane of the invading microorganisms.

Regulation of inflammation. The onset of inflammatory responses outlined above may have potentially damaging influence on the host tissues as evident in hypersensitivity conditions. Such self-damaging effects are kept in check by the host mechanisms so as to resolve inflammation. These include the following mechanisms:

i) *Acute phase proteins.* A variety of acute phase proteins (APP) are released in plasma in response to tissue trauma and infection. These include: α 1-anti-trypsin, α 1-acid glycoprotein, protease inhibitor, haptoglobin, C-reactive protein, serum amyloid A and P component etc. The APP are synthesised in the liver and their release occurs in response to circulating

cytokines produced during inflammation. APP combined with systemic features of fever and leucocytosis is termed 'acute phase response'. Deficient synthesis of APP leads to severe form of disease in chronic and repeated inflammatory responses.

ii) *Corticosteroids*. The endogenous glucocorticoids act as anti-inflammatory agents. Their levels are raised in infection and trauma by self-regulating mechanism.

iii) *Free cytokine receptors*. The presence of free receptors for cytokines in the serum correlates directly with disease activity.

iv) *Suppressor T cells*. A prohibition of suppressor T cells is seen which inhibits the function of T and B cells.

v) *Anti-inflammatory chemical mediators*. As already described, PGE₂ and prostacyclin have both pro-inflammatory as well as anti-inflammatory actions.

Inflammatory cells. The cells participating in acute and chronic inflammation are circulating leucocytes, plasma cells and tissue macrophages.

1. *Polymorphonuclear neutrophils (PMN)*. Commonly called as neutrophils or polymorphs, these cells along with basophils and eosinophils are known as granulocytes due to the presence of granules in the cytoplasm. These granules contain many substances like proteases, myeloperoxidase, lysozyme, esterase, arylsulfatase, acid and alkaline phosphatase, and cationic proteins. The diameter of neutrophils ranges from 10 to 15 μm and are actively motile. These cells comprise 40-75% of circulating leucocytes and their number is increased in blood (neutrophilia) and tissues in acute bacterial infections. These cells arise in the bone marrow from stem cells.

The functions of neutrophils in inflammation are as follows:

i) Initial phagocytosis of microorganisms as they form the first line of body defense in bacterial infection. The steps involved are adhesion of neutrophils to vascular endothelium, emigration through the vessel wall, chemotaxis, engulfment, degranulation, killing and degradation of the foreign material.

ii) Engulfment of antigen-antibody complexes and non-microbial material.

iii) Harmful effect of neutrophils is destruction of the basement membranes of glomeruli and small blood vessels.

2. *Eosinophils*. These are larger than neutrophils but are fewer in number, comprising 1 to 6% of total blood leucocytes. Eosinophils share many structural and functional similarities with neutrophils like their production in the bone marrow, locomotion, phagocytosis, lobed nucleus and presence of granules in the cytoplasm containing a variety of enzymes, of which major basic protein and eosinophil cationic protein are the most important which have bactericidal and toxic action against helminthic parasites. However, granules of eosinophils are richer in myeloperoxidase than neutrophils and lack lysozyme. High level of steroid hormones leads to fall in number of eosinophils and even disappearance from blood.

The absolute number of eosinophils is increased in the following conditions and, thus, they partake in inflammatory responses associated with these conditions:

- i) allergic conditions;
- ii) parasitic infestations;
- iii) skin diseases; and
- iv) certain malignant lymphomas.

3. *Basophils (Mast Cells)*. The basophils comprise about 1% of circulating leucocytes and are morphologically and pharmacologically similar to mast cells of tissue. These cells contain coarse basophilic granules in the cytoplasm and a polymorphonuclear nucleus. These granules are laden with heparin and histamine. Basophils and mast cells have receptors for IgE and degranulate when cross-linked with antigen.

The role of these cells in inflammation is:

- i) in immediate and delayed type of hypersensitivity reactions; and
- ii) release of histamine by IgE-sensitised basophils.

4. *Lymphocytes*. Next to neutrophils, these cells are most numerous of the circulating leucocytes (20-45%). Apart from blood, lymphocytes are present in large numbers in spleen, thymus, lymph nodes and mucosa-associated lymphoid tissue (MALT). They have scanty cytoplasm and consist almost entirely of nucleus.

Besides their role in antibody formation (B lymphocytes) and in cell-mediated immunity (T lymphocytes), these cells participate in the following types of inflammatory responses:

- i) In tissues, they are dominant cells in chronic inflammation and late stage of acute inflammation.

ii) In blood, their number is increased (lymphocytosis) in chronic infections like tuberculosis.

5. *Plasma Cells*. These cells are larger than lymphocytes with more abundant cytoplasm and an eccentric nucleus which has cart-wheel pattern of chromatin. Plasma cells are normally not seen in peripheral blood. They develop from lymphocytes and are rich in RNA and γ -globulin in their cytoplasm. There is interrelationship between plasmacytosis and hyper-globulinaemia. These cells are most active in antibody synthesis.

Their number is increased in:

i) prolonged infection with immunological responses e.g. in syphilis, rheumatoid arthritis, tuberculosis;

ii) hypersensitivity states; and

iii) multiple myeloma.

6. *Mononuclear-phagocyte system (Reticuloendothelial system)*. This cell system includes cells derived from 2 sources with common morphology, function and origin. These are as under:

i) Blood monocytes. These comprise 4-8% of circulating leucocytes.

ii) Tissue macrophages. These include the following cells in different tissues:

a) Macrophages in inflammation,

b) Histiocytes which are macrophages present in connective tissues,

c) Kupffer cells which are macrophages of liver,

d) Alveolar macrophages present in lungs,

e) Free and fixed macrophages and sinusoidal lining cells of spleen, lymph node and bone marrow,

f) Macrophages of serous cavities,

g) Microglial cells of nervous system,

i) Langerhans' cells of skin,

j) Dendritic cells found in lymphoid tissue.

The mononuclear phagocytes are the scavenger cells of the body as well as participate in immune system of the body.

Role of macrophages in inflammation. The functions of mononuclear-phagocyte cells are as under:

i) Phagocytosis (cell eating) and pinocytosis (cell drinking).

ii) Macrophages on activation by lymphokines released by T lymphocytes or by non-immunologic stimuli elaborate a variety of biologically active substances such as:

- a) Proteases like collagenase and elastase which degrade collagen and elastic tissue.
- b) Plasminogen activator which activates the fibrinolytic system.
- c) Products of complement.
- d) Some coagulation factors (factor V and thromboplastin) which convert fibrinogen to fibrin.
- e) Chemotactic agents for other leucocytes.
- f) Metabolites of arachidonic acid.
- g) Growth promoting factors for fibroblasts, blood vessels and granulocytes.
- h) Cytokines like interleukin-1 and tumour necrosis factor,
- i) Oxygen-derived free radicals.

7. *Giant Cells.* When the macrophages fail to deal with particles to be removed, they fuse together and form multinucleated giant cells. Various types of giant cells seen in inflammation and in certain tumours are described below:

i) Foreign body giant cells. These contain numerous nuclei (up to 100) which are uniform in size and shape and resemble the nuclei of macrophages. These nuclei are scattered throughout the cytoplasm.

These are seen in chronic infective granulomas, leprosy and tuberculosis.

ii) Langhans' giant cells. These are seen in tuberculosis and sarcoidosis. Their nuclei are like the nuclei of macrophages and epithelioid cells. These nuclei are arranged either around the periphery in the form of horse-shoe or ring, or are clustered at the two poles of the giant cell.

iii) Teuton giant cells. These multinucleated cells have vacuolated cytoplasm due to lipid content e.g. in xanthoma.

iv) Tumour giant cells. These are larger, have numerous nuclei which are hyperchromatic and vary in size and shape. These giant cells are not derived from macrophages but are formed from dividing nuclei of the neoplastic cells e.g. carcinoma of liver, various soft tissue sarcomas etc.

v) Miscellaneous types. These include presence of numerous nuclei in mesodermal cells e.g. Aschoff cells of rheumatic nodule, Reed-Sternberg

cells of Hodgkin's disease, osteoclasts. These are described later in respective chapters to which they pertain.

Factors determining variation in inflammatory response. Although acute inflammation is typically characterised by vascular and cellular events with emigration of neutrophilic leucocytes, not all examples of acute inflammation show infiltration by neutrophils, and vice versa some chronic inflammatory conditions are characterised by neutrophilic infiltration. For example, typhoid fever is an example of acute inflammatory process but the cellular response in it is lymphocytic; osteomyelitis is an example of chronic inflammation but the cellular response in this condition is mainly neutrophilic.

The morphologic variation in inflammation depends upon a number of factors and processes. These are discussed below:

1. Factors involving the organisms:

i) *Type of injury and infection.* For example, skin reacts to herpes simplex infection by formation of vesicle and to streptococcal infection by formation of boil; lung reacts to pneumococci by occurrence of lobar pneumonia while to tubercle bacilli it reacts by glaucomatous inflammation.

ii) *Virulence.* Many species and strains of organisms may have varying virulence e.g. the three strains of *C. diphtheriae* (gravis, intercedes and mites) produce the same diphtheria serotoxin but in different amount.

iii) *Dose.* The concentration of organism in small doses produces usually local lesions while larger dose results in more severe spreading infections.

iv) *Portal of entry.* Some organisms are infective only if administered by particular route e.g. *Vibrio cholerae* is not pathogenic if injected subcutaneously but causes cholera if swallowed.

v) *Product of organisms.* Some organisms produce enzymes that help in spread of infections e.g. hyaluronidase by *Cl. welchii*, streptokinase by Streptococci, staphylokinase and coagulase by Staphylococci.

2. Factors involving the host:

i) *General health of host.* For example, starvation, haemorrhagic shock, chronic debilitating diseases like diabetes mellitus, alcoholism etc render the host more susceptible to infections.

ii) *Immune state of host.* Immunodeficiency helps in spread of infections rapidly e.g. in AIDS.

iii) *Leukopenia*. Patients with low WBC count with neutropenia or agranulocytosis develop spreading infection.

iv) *Site or type of tissue involved*. For example, the lung has loose texture as compared to bone and, thus, both tissues react differently to acute inflammation.

v) *Local host factors*. For instance, ischaemia, presence of foreign bodies and chemicals cause necrosis and are thus harmful.

3. Type of exudation:

The appearance of escaped plasma determines the morphologic type of inflammation. These are:

i) *Catarrhal*, when the surface inflammation of epithelium produces increased secretion of mucus e.g. common cold. Thus it develops only in the mucous membranes.

ii) *Serous*, when the fluid exudate resembles serum or is watery e.g. pleural effusion in tuberculosis, blister formation in burns.

iii) *Fibrinous*, when the fibrin content of the fluid exudate is high e.g. in pneumococcal and rheumatic pericarditis. Depending on the type of epithelium (monolayer or multi-layer) and depth of necrosis two forms of fibrinous inflammation are discussed: croupous, or simple, and diphtheritic.

iv) *Purulent, or suppurative* exudate is formation of creamy pus

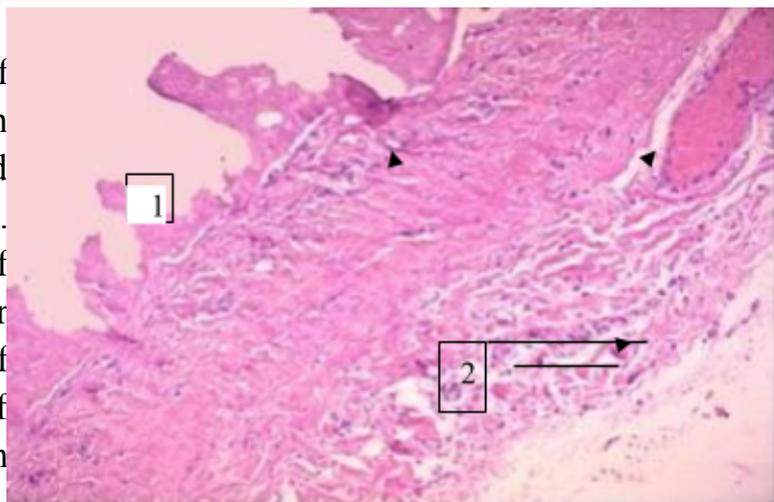


Fig. 21. Fibrinous pericarditis. H and E stained section, x 200.

1 – deposition of fibrin, 2- inflammatory infiltration and hyperemia of pericardium.

as seen in infection with pyogenic bacteria e.g. abscess, acute appendicitis. There are several types of purulent inflammation:

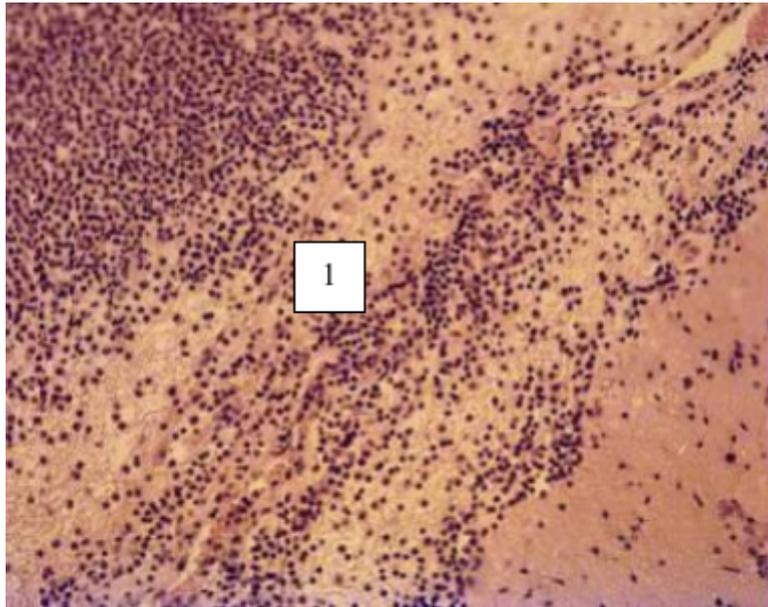


Рис. 22. Purulent meningitis. H and E stained section, x 200.

1 - inflammatory infiltration and hyperemia of meningeal coat.

Abscess is a localized collection of pus in a tissue or organ. Abscess is bounded purulent inflammation in which a cavity with pus is formed.

The bacteria which cause suppuration are called pyogenic. Pus is creamy or opaque in appearance and is composed of numerous dead as well as living neutrophils, some red cells, fragments of tissue debris and fibrin. In old pus, macrophages and cholesterol crystals are also present. The wall of abscess is called pyogenic membrane. An abscess

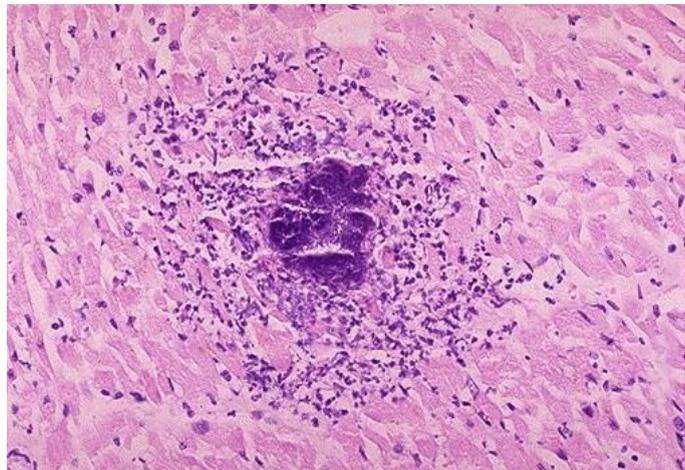


Fig. 23. Abscess in the myocardium. H and E stained section, x 200.

may be discharged to the surface due to increased pressure inside or may require drainage by the surgeon. Due to tissue destruction, resolution does not occur but instead healing by fibrous scarring takes place.

Phlegmon is unbounded purulent inflammation in which pus spreads diffusely between different components of tissue owing to fusion and tissue

lysis. Phlegmon frequently occurs along the muscular bands, tendons, fascias, vascular-nerves bands and in subcutaneous fat. Two types of phlegmon have been described: soft and dense.

If purulent exudate appears in the human cavities it is called *empyema*. tissues.

Furuncle is an acute inflammation via hair follicles in the dermal

Carbuncle is seen in untreated diabetics and occurs as a located abscess in the dermis and soft tissues of the neck.

v) Haemorrhagic, when there is a vascular damage e.g. acute haemorrhagic pneumonia in influenza.

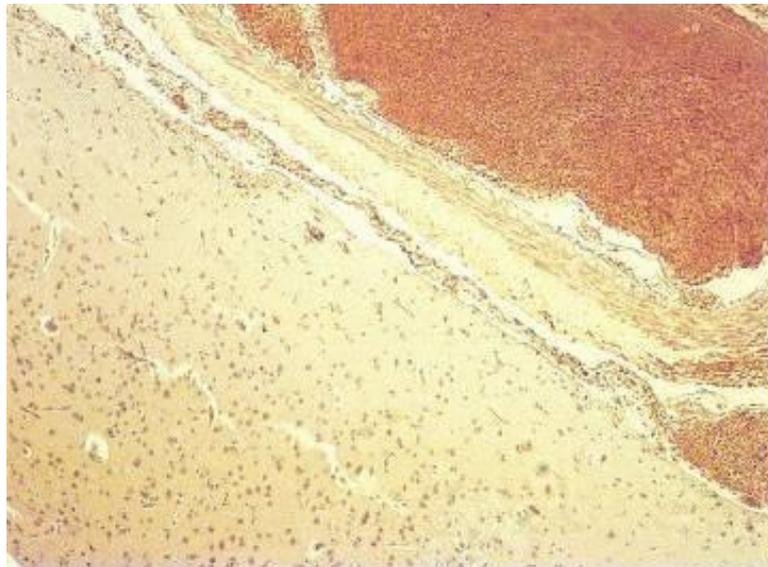


Рис. 24. acute haemorrhagic inflammation of meningeal coat in influenza. H and E stained section, x 200.

vi) Putrefactive, when inflammatory reaction results from action of putrefactive bacteria.

vii) Mixed, when exudate contains different components.

4. Cellular proliferation

Variable cellular proliferation is seen in different types of inflammations.

i) There is no significant cellular proliferation in acute bacterial infections except in typhoid fever in which there is intestinal lymphoid hyperplasia.

ii) Viral infections have the ability to stimulate cellular proliferation e.g. epidermal cell proliferation in herpes simplex, chickenpox and smallpox.

iii) In rapidly progressive glomerulonephritis, there is proliferation of glomerular capsular epithelial cells resulting in formation of 'crescents'.

iv) In chronic inflammation, cellular proliferation of macrophages, fibroblasts and endothelial cells occurs.

5. *Necrosis*

The extent and type of necrosis in inflammation is variable.

i) In gas gangrene, there is extensive necrosis with discoloured and foul smelling tissues.

ii) In acute appendicitis, there is necrosis as a result of vascular obstruction.

iii) In chronic inflammation such as tuberculosis, there is characteristic caseous necrosis.

Morphology of acute inflammation. Inflammation of an organ is usually named by adding the suffix “*itis*” to its Latin name e.g. appendicitis, hepatitis, cholecystitis, meningitis etc. A few morphologic varieties of acute inflammation are described below:

1. *Pseudomembranous inflammation.* It is inflammatory response of mucous surface (oral, respiratory, bowel) to toxins of diphtheria or irritant gases. As a result of denudation of epithelium, plasma exudes on the surface where it coagulates, and together with necrosed epithelium, forms false membrane that gives this type of inflammation its name.

2. *Ulcer.* Ulcers are local defects on the surface of an organ produced by inflammation. Common sites for ulcerations are the stomach, duodenum, intestinal ulcers in typhoid fever, intestinal tuberculosis, bacillary and amoebic dysentery, ulcers of legs due to varicose veins etc. In the acute stage, there is infiltration by polymorphs with vasodilatation while long-standing ulcers develop infiltration by lymphocytes, plasma cells and macrophages with associated fibroblastic proliferation and scarring.

3. *Suppuration (abscess formation).* When acute bacterial infection is accompanied by intense neutrophilic infiltrate in the inflamed tissue, it results in tissue necrosis. A cavity is formed which is called an abscess and contains purulent exudate or pus and the process of abscess formation is known as suppuration. The bacteria which cause suppuration are called pyogenic.

Microscopically, pus is creamy or opaque in appearance and is composed of numerous dead as well as living neutrophils, some red cells, fragments of tissue debris and fibrin.

An abscess may be discharged to the surface due to increased pressure inside or may require drainage by the surgeon. Due to tissue destruction, resolution does not occur but instead healing by fibrous scarring takes place.

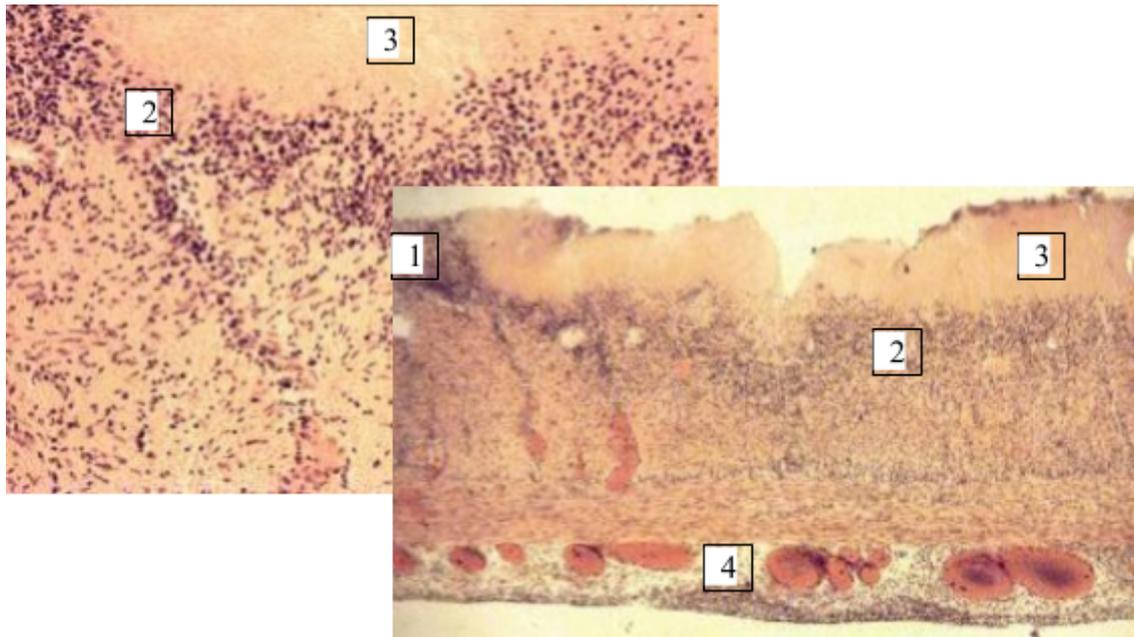


Рис. 25. Changes of the gut wall at necrotic stage. H and E stained section, x 200, x100.

1. border of ulcer, 2. diffuse inflammatory infiltration, 3. necrotic zone, 4. hyperemia of blood vessels.

Some of the common examples of abscess formation are as under:

i) Boil or furuncle which is an acute inflammation via hair follicles in the dermal tissues.

ii) Carbuncle is seen in untreated diabetics and occurs as a loculated abscess in the dermis and soft tissues of the neck.

4. *Cellulitis*. It is a diffuse inflammation of soft tissues resulting from spreading effects of substances like hyaluronidase released by some bacteria.

5. *Bacterial infection of the blood*. This includes the following 3 conditions:

i) Bacteraemia is defined as presence of small number of bacteria in the blood which do not multiply significantly. They are commonly not detected by direct microscopy. Blood culture is done for their detection e.g. infection with *Salmonella typhi*, *Escherichia coli*, *Streptococcus viridans*.

ii) Septicaemia means presence of rapidly multiplying, highly pathogenic bacteria in the blood e.g. pyogenic cocci, bacilli of plague etc. Septicaemia is generally accompanied by systemic effects like toxemia, multiple small haemorrhages, neutrophilic leucocytosis and disseminated intravascular coagulation (DIC).

iii) Pyaemia is the dissemination of small septic thrombi in the blood which cause their effects at the site where they are lodged. This can result in pyaemic abscesses or septic infarcts.

a) Pyaemic abscesses are multiple small abscesses in various organs such as in cerebral cortex, myocardium, lungs and renal cortex, resulting from very small emboli fragmented from septic thrombus. Microscopy of pyaemic abscess shows a central zone of necrosis containing numerous bacteria, surrounded by a zone of suppuration and an outer zone of acute inflammatory cells.

b) Septic infarcts result from lodgement of larger fragments of septic thrombi in the arteries with relatively larger foci of necrosis, suppuration and acute inflammation e.g. septic infarcts of the lungs, liver, brain, and kidneys from septic thrombi of leg veins or from acute bacterial endocarditis.

Systemic effects of acute inflammation. The account of acute inflammation given up to now above is based on local tissue responses. However, acute inflammation is associated with systemic effects as well. These include fever, leucocytosis and lymphangitis-lymphadenitis.

1. *Fever occurs due to bacteraemia.* It is thought to be mediated through release of factors like prostaglandins, interleukin-1 and tumour necrosis factor in response to infection.

2. *Leucocytosis* commonly accompanies the acute inflammatory reactions, usually of the range of 15,000-20,000/ μ l. When the counts are higher than this with 'shift to left' of myeloid cells, the blood picture is described as leukaemoid reaction. Usually, in bacterial infections there is neutrophilia; in viral infections lymphocytosis; and in parasitic infestations, eosinophilia. Typhoid fever, an example of acute inflammation, however, induces leucopenia with relative lymphocytosis.

3. *Lymphangitis-lymphadenitis* is one of the important manifestations of localised inflammatory injury. The lymphatics and lymph nodes that drain the inflamed tissue show reactive inflammatory changes in the form of lymphangitis and lymphadenitis. This response represents either a nonspecific

reaction to mediators released from inflamed tissue or is an immunologic response to a foreign antigen. The affected lymph nodes may show hyperplasia of lymphoid follicles (follicular hyperplasia) and proliferation of mononuclear phagocytic cells in the sinuses of lymph node (sinus histiocytosis).

4. *Shock may occur in severe cases.* Massive release of cytokine TNF- α , a mediator of inflammation, in response to severe tissue injury or infection results in profuse systemic vasodilatation, increased vascular permeability and intravascular volume loss. The net effect of these changes is hypotension and shock. Systemic activation of coagulation pathway may occur leading to microthrombi throughout the body and result in disseminated intravascular coagulation (DIC), bleeding and death.

Fate of acute inflammation. The acute inflammatory process can culminate in one of the following 4 outcomes:

1. Resolution,
2. Healing by scarring,
3. Progression to suppuration,
4. Progression to chronic inflammation.

1. *Resolution.* It means complete return to normal tissue following acute inflammation. This occurs when tissue changes are slight and the cellular changes are reversible e.g. resolution in lobar pneumonia.

2. *Healing by scarring.* This takes place when the tissue destruction in acute inflammation is extensive so that there is no tissue regeneration but actually there is healing by fibrosis.

3. *Suppuration.* When the pyogenic bacteria causing acute inflammation result in severe tissue necrosis, the process progresses to suppuration. Initially, there is intense neutrophilic infiltration. Subsequently, mixture of neutrophils, bacteria, fragments of necrotic tissue, cell debris and fibrin comprise pus which is contained in a cavity to form an abscess. The abscess, if not drained, may get organised by dense fibrous tissue, and in time, get calcified.

4. *Chronic inflammation.* The acute inflammation may progress to chronic inflammation in which the processes of inflammation and healing proceed side by side.

CHRONIC (PRODUCTIVE, PROLIFERATIVE) INFLAMMATION

Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same time. Chronic inflammation can be caused by one of the following 3 ways:

1. *Chronic inflammation following acute inflammation.* When the tissue destruction is extensive, or the bacteria survive and persist in small numbers at the site of acute inflammation e.g. in osteomyelitis, pneumonia terminating in lung abscess.

2. *Recurrent attacks of acute inflammation.* When repeated bouts of acute inflammation culminate in chronicity of the process e.g. in recurrent urinary tract infection leading to chronic pyelonephritis, repeated acute infection of gall bladder leading to chronic cholecystitis.

3. *Chronic inflammation starting de novo.* When the infection with organisms of low pathogenicity is chronic from the beginning e.g. infection with *Mycobacterium tuberculosis*.

There are three types of productive inflammation:

- 1) Interstitial;
- 2) Granulomatous;
- 3) Inflammation with the formation of polyps and pointed condylomas.

General features of chronic inflammation. Though there may be differences in chronic inflammatory response depending upon the tissue involved and causative organisms, there are some basic similarities amongst various types of chronic inflammation. These general features characterise any chronic inflammation.

1. *Mononuclear cell infiltration.* Chronic inflammatory lesions are infiltrated by mononuclear inflammatory cells like phagocytes and lymphoid cells. Phagocytes are represented by circulating monocytes, tissue macrophages, epithelioid cells and sometimes, multinucleated giant cells. The macrophages comprise the most important cells in chronic inflammation. These may appear at the site of chronic inflammation from:

- i) chemotactic factors for macrophages as already described;
- ii) local proliferation of macrophages; and
- iii) longer survival of macrophages at the site of inflammation.

The blood monocytes on reaching the extravascular space transform into tissue macrophages. Besides the role of macrophages in phagocytosis,

they may get activated in response to stimuli such as cytokines (lymphokines) and bacterial endotoxins. On activation, macrophages release several biologically active substances e.g. acid and neutral proteases, oxygen-derived reactive metabolites and cytokines. These products bring about tissue destruction, neovascularisation and fibrosis.

Other chronic inflammatory cells include lymphocytes, plasma cells, eosinophils and mast cells. In chronic inflammation, lymphocytes and macrophages influence each other and release mediators of inflammation.

2. *Tissue destruction or necrosis.* Tissue destruction and necrosis are common in many chronic inflammatory lesions and are brought about by activated macrophages by release of a variety of biologically active substances.

3. *Proliferative changes.* As a result of necrosis, proliferation of small blood vessels and fibroblasts is stimulated resulting in formation of inflammatory granulation tissue. Eventually, healing by fibrosis and collagen laying takes place.

Types of chronic inflammation. Conventionally, chronic inflammation is subdivided into 2 types:

1. *Nonspecific*, when the irritant substance produces a non-specific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.

2. *Specific*, when the injurious agent causes a characteristic histological tissue response e.g. tuberculosis, leprosy, syphilis.

However, for a more descriptive classification, histological features are used for classifying chronic inflammation into 2 corresponding types:

1. *Chronic nonspecific interstitial inflammation.* It is characterised by nonspecific inflammatory cell infiltration e.g. chronic osteomyelitis, lung abscess. A variant of this type of chronic inflammatory response is chronic suppurative inflammation in which infiltration by polymorphs and abscess formation are additional features e.g. actinomycosis. The inflammatory cell infiltration consists of lymphocytes, monocytes, plasmocytes, eosinophils and other cells.

2. *Chronic granulomatous inflammation.* It is characterised by formation of granulomas e.g. tuberculosis, leprosy, syphilis, actinomycosis, sarcoidosis etc.

GRANULOMATOUS INFLAMMATION

Granuloma is defined as a circumscribed, tiny lesion, about 1 mm in diameter, composed predominantly of collection of modified macrophages called epithelioid cells, and rimmed at the periphery by lymphoid cells. The word 'granuloma' is derived from granule meaning circumscribed granule-like lesion, and -oma which is a suffix commonly used for true tumours but here indicates inflammatory mass or collection of macrophages. Epithelioid cells, so called because of their epithelial cell-like appearance, are modified macrophages which are somewhat elongated, having pale-staining abundant cytoplasm, lightly-staining slipper-shaped nucleus and the cell membrane of adjacent epithelioid cells is closely apposed. Epithelioid cells are weakly phagocytic.

Besides the presence of epithelioid cells and lymphoid cells, granulomas may have giant cells, necrosis and fibrosis:

1. The giant cells are formed by fusion of adjacent epithelioid cells and may have 20 or more nuclei. These nuclei may be arranged at the periphery like horseshoe or ring or clustered at the two poles (Langhans' giant cells), or they may be present centrally (foreign body giant cells). The former are commonly seen in tuberculosis while the latter are common in foreign body tissue reactions. Like epithelioid cells these giant cells are weakly phagocytic but produce secretory products which help in removing the invading agents.

2. Necrosis may be a feature of some granulomatous conditions e.g. central caseation necrosis of tuberculosis, so called because of cheese-like appearance and consistency of necrosis.

3. Fibrosis is due to proliferation of fibroblasts at the periphery of granuloma.

The following two factors favour the formation of granulomas:

- i) Presence of poorly digestible irritant which may be organisms like Mycobacterium tuberculosis, particles of talc etc.

- ii) Presence of cell-mediated immunity to the irritant, implying thereby the role of hypersensitivity in granulomatous inflammation.

A fully-developed tubercle is about 1 mm in diameter with central area of caseation necrosis, surrounded by epithelioid cells and one to several multi-nucleated giant cells (commonly Langhans' type), surrounded at the periphery by lymphocytes and bounded by fibroblasts and fibrous tissue.

Granulomatous inflammation is typical of reaction to poorly digestible agents elicited by tuberculosis, leprosy, fungal infections, schistosomiasis, foreign particles etc.

TUBERCULOSIS



Рис. 26. Tuberculosis of lungs.
1 – large tuberculosis cavity.

Tissue response in tuberculosis represents classical example of chronic granulomatous inflammation in humans.

Causative organism:

Tubercle bacillus or Koch's bacillus (named after discovery of the organism by Robert Koch in 1882) called *Mycobacterium tuberculosis* causes tuberculosis in the lungs and other tissues of the human body. The organism is a strict aerobe and thrives best in tissues with high oxygen tension like in the apex of the lung.

The organism has 5 distinct pathogenic strains: *hominis*, *bovis*,

avium, murine, and cold-blooded vertebrate strain. A sixth non-pathogenic strains, *M. smegmatis*, is found in the smegma and as contaminant in the urine of both men and women. Out of these, the first two strains (*hominis* and *bovis*) are definitely infective to human beings while infection with *avium* strain (*M. avium intracellulare*) is common in patients with AIDS.

Hypersensitivity or allergy, and immunity or resistance, play a major role in the development of lesions in tuberculosis. Tubercle bacilli as such do not produce any toxins. Tissue changes seen in tuberculosis are not the result of any exotoxin or endotoxin but are instead the result of host response to the organism which is in the form of development of cell-mediated hypersensitivity (or type IV hypersensitivity) and immunity.

Evolution of tubercle: The sequence of events which take place when tubercle bacilli are introduced into the tissue. These are as under:

1. When the tubercle bacilli are injected intravenously into the guinea pig, the bacilli are lodged in pulmonary capillaries where an initial response of neutrophils is evoked which are rapidly destroyed by the organisms.

2. After about 12 hours, there is progressive infiltration by macrophages which dominate the picture throughout the remaining life of the lesions. If the tubercle bacilli are, however, inhaled into the lung alveoli, macrophages predominate the picture from the beginning.

3. The macrophages start phagocytosing the tubercle bacilli. In 2-3 days, the macrophages undergo structural changes as a result of immune mechanisms – the cytoplasm becomes pale and eosinophilic and their nuclei become elongated and vesicular. These modified macrophages resemble epithelial cells and are called epithelioid cells.

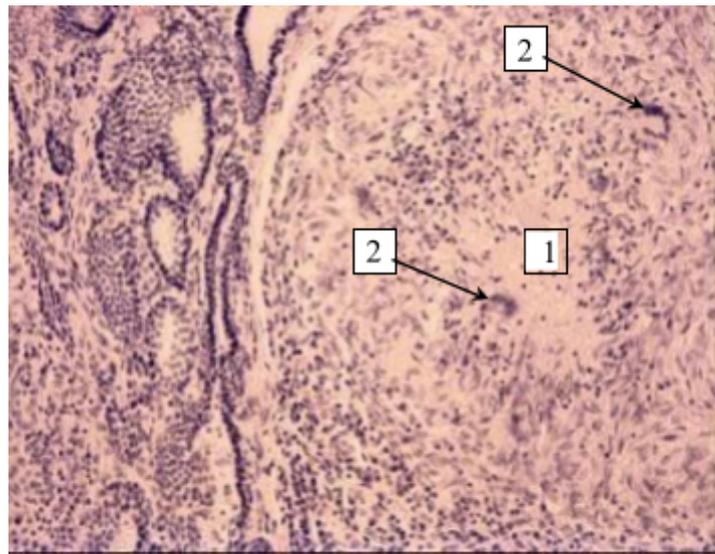


Рис. 27. Tuberculosis of thyroid gland. H and E stained section, x 200.

1) area of caseous necrosis, 2) Pyrogov-Langhans' cells

4. The macrophages continue to enter the tissue

either from circulating monocytes or from local proliferation and undergo changes to form more epithelioid cells. The epithelioid cells in time aggregate into tight clusters or granulomas.

Release of cytokines in response to sensitised CD 4 + T cells and some constituents of mycobacterial cell wall play a role in formation of granuloma.

5. Some of the macrophages form multinucleated giant cells by fusion of adjacent cells. The giant cells may be Langhans' type having peripherally arranged nuclei in the form of horseshoe or ring, or clustered at the two poles of the giant cell; or they may be foreign body type having centrally-placed nuclei.

6. Around the mass of epithelioid cells and giant cells is a zone of lymphocytes, plasma cells and fibroblasts. The lesion at this stage is called

hard tubercle due to absence of central necrosis.

7. Within 10 -14 days, the centre of the cellular mass begins to undergo caseation necrosis, characterised by cheesy appearance and high lipid content. This stage is called soft tubercle which is the hallmark of tuberculous lesions. The development of caseation necrosis is possibly due to interaction of mycobacteria with activated T cells (CD 4 + helper T cells via IF- γ and CD 8 + suppressor T cells directly) as well as by direct toxicity of mycobacteria on macrophages. Microscopically, caseation necrosis is structureless, eosinophilic and granular material with nuclear debris.

8. The soft tubercle which is a fully-developed granuloma with caseous centre does not favour rapid proliferation of tubercle bacilli. Acid fast bacilli are difficult to find in these lesions and may be demonstrated at the margins of recent necrotic foci and in the walls of the cavities.

The fate of a granuloma is variable:

i) The caseous material may undergo liquefaction and extend into surrounding soft tissues, discharging the contents on the surface. This is called cold abscess although there are no pus cells in it.

ii) In tuberculosis of tissues like bones, joints, lymph nodes and epididymis, sinuses are formed and the sinus tracts are lined by tuberculous granulation tissue,

iii) The adjacent granulomas may coalesce together enlarging the lesion which is surrounded by progressive fibrosis.

iv) In the granuloma enclosed by fibrous tissue, calcium salts may get deposited in the caseous material (dystrophic calcification) and sometimes the lesion may even get ossified over the years.

LEPROSY

Leprosy or Hansen's disease (after discovery of the causative organism by Hansen in 1874) is a chronic infectious disease affecting mainly the cooler parts of the body such as the skin, mouth, respiratory tract, eyes, peripheral nerves, superficial lymph nodes and testis. Though the earliest and main involvement in leprosy is of the skin and nerves but in bacteraemia from endothelial colonisation or by bacilli filtered from blood by reticuloendothelial system, other organs such as the liver, spleen, bone marrow and regional lymph nodes are also involved. Advanced cases may develop secondary amyloidosis and renal disease, both of which are of immunologic origin.

Causative organism. The disease is caused by *Mycobacterium leprae* which closely resembles *Mycobacterium tuberculosis* but is less acid-

fast. The organisms in tissues appear as compact rounded masses (globi) or are arranged in parallel fashion like cigarettes-in-pack.

Incidence. The disease is endemic in areas with hot and moist climates and in poor tropical countries. According to the WHO, five countries – India, Brazil, Indonesia, Myanmar (Burma) and Nigeria, together constitute vast majority of leprosy cases, of which India accounts for about one-third of all registered leprosy cases globally. In India, the disease is seen more commonly in regions like Tamil Nadu, Bihar, Pondicherry, Andhra Pradesh, Orissa, West Bengal and Assam. Very few cases are now seen in Europe and the United States.

Mode of transmission. Leprosy is a slow communicable disease and the incubation period between first exposure and appearance of signs of disease varies from 2 to 20 years (average about 3 years). The infectivity may be from the following sources:

1. Direct contact with untreated leprosy patients who shed numerous bacilli from damaged skin, nasal secretions, mucous membrane of mouth and hair follicles.
2. Materno-foetal transmission across the placenta.
3. Transmission from milk of leprosy patient to infant.

Classification. Leprosy is broadly classified into 2 main types:

- Lepromatous type representing low resistance; and
- Tuberculoid type representing high resistance.

There may be two types of lepra reaction: type I (borderline reactions), and type II (erythema nodosum leprosum).

Type I: Borderline reactions. The polar forms of leprosy do not undergo any change in clinical and histopathological picture. The borderline groups are unstable and may move across the spectrum in either direction with upgrading or downgrading of patient's immune state. Accordingly, there may be two types of borderline reaction:

1. Upgrading reaction is characterised by increased cell-mediated immunity and occurs in patients of borderline lepromatous (BL) type on treatment who upgrade or shift towards tuberculoid type.

Histologically, the upgrading reaction shows an increase of lymphocytes, oedema of the lesions and reduced B.I.

2. Downgrading reaction is characterised by lowering of cellular immunity and is seen in borderline tuberculoid (BT) type who downgrade or shift towards lepromatous type.

Histologically, the lesions show dispersal and spread of the granulomas and increased presence of lepra bacilli.

Type II: Erythema nodosum leprosum (ENL). ENL occurs in lepromatous patients after treatment. It is characterised by tender cutaneous nodules, fever, iridocyclitis, synovitis and lymph node involvement.

Histologically, the lesions in ENL show infiltration by neutrophils and prominence of vasculitis. Secondary amyloidosis may follow repeated attacks of ENL in leprosy.

Histopathology of Leprosy. Usually, skin biopsy from the margin of lesions is submitted for diagnosis and for classification of leprosy. The histopathologic diagnosis of multibacillary leprosy like LL and BL offers no problem while the indeterminate leprosy and tuberculoid lesions are paucibacillary and their diagnosis is made together with clinical evidence.

In general, for histopathologic evaluation in all suspected cases of leprosy the following broad guidelines should be followed:

- cell type of granuloma;
- nerve involvement; and
- bacterial load.

The main features in various groups are given below.

1. Lepromatous leprosy:

The following features characterise lepromatous polar leprosy:

i) In the dermis, there is proliferation of macrophages with foamy change, particularly around the blood vessels, nerves and dermal appendages. The foamy macrophages are called 'lepra cells' or Virchow cells.

ii) The lepra cells are heavily laden with acid-fast bacilli demonstrated with AFB staining. The AFB may be seen as compact globular masses (globi) or arranged in parallel fashion like 'cigarettes in a pack'.

iii) The dermal infiltrate of lepra cells characteristically does not encroach upon the basal layer of epidermis and is separated from epidermis by a subepidermal uninvolved clear zone.

iv) The epidermis overlying the lesions is thinned out, flat and may even ulcerate

2. Tuberculoid leprosy:

tures:

The polar tuberculoid form presents the following histological fea-

i) The dermal lesions show granulomas resembling hard tubercles composed of epithelioid cells, Langhans' giant cells and peripheral mantle of lymphocytes.

ii) Lesions of tuberculoid leprosy have predilection for dermal nerves which may be destroyed and infiltrated by epithelioid cells and lymphocytes.

iii) The granulomatous infiltrate erodes the basal layer of epidermis i.e. there is no clear zone.

iv) The lepra bacilli are few and seen in destroyed nerves.

3. *Borderline leprosy:*

The histopathologic features of the three forms of borderline leprosy are as under:

i) Borderline tuberculoid (BT) form shows epithelioid cells and plentiful lymphocytes. There is a narrow clear subepidermal zone. Lepra bacilli are scanty and found in nerves.

ii) Borderline lepromatous (BL) form shows predominance of histiocytes, a few epithelioid cells and some irregularly dispersed lymphocytes. Numerous lepra bacilli are seen.

iii) Midborderline (BB) form shows sheets of epithelioid cells with no giant cells. Some lymphocytes are seen in the perineurium. Lepra bacilli are present, mostly in nerves.

4. *Indeterminate leprosy:*

The histopathologic features are nonspecific so that the diagnosis of nonspecific chronic dermatitis may be made. However, a few features help in suspecting leprosy like:

i) Lymphocytic or mononuclear cell infiltrate, focalised particularly around skin adnexal structures like hair follicles and sweat glands or around blood vessels.

ii) Nerve involvement, if present, is strongly supportive of diagnosis.

iii) Confirmation of diagnosis is made by finding of lepra bacilli.

ACTINOMYCOSIS

Actinomycosis is a chronic suppurative disease caused by anaerobic bacteria, *Actinomyces israelii*. The disease is conventionally included in

mycology though the causative organism is filamentous bacteria and not true fungus. The disease is worldwide in distribution. The organisms are commensals in the oral cavity, alimentary tract and vagina. The infection is always endogeneous in origin and not person-to-person. The organisms invade, proliferate and disseminate in favourable conditions like break in mucocutaneous continuity, some underlying disease etc.

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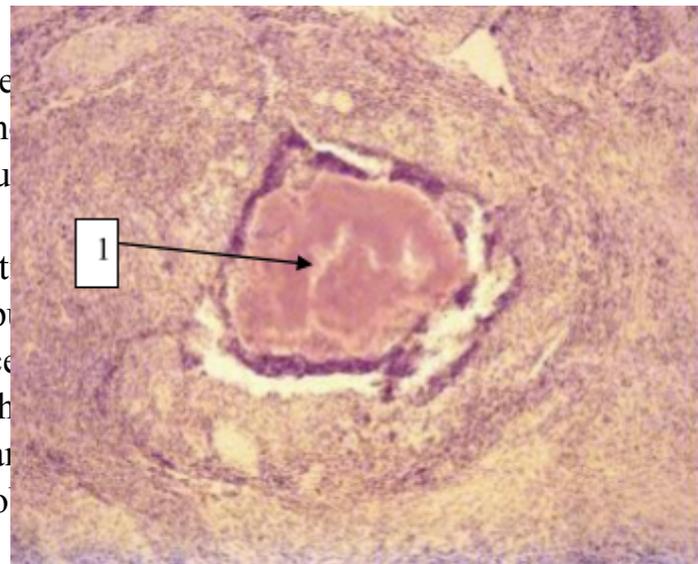


Fig. 28. Thoracic actinomycosis. H and E stained section, x 200.

1 - the bacterial colony with characterised radiating filaments in centre of abscess.

abscess contain the bacterial colony, 'sulfur granule', characterised by radiating

filaments (hence previously known as ray fungus) with hyaline, eosinophilic, club-like ends representative of secreted immunoglobulins.

iii) Bacterial stains reveal the organisms as gram-positive filaments, non-acid fast, which stain positively with Gomori's methenamine silver (GMS) staining.

SARCOIDOSIS (BOECK'S SARCOID)

Sarcoidosis is a systemic disease of unknown etiology. It is worldwide in distribution and affects adults from 20-40 years of age. The disease is characterised by the presence of non-caseating epithelioid cell granulomas ('sarcoid granuloma') in the affected tissues and organs, notably lymph nodes and lungs. Other sites are skin, spleen, uvea of the eyes, salivary glands, liver and bones of hands and feet. The histologic diagnosis is generally made by exclusion.

Etiology and pathogenesis: The cause of sarcoidosis remains unknown. Since the disease is characterised by granulomatous tissue reaction, possibility of cell-mediated immune mechanisms has been suggested. The following observations point towards a possible immune origin of sarcoidosis:

1. Cells lavaged from lung lesions show increased helper T cells that elaborate lymphokines which initiate the formation of non-caseating granulomas.

2. The peripheral blood, however, shows lymphopenia with reduction in helper T cells and increase in suppressor T cells, accounting for cutaneous unresponsiveness to tuberculin and dinitrochlorobenzene (DNCB).

3. Circulating B cells are normal in number but there is hyperglobulinaemia due to B cell hyperplasia in lymphoid tissue.

Pathologic changes: The lesions in sarcoidosis are generalised and may affect various organs and tissues at sometime in the course of disease, but brunt of the disease is borne by lungs and lymph nodes:

Microscopically, the following features are present:

1. The diagnostic feature in sarcoidosis of any organ or tissue is the non-caseating sarcoid granuloma, composed of epithelioid cells, Langhans' and foreign body giant cells and surrounded peripherally by fibroblasts.

2. Typically, granulomas of sarcoidosis are 'naked' i.e. either devoid of peripheral rim of lymphocytes or there is paucity of lymphocytes.

3. In late stage, the granuloma is either enclosed by hyalinised fibrous tissue or is replaced by hyalinised fibrous mass.

4. The giant cells in sarcoid granulomas contain certain cytoplasmic inclusions like:

i) Asteroid bodies which are eosinophilic and stellate-shaped structures.

ii) Schaumann's bodies or conchoid (conch like) bodies which are concentric laminations of calcium and of iron salts, complexed with proteins.

iii) Birefringent cytoplasmic crystal which are colourless.

Similar types of inclusions are also observed in chronic berylliosis.

Kviem's test: it is a useful intradermal diagnostic test. The antigen prepared from involved lymph node or spleen is injected intradermally. In a positive test, nodular lesion appears in 3-6 weeks at the inoculation site

which on microscopic examination shows presence of non-caseating granulomas.

RHINOSCLEROMA

The nose, the granuloma (scleroma) consists of the plasma cells, epithelioid cells, lymphocytes, and hyaline spheres. Large macrophages with light cytoplasm containing Klebsiella Rhinoscleromatis are called Mikulicz's cells, sclerosis and hyalinosis take place.

GLANDERS

Glanders can be acute and chronic. Acute glanders is characterized by the appearance of granulation tissue nodules comprised of macrophages, epithelioid cells and neutrophils. These nodules undergo the necrosis and suppuration. Thus there is formation of abscesses in different organs. Chronic glanders is characterized by the proliferative nodules formation in different organs which resemble tuberculous granulomas.

PROLIFERATIVE INFLAMMATION WITH THE FORMATION OF POLYPS AND POINTED CONDYLOMAS

This kind of inflammation is characterized by chronic course and it is accompanied by formation of polyps and pointed condylomas on the mucous membranes. Integumentary epithelium is underwent to hyperplasia, thus there is a growth of it in the form of polyps, connective tissue. Basis of polyps is diffusely infiltrated by lymphocytes, plasmocytes, macrophages and other cells. Similar formations arise in nasal cavity, sinus of Highmore, bronchial tubes, on the mucous membrane of stomach, intestine, and uterus.

If similar process develops in the area of junction of squamous and columnar epithelium it is a condyloma. The surface of condyloma is covered by squamous epithelium. Such outgrowths frequently arise in anus, genitals, and typical for syphilis and gonorrhoea.

IMMUNOPATHOLOGICAL PROCESSES

Immunopathological processes are pathological states which are associated with disturbances of structure and function of the lymphoid tissue.

THYMUS

Thymus is the central organ of immune system. In case of disturbances immunogenesis usually the following processes and pathology are seen in the thymus.

1. *Accidental thymus transformation* (involution), that is reduction in the size and mass due to thymocytes migration to the peripheral immune organs and blood as well as due to their partial decomposition and engulfment by macrophages (this is called apoptosis).

According to T. Ivanovskaya (1976), there are 5 stages of accidental thymus evolution.

Stage 1 – «holey clearing» – accumulation of lymphocytes around the macrophages. It occurs in the cortex.

Stage 2 – transition of the lymphocytes from the cortex to the medullar substance. The boundary between the layers is either poorly seen or not seen at all.

Stage 3 – «layer inversion», when the cortex layer looks light, and medullar layer looks dark as a result of transition of lymphocytes from the cortex to the medullar substance.

Stage 4 – reduction in the lymphocyte amount in the both layers, reticular stroma growth.

Stage 5 – collapse of the thymus lobe, sclerosis and atrophy.

Accidental transformation more often occurs in the newborn suffering from stress factors. The more powerful the stimulus, the more pronounced the degree of involution. Accidental involution occurs in severe infections, intoxications, in children born from sick mothers. The process is reversible. Elimination of pathological agent results in thymus normalization.

2. *Thymus hyperplasia* (thymolymphatic state, thymomegaly). The weight and the size of thymus are considerably increased. Microscopic examination reveals a large number of immature lobules (zones are not distinct). The density of the thymocytes is high. If this condition is accompanied by hypoplasia of adrenal and sexual glands as well as narrowing of the

aorta and arteries, this pathological process is called «thymo-lymphatic state».

3. *Thymus hypoplasia* is characterized by absence of lobule division into cortical and medullar layers, poor development of reticuloepithelial component responsible for hormonal function, as well as lymphocyte component. As a rule thymus hypoplasia is typical for congenital immune deficiency.

4. *Aplasia* – total congenital lack of organ or its part.

5. *Agenesis* – congenital disorder which is characterized by absence of thymus germ.

6. *Dysplasia* of thymus means morphological structure disorders: there is no cortex and medulla in lobules, the boundary between lobules is poorly seen, etc.

Thymus hypoplasia, aplasia, agenesis and dysplasia are congenital disturbances.

7. *Thymus hyperplasia* is characterized by increase of lymphoid follicles.

CHANGES IN LYMPHOID TISSUE AT ANTIGEN STIMULATION

At first in the bone marrow hyperplasia of B-lymphocytes is observed, and then it becomes empty, as a result of increased transition of lymphocytes.

At antigen stimulation the morphologic processes developing in lymph nodes and spleen of healthy person and sick man are similar. Nevertheless they differ by the quantity of the involved in pathological process cells, as well as by the character and grade of immune inflammatory reactions.

These changes are characterized by: macrophage reactions; hyperplasia of B-lymphocytes with following transformation of them into plasma cells.

The number of plasma cells shows the intensity of immunogenesis and corresponds to the level of antibodies production.

Lymph nodes are: hyperplastic, edematous; follicular centers are pale (germinal centers are enlarged), contain numerous blasts and macrophages; macrophage aggregations are seen; there are a lot of plasma cells and macrophages in sinuses.

If cellular immune reaction develops in response to antigen the following morphologic changes occur: proliferation of sensitized T-lymphocytes, enlargement of T-dependent areas in lymph nodes (paracortical zones).

HYPERSENSITIVITY REACTION

A state of balance in the immune response (humoral or cell-mediated) is essential for protection against endogenous and exogenous antigens. Hypersensitivity is defined as a state of exaggerated immune response to an antigen. The lesions of hypersensitivity (immunologic tissue injury) are produced due to interaction between antigen and product of the immune response.

Depending upon the rapidity and duration of the immune response, four distinct forms of hypersensitivity reactions are recognized:

Hypersensitivity of immediate type. Immediate type of hypersensitivity is further of three types – type I, II and III.

Immediate hypersensitivity reaction morphologically manifests by the picture of acute immune inflammation which develops rapidly, alteration and exudation stages prevail, and proliferation increases slowly. The vessels and connective tissues are involved first. Alteration manifests by mucoid, fibrinoid swelling and fibrinoid necrosis. The exudate is either fibrinous or fibrino-hemorrhagic. Acute immune inflammation is observed in some forms of leprosy and syphilis. It is responsible for vascular reaction in lupus erythematosus, glomerulonephritis, and nodular periarteritis.

Hypersensitivity of delayed type. Morphologically it manifests by chronic immune inflammation characterized by lymphocyte-macrophage infiltration. When the lymphocyte-macrophage infiltration accompanied by vascular plasmorrhagic and degenerative processes is seen the conclusion of immune inflammation can be made. The condition occurs in autoimmune diseases, tuberculosis, brucellosis, dermatitis.

Type I reaction, or immediate-type, hypersensitivity reactions, IgE antibody is formed and binds to receptors on mast cells and basophils. The binding of antigen that reacts with the IgE releases products from these cells and results in characteristic symptoms of diseases such as asthma or anaphylaxis (anaphylactic shock). Immediate type in which on administration

of antigen, the reaction occurs immediately (within seconds to minutes). Immune response this type is mediated largely by humoral antibodies.

Type II reaction (Cytotoxic Type): antibody-mediated cytotoxicity. In this type, IgG or IgM antibody is formed against an antigen, usually a protein on a cell surface or (less commonly) a component of extracellular matrix, such as basement membrane. This antigen-antibody coupling leads to complement activation, which in turn is responsible for the lysis (cytotoxicity) of the cell or damage to the extracellular matrix (e.g. Autoimmune hemolytic anemias, Goodpasture disease).

Type III reaction: immune complex disease. In this type of reaction, the antibody responsible for tissue injury is also IgM or IgG but here the mechanism of tissue injury differs. The antigen is usually not fixed to the cell surface but circulates in the vascular compartment and is eventually deposited in tissues. Circulating antigen-antibody (immune) complexes (which normally are removed by the reticuloendothelial system) are deposited in tissues, leading to complement activation. Complement activation at sites of antigen localization leads to recruitment of leukocytes, which are responsible for the subsequent tissue injury. (e.g. Autoimmune diseases (SLE - lupus erythematosus, rheumatoid arthritis), most types of glomerulonephritis). Immune complexes may also develop in situ (i.e., antibodies are directed against antigens that are endogenous to the tissues or have been planted there), thus triggering localized tissue damage.

Type IV hypersensitivity: Type IV reactions, also known as cell-mediated or delayed hypersensitivity reactions, do not require the formation of an antibody. Rather, antigenic activation of T lymphocytes, usually with the help of macrophages, causes the release of products by these cells, thus leading to subsequent tissue injury (e.g. Granulomatous diseases (tuberculosis, sarcoidosis). Granulomatosis is morphological manifestation of delayed hypersensitivity reaction.

Reaction of transplant rejection resembles slow hypersensitivity reaction. Transplant antigens induce the production of antibodies and sensitized lymphocytes which infiltrate the transplant.

Microscopically, lymphohistiocyte infiltration is observed in the transplant. Cellular infiltration causes the disturbance of blood circulation and edema; as a result degenerations and necrosis of transplant develop. The neutrophils and macrophages appear in the transplant. Enzymatic destruction of the transplant begins which is followed by its rejection.

AUTOIMMUNE DISEASES

Immunologic tolerance and autoimmunity. An immune response generated against self antigens is an aberrancy that implies the loss of immunologic ability to distinguish self and alien antigens. The normal status of immunologic unresponsiveness to self antigens is termed **tolerance**. Tolerance probably represents an active process involving continuous generation of cellular and humoral inhibitory regulators. Loss of tolerance to self antigen is referred to as **autoimmunity**. The mechanisms by which tolerance is generated and lost are poorly understood. Theories of autoimmunity include:

1. Recognition of previously hidden (physiological isolation) or sequestered antigen.
2. Diminution of suppressor T-cell function.
3. Increase in helper T-cell activity.
4. T-cell-independent polyclonal B-cell activation by complex antigens.
5. Modification of self antigens by drugs or microorganisms.
6. Cross-reactivity between autologous antigens and microbial antigens.

Autoimmune diseases are those occurring as a result of the reaction of autoantibodies and sensitized lymphocytes against normal antigens of the own tissue. The causes of autoimmune diseases are not clearly known. Chronic viral infections, radiation and genetic factors may be responsible for them.

In the pathogenesis of autoimmune diseases the following factors are distinguished:

- predisposing (HLA genes, hormonal background, genetically dependent features of the target cells);
- initiating (viral and bacterial infections, exposure of immune system and target organs to chemical and physical factors);
- contributing (dysfunction of immune system, T-lymphocyte suppressor activity).

In the pathogenesis 2 mechanisms can be distinguished; therefore all the autoimmune diseases can be divided into 2 groups.

Group 1. Organ specific diseases. They are characterized by disturbance of physiological isolation of the organs and tissues due to absence of

immune tolerance. Lymphohistiocyte infiltration occurs in the tissues (like at delayed hypersensitivity reaction). The main organ specific diseases are:

1. Endocrine glands:

Hashimoto's (autoimmune) thyroiditis.

Graves' disease.

Insulin-dependent diabetes mellitus.

Idiopathic Addison's disease.

2. Alimentary tract:

Autoimmune atrophic gastritis in pernicious anemia.

Ulcerative colitis.

Crohn's disease.

3. Blood cells:

Autoimmune hemolytic anemia.

Autoimmune thrombocytopenia.

4. Others:

Myasthenia gravis.

Autoimmune orchitis.

Autoimmune encephalomyelitis.

Goodpasture's syndrome.

Primary biliary cirrhosis.

Chronic active hepatitis.

Membranous glomerulonephritis.

Autoimmune skin diseases.

Group 2. Organ non-specific diseases. Primary disturbances in the immune system causing the loss of ability to distinguish «own» and «foreign» antigens, they are:

Systemic lupus erythematosus.

Rheumatoid arthritis.

Scleroderma (Progressive systemic sclerosis).

Polymyositis-Dermatomyositis.

Polyarteritis nodosa (PAN).

Sjogren's syndrome.

Reiter's syndrome.

Mixed connective tissue disease.

The diseases with autoimmune disturbances. In these diseases antigenic properties of the tissues are changed, that causes immune reaction development. Autoimmunization is responsible not for the beginning but the

progress of the disease as autoimmune antibodies appear during the disease. It is observed in glomerulonephritis, hepatitis, chronic gastritis, burn disease, rheumatism, liver cirrhosis.

IMMUNODEFICIENCY SYNDROMES (IDS)

Immune deficiency syndromes result from immune system insufficiency.

All immune deficiencies are divided into 2 groups: primary or congenital immune deficiencies and secondary or acquired immune deficiencies.

Primary IDS may be understood as primary defects in development of the immune system. Secondary ones result from diseases or drugs that affect immune system.

Primary IDS may be classified into the following 4 general groups depending on the stage in development at which the defect occurs:

- T-cell deficiencies;
- B-cell deficiencies;
- Combined (T-, B-cell) deficiencies;
- Deficiency in inflammatory cells (agranulocytosis).

T-cell deficiencies manifests by agenesis, hypoplasia of the thymus and T-dependent zones of the immune system. They are inherited according to autosomal dominant type, e.g. *MacCusic syndrome*. Except for the pathology of thymus and primary lymphatic tissue, defects of development occur.

DiGeorge syndrome is a selective deficiency of T-cells. This lack results from failure of the third and fourth pharyngeal pouches to develop and become thymus and parathyroid glands. DiGeorge syndrome is thought to be the result of an early intrauterine growth defect. It is not genetically linked. Affected infants have total absence of T-cell immunity, in association with hypocalcemia and tetany. All lymphocytes are B-cells. Plasma cells are present in normal numbers. T-cell areas, such as paracortical areas of lymph nodes and periarteriolar sheaths are depressed.

Chronic mucocutaneous candidiasis is a selective defect of T-cell immunity characterized by recurrent candidal infections involving the skin and mucous membranes. The remainder of T-cell functions are intact, as is B-cell immunity. The defect may be inherited as an autosomal recessive gene. Chronic mucocutaneous candidiasis is associated with endocrinopa-

thies. Hypoparathyroidism is most common. Addison's disease, hypothyroidism, diabetes mellitus, and pernicious anemia are also seen. This association suggests a multiorgan endocrinopathy with selective thymic dysfunction.

B-cell deficiencies. This type of inheritance is connected with X-chromosome, e.g. agammaglobulinemia – Bruton's syndrome. The thymus is preserved. B-zones in the peripheral lymphatic organs are absent. Immunoglobulins synthesis is absent.

Bruton's (X-Linked) agammaglobulinemia

In 1952, Bruton described an X-linked deficiency immunoglobulin production. These patients suffered from recurrent bacteria-related bronchitis, otitis, and skin infections. Infections usually began around the 6th month of life, as circulating maternal antibodies in the infants subsided. All immunoglobulins were either markedly decreased or absent. Circulating mature B-cells were also absent.

It is now recognized that pre-B-cells are present in patients with Bruton's agammaglobulinemia, and there seems to be a defect in the B-cell maturation sequence. Morphologically, there are no germinal centers in lymph nodes, the spleen, and the tonsils. Plasma cells are absent.

Transient hypogammaglobulinemia of infancy is characterized by diminished levels of immunoglobulin but the ability to produce certain antibody. The disorder appears to be related to an abnormally long delay in the production of serum immunoglobulin. (Maternal antibodies normally decline in the infant over the first 6 months of life.) Defects in helper T-cell function are thought to be responsible.

Common variable immunodeficiency is a somewhat poorly defined entity characterized by hypogammaglobulinemia despite normal numbers of circulating B-cells. In most cases, there is no clear-cut genetic predisposition. Patients range in age from very young to elderly, although young adults are most commonly affected.

Selective IgA deficiency is the most common immunodeficiency disorder, occurring in about 1 in 700 people. Serum IgA levels are low, but the numbers of circulating IgA-cells are normal. However, these IgA-cells possess an immature phenotype that coexpresses IgD and IgM. Thus, the defect seems to be in the maturation of IgA-bearing cells. Similar selective deficiencies in IgM are reported but rare.

Combined syndromes – insufficiency of cellular and humoral immunity (T-, B-cell insufficiency). This is inherited according to autosomal-recessive type, e.g. Glanzmann-Riniker syndrome (agammaglobulinemia of Swiss type); severe combined immunodeficiency (SCID); hypoplasia of thymus and peripheral lymphatic tissue.

Severe combined immunodeficiency (SCID) is a severe disorder characterized by nearly total absence of both T-cell and B-cell immunity. Infants present early with recurrent opportunistic infections. SCID may be transmitted as either an X-linked or autosomal recessive way.

Morphologically, there is a virtual absence of lymphoid tissue in the form of lymph nodes, spleen, and tonsils. The thymus gland fails to descend from the neck into the mediastinum and lacks lymphoid cells and Hassall's corpuscles.

These patients appear to have a stem-cell defect; deficiency in the enzyme adenosine deaminase (ADA) is found in the cells of many patients with the autosomal recessive form of SCID. ADA converts adenosine to inosine, or deoxyadenosine to deoxyinosine. Without this enzyme, there is an accumulation of adenosine, deoxyadenosine, and deoxyadenosine triphosphate (dATP). This latter compound inhibits ribonucleotide reductase, causing depletion of deoxyribonucleotide triphosphates and abnormal lymphocyte function. SCID with ADA deficiency may be diagnosed prenatally by amniocentesis.

Secondary deficiencies occur after full development of the immune system. Some of these are secondary to immunosuppressive therapy, e.g. in tumors, autoimmune diseases, glomerulonephritis, etc. Chronic virus infections and HIV (human immunodeficiency virus) may cause secondary deficiencies.

NEOPLASIA

The term “neoplasia”, “neoplasm” or “tumor” means new growth. However, all “new growths” are not neoplasms since examples of new growth of tissues and cells also exist in the processes of embryogenesis, regeneration and repair, hyperplasia and hormonal stimulation. The proliferation and maturation of cells in normal adults is controlled as a result of which some cells proliferate throughout life (labile cells), some have limited proliferation (stable cells), while others do not replicate (permanent cells). On the other hand, neoplastic cells lose control and regulation of replication and form an abnormal mass of tissue.

Therefore, satisfactory definition of a neoplasm or tumor is “a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells”. The branch of science dealing with the study of neoplasms or tumors is called oncology (oncos = tumor, logos = study). Neoplasms may be benign when they are slow-growing and localized without causing much difficulty to the host, or malignant when they proliferate rapidly, spread throughout the body and may eventually cause death of the host. The common term used for all malignant tumors is cancer. Hippocrates (460-377 BC) proposed the term “karkinos” for cancer of the breast. The word “cancer” means crab, thus reflecting the true character of cancer since it sticks to the part stubbornly like a “crab”.

All tumors, benign as well as malignant, have 2 basic components:

- *Parenchyma* comprised by proliferating tumour cells; parenchyma determines the nature and evolution of the tumour.
- *Supportive stroma* composed of fibrous connective tissue and blood vessels; it provides the framework on which the parenchymal tumour cells grow.

The tumours derive their nomenclature on the basis of the parenchymal component comprising them. The suffix “-oma” is added to denote benign tumours. Malignant tumours of epithelial origin are called carcinomas, while malignant mesenchymal tumours are named sarcomas (sarcos = fleshy). However, some cancers are composed of highly undifferentiated cells and are referred to as undifferentiated malignant tumours. Rarely, combinations of carcinoma and sarcoma are encountered called as carcinosarcoma. Although, this broad generalization regarding nomenclature of tumours usually holds true in majority of instances, some examples contrary to

this concept are: melanoma for carcinoma of the melanocytes, hepatoma for carcinoma of the hepatocytes, lymphoma for malignant tumour of the lymphoid tissue, and seminoma for malignant tumour of the testis.

Tumours composed of a single type of parenchymal cells that differentiate towards more than one cell line are called mixed tumours. Teratomas, on the other hand, are made up of a number of parenchymal cell types arising from totipotent cells derived from more than one germ cell layer. Choristoma refers to the ectopic rests of normal tissue. Hamartoma is a mass of disorganized but mature cells of tissues indigenous to the particular site.

CLASSIFICATION OF TUMOURS

The currently used classification of tumours is based on the histogenesis (i.e. tissue of origin) and on the anticipated behaviour. However, it must be mentioned here that the classification described here is only a summary. Detailed classifications of benign and malignant tumours pertaining to different tissues and body systems along with morphologic features of specific tumours appear in the specific chapters of Systemic Pathology later.

HISTOGENETIC CLASSIFICATION OF TUMORS:

1. Epithelial tumors without specific localization (organ-nonspecific),
2. Epithelial tumors of exo- and endocrine glands, epithelial coats (organ-specific),
3. Mesenchymal tumors,
4. Tumors of melanin forming tissue,
5. Tumors of nerve system and coats,
6. Tumors of blood system,
7. Tumors of APUD- system,
8. Teratomas.

CHARACTERISTICS OF TUMOURS

Majority of neoplasms can be categorized morphologically into benign and malignant on the basis of certain characteristics, the most important being the degree of differentiation of the tumour cells. However, there are exceptions. A small proportion of tumours have some features suggesting innocent growth while other features point towards a more ominous behaviour. Therefore, it must be borne in mind before describing char-

acteristics of neoplasm that there is a wide variation in the degree of deviation from the normal in all the tumours.

The characteristics of tumours are described under the following headings:

- I. Macroscopic features
- II. Microscopic features
- III. Growth rate
- IV. Local invasion (Direct spread)
- V. Metastasis (Distant spread)

I. MACROSCOPIC FEATURES

The macroscopic appearance of tumours within the same group may be quite variable and the features may not be diagnostic on the basis of gross appearance alone. However, certain distinctive features characterize almost all tumours – they have a different colour, texture and consistency as compared to the surrounding tissue of origin. Gross terms such as papillary, fungating, infiltrating, haemorrhagic, ulcerative and cystic are used to describe the macroscopic appearance of the tumours.

- **Benign tumours** are generally spherical or ovoid in shape. They are encapsulated or well-circumscribed, freely movable, more often firm and uniform, unless secondary changes like haemorrhage or infarction supervene.

- **Malignant tumours**, on the other hand, are usually irregular in shape, poorly-circumscribed and extend into the adjacent tissues. Secondary changes like haemorrhage, infarction and ulceration are seen more often. Sarcomas typically have fish-flesh like consistency while carcinomas are generally firm.

There are 3 types of tumor growth:

1. *Expansive growth* – tumor increases in size and squeezes the surrounding tissues. It mainly occurs in benign tumors.

2. *Opposition growth* – tumor increases in size by the transformation of adjacent normal cells into tumor cells.

3. *Infiltrative, or invasive growth* – in this type tumor grows and leads destruction of the surrounding tissues. It mainly occurs in malignant tumors with rapid growth.

In tube organs, tumors can grow into organ lumen (exophytic growth), or in the organ wall (endophytic growth), or in both directions (mixed growth).

II. MICROSCOPIC FEATURES

1. Microscopic Pattern

The tumour cells may be arranged in a variety of patterns in different tumours. For example:

- The epithelial tumours generally consist of acini, sheets, columns or cords of epithelial tumour cells that may be arranged in solid or papillary pattern.

- The mesenchymal tumours have mesenchymal tumour cells lying separated from each other usually by the intercellular substance such as cartilaginous matrix in chondroma, osteoid in osteosarcoma, reticulin network in soft tissue sarcomas etc.

- Haematopoietic tumours such as leukaemias and lymphomas often have none or little stromal support.

- Generally, most benign tumours and low-grade malignant tumours reduplicate the normal structure of origin more closely so that there is little difficulty in identifying and classifying such tumours. However, anaplastic tumours differ greatly from the arrangement in normal tissue of origin of the tumour and may occasionally pose problems in classifying the tumour.

- Other cellular deviations from the normal cellular arrangement in malignant tumours are: loss of basal orientation (polarity), altered alignment of tumour cells to each other, and stromal invasion by tumour cells.

- Certain tumours have mixed patterns, e.g. teratoma arising from totipotent cells, pleomorphic adenoma of salivary gland (mixed salivary tumour), fibroadenoma of the breast, carcinosarcoma of the uterus and various other combinations of tumour types.

2. Tumour Cytomorphology (Differentiation and Anaplasia)

The neoplastic cell is characterized by morphologic and functional alterations, the most significant of which are “differentiation” and “anaplasia”.

- **Differentiation** is defined as the extent of morphological and functional resemblance of parenchymal tumour cells to corresponding normal cells. If the deviation of neoplastic cell in structure and function is minimal as compared to normal cell, the tumour is described as “well-differentiated”

such as most benign and low-grade malignant tumours. “Poorly differentiated”, “undifferentiated” or “dedifferentiated” are synonymous terms for poor structural and functional resemblance to corresponding normal cell.

• **Anaplasia (atypia)** is lack of differentiation and is a characteristic feature of most malignant tumours.

There are 2 types of morphological atypia – tissue and cellular.

Tissue atypia is characterized by conservation of cells structure (externally they look like normal) but changes of their mutual position in tissue. And in whole tissue looks unusually.

Cellular atypia means changes of cell structure. Cells are polymorphic, contain hyperchromic polymorphic nucleus, multiple mitosis (pathological too), and have increased nucleo-cytoplasm relation.

As a result of anaplasia, noticeable morphological and functional alterations in the neoplastic cells are observed. These are considered below:

i) **Pleomorphism.** The term pleomorphism means variation in size and shape of the tumour cells. The extent of cellular pleomorphism generally correlates with the degree of anaplasia. Tumour cells are often bigger than normal but in some tumours they can be of normal size or smaller than normal.

ii) **Nucleocytoplasmic changes.** The nuclei of tumour cells show most conspicuous changes compared to normal cells:

a) Generally, the nuclei of malignant tumour cells are enlarged, disproportionate to the cell size so that the nucleocytoplasmic ratio is increased from normal 1:5 to 1:1.

b) Just like cellular pleomorphism, the nuclei too, show variation in size (anisonucleosis) and shape in malignant tumour cells.

c) Characteristically, the nuclear chromatin of malignant cell is increased and coarsely clumped. This is due to increase in the amount of nucleoprotein resulting in dark-staining nuclei, referred to as hyperchromatism. Besides, a prominent nucleolus or nucleoli may be present in these nuclei reflecting increased nucleoprotein synthesis. Nuclear shape may vary and nuclear chromatin is clumped along the nuclear membrane.

d) The parenchymal cells of poorly-differentiated tumours often show large number of mitoses as compared with benign tumours and well-differentiated malignant tumours. However, it is most important to identify abnormal and atypical mitotic figures such as tripolar, quadripolar and multipolar spindles in malignant tumour cells because increased number of normal

mitoses may be present in non-neoplastic proliferations such as in haematopoietic cells of the bone marrow, intestinal epithelium, hepatocytes etc.

e) Multinucleate tumour giant cells or giant cells containing a single large and bizarre nucleus, possessing nuclear characters of the adjacent tumour cells, are another important feature of anaplasia in malignant tumours.

f) The cytoplasm of tumour cells in better-differentiated cancers and in benign tumours may show the normal constituents from which the tumour is derived, e.g. the presence of mucus, keratin, cross striations etc. But the more anaplastic tumour cells lose such features.

iii) **Genetic abnormalities.** All tumour cells have abnormal genetic composition and on division they transmit the genetic abnormality to their progeny. The first tumour cell is perhaps produced by mutation in the original normal cell resulting in abnormal gene/genes. Other possible hypotheses for genetic abnormality in neoplastic cells are the activation of a normally suppressed oncogene present in all the cells of the body, or a virus adding new genetic material to the cell (discussed later). The chromosomal abnormalities are more marked in more malignant tumours which include deviations in both morphology and number of chromosomes. Most malignant tumours show aneuploidy, often in the form of an increase in the number of chromosomes, reflected morphologically by the increase in the size of nuclei. One of the most important examples of a consistent chromosomal abnormality in human malignancy is the presence of Philadelphia chromosome (named after the city in which it was first described) in 95% cases of chronic myeloid leukaemia. In this, part of the long arm of chromosome 9 is translocated to part of the long arm of chromosome 22 (t 9; 22). Other examples of neoplasms showing chromosomal abnormalities are Burkitt's lymphoma, acute lymphoid leukaemia, multiple myeloma, retinoblastoma, oat cell carcinoma, Wilm's tumour etc.

iv) **Functional changes.** Structural anaplasia in tumours is accompanied with functional anaplasia. The functional abnormality in neoplasms may be quantitative, qualitative, or both.

Generally, benign tumours and better-differentiated malignant tumours continue to function well qualitatively, though there may be quantitative abnormality in the product, e.g. large or small amount of collagen produced by benign tumours of fibrous tissue, keratin formation in well-differentiated squamous cell carcinoma. In more anaplastic tumours, there is

usually quantitative fall in the product made by the tumour cells, e.g. absence of keratin in anaplastic squamous cell carcinoma.

There may be both qualitative and quantitative abnormality of the cellular function in some anaplastic tumours, e.g. multiple myeloma producing abnormal immunoglobulin in large quantities.

Endocrine tumours may cause excessive hormone production leading to characteristic clinical syndromes. Besides the production of hormones by endocrine tumours, hormones or hormone-like substances may be produced by certain tumours quite unrelated to the endocrine glands. This property of tumours is called ectopic hormone production, e.g. oat cell carcinoma of the lung can secrete ACTH and ADH; less often it may produce gonadotropin, thyrotropin, parathormone, calcitonin and growth hormone. Ectopic erythropoietin may be produced by carcinoma of kidneys, hepato-cellular carcinoma and cerebellar haemangioblastoma.

3. Angiogenesis and Tumour Stroma

The connective tissue along with its blood supply forms the supportive framework on which the parenchymal tumour cells grow and receive nourishment. In addition to variable amount of connective tissue and vascularity, the stroma may have nerves and metaplastic bone or cartilage but no lymphatics. In order to provide nourishment to growing tumour, new blood vessels are formed from pre-existing ones (angiogenesis) that is probably stimulated by secretion of tumour angiogenesis factors from the parenchymal tumour cells such as vascular endothelial growth factor (VEGF). However, if the tumour outgrows its blood supply as occurs in rapidly growing tumours, its core undergoes ischaemic necrosis. The collagenous tissue in the stroma may be scanty so that the tumour is soft and fleshy such as in sarcomas, lymphomas etc. If the tumour is almost entirely composed of parenchymal cells, it is called medullary, if there is excessive connective tissue stroma, it is referred to as desmoplasia and the tumour is hard or scirrhous. Growth of fibrous tissue in tumour is stimulated by basic fibroblast growth factor (bFGF) elaborated by tumour cells.

4. Inflammatory Reaction

At times, prominent inflammatory reaction is present in and around the tumours. It could be the result of ulceration in the cancer when there is secondary infection. The inflammatory reaction in such instances may be acute or chronic. However, some tumours show chronic inflammatory reac-

tion, chiefly of lymphocytes, plasma cells and macrophages, and in some instances, granulomatous reaction, in the absence of ulceration. This could possibly be due to cell-mediated immunologic response by the host in an attempt to destroy the tumour.

The examples of such reaction are: seminoma testis, malignant melanoma of the skin, lymphoepithelioma of the throat, medullary carcinoma of the breast, Warthin's tumour of salivary glands etc. In some cases, such an immune response improves the prognosis.

III. GROWTH RATE

The tumour cells generally proliferate more rapidly than the normal cells. In general, benign tumours grow slowly and malignant tumours rapidly. However, there are exceptions to this generalization. The rate at which the tumour enlarges depends upon 3 main factors:

1. Rate of division and destruction of tumour cells
2. Non-neoplastic elements in the tumour
3. Degree of differentiation of the tumour.

1. Rate of division and destruction of tumour cells.

The rate of division of tumour cells depends upon 2 factors – proportion of cells undergoing mitosis (mitotic index), and the duration taken to complete the mitotic cell cycle. If the rate of cell division is high, it is likely that the cells in the centre of the tumour are not adequately nourished and may undergo ischaemic necrosis. Thereafter, the rate of tumour enlargement slows in spite of rapid division of tumour cells.

2. Non-neoplastic elements within the tumours.

These are the connective tissue stroma, abundant mucoid material, cartilaginous matrix etc all of which add to the bulk of the tumours.

3. **Degree of differentiation.** In general, rate of growth of malignant tumour is directly proportionate to the degree of differentiation. Some tumours growing steadily for years may suddenly show spurt in their growth which is due to appearance of aggressive clone of malignant cells in the tumour. On the other hand, some tumours may cease to grow after sometime. Rarely, a malignant tumour such as choriocarcinoma and malignant melanoma may disappear spontaneously from the primary site, possibly due to necrosis caused by good host immune attack, only to reappear as secondaries elsewhere in the body.

The regulation of tumour growth is under the control of growth factors secreted by the tumour cells. Out of more than 100 growth factors described so far, important ones modulating tumour biology are:

- i) Epidermal growth factor (EGF)
- ii) Fibroblast growth factor (FGF)
- iii) Platelet-derived growth factor (PDGF)
- iv) Colony stimulating factor (CSF)
- v) Transforming growth factors- β (TGF- β)
- vi) Interleukins (IL).

IV. LOCAL INVASION (DIRECT SPREAD)

Most benign tumours form encapsulated or circumscribed masses that push aside the surrounding normal tissues without actually invading, infiltrating or metastasising. Malignant tumours also enlarge by expansion and some well-differentiated tumours may be partially encapsulated as well. But characteristically, they are distinguished from benign tumours by invasion, infiltration and destruction of the surrounding tissue, besides distant metastasis (described below). In general, tumours invade via the route of least resistance, though eventually most cancers recognize no anatomic boundaries. Often, cancers extend through tissue spaces, permeate lymphatics, blood vessels, perineural spaces and may penetrate a bone by growing through nutrient foramina. More commonly, the tumours invade thin-walled capillaries and veins than thick-walled arteries. Dense compact collagen, elastic tissue and cartilage are some of the tissues which are sufficiently resistant to invasion by tumours. Certain tumours such as carcinoma of the cervix arise from pre-invasive stage called carcinoma in situ when the cancer cells are confined to layers superficial to the basement membrane. When the tumour cells cross the basement membrane, it is referred to as invasive cancer.

Mechanism of local invasion by malignant tumours is discussed in the next section along with mechanism of metastasis.

V. METASTASIS (DISTANT SPREAD)

Metastasis (meta = transformation, stasis = residence) is defined as spread of tumour by invasion in such a way that discontinuous secondary tumour mass/masses are formed at the site of lodgement. Metastasis is the most important feature to distinguish malignant from benign tumours. Benign tumours do not metastasize while all the malignant tumours with a few exceptions like gliomas of the central nervous system and basal cell carci-

noma of the skin can metastasize. Generally, larger, more aggressive and rapidly-growing tumours are more likely to metastasize but there are numerous exceptions.

Metastasis is a common event in malignant tumours which greatly reduces the survival of patient. In the biology of tumour, metastasis is a form of unusual cell differentiation in which the tumour cells form disorderly masses at ectopic sites and start growing there. This random phenomenon takes place in a stepwise manner involving only a subpopulation of tumour cells selectively. The process is governed by inappropriate expression of genes which normally partake in physiologic processes, i.e. it is a genetically programmed phenomenon.

Recent evidence has shown that in metastatic tumours, survival of host is correlated with some clinical and molecular features of tumours which act as prognostic markers. These are as under:

i) **Clinical prognostic markers:** Size, grade, vascular invasion and nodal involvement by the tumour.

ii) **Molecular prognostic markers:** Molecular markers indicative of poor prognosis in certain specific tumours are:

- a) expression of an oncogene by tumour cells (C-met);
- b) CD 44 molecule;
- c) oestrogen receptors;
- d) epidermal growth factor receptor;
- e) angiogenesis factors and degree of neovascularisation; and
- f) expression of metastasis associated gene or nucleic acid (MAGNA) in the DNA fragment in metastasising tumour.

ROUTES OF METASTASIS

Cancers may spread to distant sites by following pathways:

1. Lymphatic spread
2. Haematogenous spread
3. Other routes (transcoelomic spread, spread along epithelium-lined surfaces, spread via cerebrospinal fluid, implantation).

1. **LYMPHATIC SPREAD.** In general, carcinomas metastasise by lymphatic route while sarcomas favour haematogenous route. However, sarcomas may also spread by lymphatic pathway. The walls of lymphatics are readily invaded by cancer cells and may form a continuous growth in the lymphatic channels called lymphatic permeation, or may detach to form tumour emboli-

li so as to be carried along the lymph to the next draining lymph node. The tumour emboli enter the lymph node at its convex surface and are lodged in the subcapsular sinus. Later, of course, the whole lymph node may be replaced and enlarged by the metastatic tumour. Generally, regional lymph nodes draining the tumour are invariably involved, but sometimes lymphatic metastases do not develop first in the lymph node nearest to the tumour because of venous-lymphatic anastomoses or due to obliteration of lymphatics by inflammation or radiation, so called skip metastasis. Other times, due to obstruction of the lymphatics by tumour cells, the lymph flow is disturbed and retrograde metastases may be seen at unusual sites e.g. metastasis of carcinoma prostate to the supraclavicular lymph nodes, metastatic deposits in the adrenals from carcinoma lung etc.

It is believed that lymph nodes in the vicinity of tumour perform multiple roles – as initial barrier filter, and in destruction of tumour cells, while later provide fertile soil for growth of tumour cells. Detailed mechanism of lymphatic route of metastasis is given at the end of this section under biology of invasion and metastasis.

2. **HAEMATOGENOUS SPREAD.** Metastasis through blood vessels is the common route for sarcomas but certain carcinomas also frequently metastasise by this mode, especially those of the lung, breast, thyroid, kidney and prostate. The common sites for blood-borne metastasis are the liver, lungs, kidneys, brain and bones, all of which provide “good soil” for the growth of “good seeds” (seed-soil theory) than are the unfavourable sites like the spleen and muscles. Only a proportion of cancer cells are capable of clonal proliferation in the proper environment; others die without establishing a metastasis. The cancer cells readily invade the walls of capillaries, venules and veins than the arteries which are thick-walled and contain elastic tissue resistant to invasion. Nevertheless, arterial spread may occur in some instances such as when tumour cells pass through pulmonary capillary bed or through pulmonary arterio-venous shunts.

The tumour embolus may occlude a small vessel in the microcirculation, extend through the vessel wall and then establish a metastasis at the new site of lodgement. Thus, cancers of the organs draining into portal veins frequently establish metastasis in the liver, while cancers of organs draining into caval veins metastasise to the lungs. As with lymphatic metastases, haematogenous metastases may also occur at unusual sites due to ret-

rograde spread after venous obstruction. Important examples of this type of spread are seen in vertebral metastases in cancers of the thyroid and prostate.

Macroscopically, blood-borne metastases in an organ appear as multiple, rounded nodules of varying size, scattered throughout the organ. Sometimes, the metastasis may grow bigger than the primary tumour. Metastatic deposits just like primary tumour may cause further dissemination via lymphatics and blood vessels.

Microscopically, the secondary deposits generally reproduce the structure of primary tumour.

3. **OTHER ROUTES OF METASTASIS.** Some uncommon routes of distant spread are as under:

i) **Transcoelomic spread.** Certain cancers invade through the serosal wall of the coelomic cavity so that tumour fragments or clusters of tumour cells break off to be carried in the coelomic fluid and are implanted elsewhere in the body cavity. Peritoneal cavity is involved most often, but occasionally pleural and pericardial cavities are also affected. Transcoelomic spread in peritoneum by carcinoma of the stomach and ovary are important examples, while pleura and pericardium are often involved by carcinoma of the bronchus and breast.

ii) **Spread along epithelium-lined surfaces.** It is unusual for a malignant tumour to spread along the epithelium-lined surfaces because intact epithelium and mucus coat are quite resistant to penetration by tumour cells. However, exceptionally a malignant tumour may spread through the fallopian tube from the endometrium to the ovaries or vice-versa, through the bronchus into alveoli, and through the ureters from the kidneys into lower urinary tract.

iii) **Spread via cerebrospinal fluid.** Malignant tumour of the ependyma and leptomeninges may spread by release of tumour fragments and tumour cells into the CSF and produce metastases at other sites in the central nervous system.

iv) **Implantation.** Rarely, a tumour may spread by implantation by surgeon's scalpel, needles, sutures, or be implanted by direct contact such as transfer of cancer of the lower lip to the apposing upper lip.

MECHANISM AND BIOLOGY OF INVASION AND METASTASIS

Malignant tumours are distinguished from benign tumours by two of the most characteristic features:

- development of metastasis; and
- invasiveness.

The process of local invasion and distant spread (lymphatic and haematogenous) involves the following steps:

1. Invasion of the basement membrane
2. Passage through the extracellular matrix
3. Invasion of lymphatic or vascular channels
4. Extravasation of tumour cells

5. Survival and growth of metastatic deposit. However, malignant cells in large numbers are released into circulation from the primary tumour while only a few are able to establish the metastasis. This is explained on the basis of tumour heterogeneity, i.e. in the population of monoclonal tumours cells, a subpopulation or clone of tumour cells has the right biologic characteristics to complete the above steps involved in the development of metastasis.

1. **Invasion of the basement membrane.** The tumour is termed “invasive” when the epithelial tumour cells have breached through the underlying basement membrane, while malignant cells confined to the layers above the basement membrane is termed “carcinoma in situ”.

Normal epithelial cells adhere to each other and to other components by various adhesion molecules. These include the following:

i) *Epithelial (E) cadherins (cell-cell adhesion molecule) and catenins (E cadherin-cytoskeleton interaction)*, both suppress invasion and metastasis. In most carcinomas, the expression of E-cadherin and catenin is reduced or even lost which allows individual malignant cells to “loosen up” and leave the primary tumour.

ii) *Integrins* are transmembrane receptors causing adhesive interaction between individual tumour cells and in tumour cell-components of extracellular matrix interaction. The expression of integrins on the cell surface in human melanoma cells has been highlighted in invasion and metastasis of this tumour.

iii) *Immunoglobulin supergene family* includes several intercellular adhesion molecules which include intercellular adhesion molecule-1 (ICAM-1), MUC-18, CD44 and vascular cell adhesion molecule (VCAM-

1). Expression of ICAM-1, MUC-18 and CD44 is directly correlated with invasion and metastatic potential of tumours, while VCAM-1 (adhesion molecule between tumour cell-endothelium) is downregulated and hence results in detachment of tumour cells in invasion and metastatic clone of tumour.

2. **Passage through the extracellular matrix.** The tumour cells after attachment to the basement membrane migrate through it by the following mechanisms:

i) *Autocrine motility factor (AMF)*, a type of tumour cell cytokine, stimulates receptor-mediated motility of tumour cells, increased adhesion of tumour cells to the matrix and release of lysosomal enzyme,

ii) *Proteolytic enzymes* such as urokinase type plasminogen activator and matrix metalloproteins (MMP) of collagenase family are elaborated by malignant cells which bring about degradation of basement membrane components such as collagen type IV, laminin and proteoglycans.

3. **Invasion of lymphatic and vascular channels.** After the malignant cells have breached the basement membrane and invaded the extracellular matrix, these cells penetrate the lymphatic and vascular channels and hence reach the systemic circulation. However, a very small proportion of malignant cells (less than 0.1%) in the blood stream actually survive to develop into metastasis.

4. **Extravasation of tumour cells.** Tumour cells in the circulation (capillaries, venules, lymphatics) may mechanically block these vascular channels and attach to vascular endothelium. In this way, the sequence similar to local invasion is repeated and the basement membrane is exposed.

5. **Survival and growth of metastatic deposit.** The extravasated malignant cells on lodgement in the right environment grow further under the influence of growth factors produced by host tissues, tumour cells and by cleavage products of matrix components. These growth factors in particular include: PDGF, FGF, and TGF- β . The last one, TGF- β stimulates growth of newer vessels in the host tissue termed angiogenesis. The metastatic deposits grow further if the host immune defense mechanism fails to eliminate it. Metastatic deposits may further metastasise to the same organ or to other sites by similar mechanisms.

GRADING AND STAGING OF CANCER

“Grading” and “staging” are the two systems to determine the prognosis and choice of treatment after a malignant tumour is detected. Grading is defined as the macroscopic and microscopic degree of differentiation of the tumour, while staging means extent of spread of the tumour within the patient.

GRADING

Cancers may be graded grossly and microscopically. Gross features like exophytic or fungating appearance are indicative of less malignant growth than diffusely infiltrating tumours. However, grading is largely based on 2 important histologic features: the degree of anaplasia, and the rate of growth. Based on these features, cancers are categorized from grade I as the most differentiated, to grade III or IV as the most undifferentiated or anaplastic. Many systems of grading have been proposed but the one described by Broders for dividing squamous cell carcinoma into 4 grades depending upon the degree of differentiation is followed for other malignant tumours as well. Broders' grading is as under:

Grade I: Well-differentiated (less than 25% anaplastic cells).

Grade II: Moderately-differentiated (25-50% anaplastic cells).

Grade III: Moderately-differentiated (50-75% anaplastic cells).

Grade IV: Poorly-differentiated or anaplastic (more than 75% anaplastic cells).

However, grading of tumours has several short-comings. It is subjective and the degree of differentiation may vary from one area of tumour to the other. Therefore, it is common practice with pathologists to grade cancers in descriptive terms (e.g. well-differentiated, undifferentiated, keratinizing, non-keratinizing etc) rather than giving the tumours grade numbers.

STAGING

The extent of spread of cancers can be assessed by 3 ways – by clinical examination, by investigations, and by pathologic examination of the tissue removed. Two important staging systems currently followed are: TNM staging and AJC staging.

TNM staging. (T for primary tumour, N for regional nodal involvement, and M for distant metastases) was developed by the UICC (Union In-

ternationale Centre Cancer, Geneva). For each of the 3 components namely T, N and M, numbers are added to indicate the extent of involvement, as under:

T₀ to T₄: In situ lesion to largest and most extensive primary tumour.
 N₀ to N₃: No nodal involvement to widespread lymph node involvement. M₀ to M₂: No metastasis to disseminated haematogenous metastases.

Staging of Malignant Neoplasms

Stage	Definition
Tis	In situ, non-invasive (contined epithelium)
T1	Small, minimally invasive within primary organ site
T2	Larger, more invasive within the primary organ site
T3	Larger and/or invasive beyond margins of primary organ site
T4	Very larger and/or very invasive, spread to adjacent organs
N0	No lymph node involvement
N1	Regional lymph node involvement
N2	Extensive regional lymph node involvement
N3	More distant lymph node involvement
M0	No distant metastases
M1	Distant metastases present

AJC staging. (American Joint Committee staging) divides all cancers into stage 0 to IV, and takes into account all the 3 components of the preceding system (primary tumour, nodal involvement and distant metastases) in each stage.

TNM and AJC staging systems can be applied for staging most malignant tumours.

ETIOLOGY AND PATHOGENESIS OF NEOPLASIA

A lot of clinical and experimental research and epidemiological studies have been carried out in the field of oncology so as to know the possible causes of cancer and mechanisms involved in transformation of a normal

cell into a neoplastic cell. It is widely known that no single factor is responsible for development of tumours. The role of some factors in production of

neoplasia is established while that of others is epidemiological and many others are still unknown. Based on the current state of knowledge, these factors are broadly described under 2 main headings:

I. Predisposing epidemiologic factors or cofactors in carcinogenesis, which include a number of endogenous host factors and exogenous environmental factors.

II. Carcinogenesis, that encompasses exogenous agents like chemical, physical, hormonal and biological substances.

EPIDEMIOLOGIC FACTORS

Cancer incidence. The overall incidence of cancer in a population or country is known by registration of all cancer cases (cancer registry) and by rate of death from cancer. It is estimated that about 20% of all deaths are cancer-related. There have been changing patterns in incidence of cancers in both the sexes and in different geographic locations as outlined below.

1. **Familial and genetic factors.** It has long been suspected that familial predisposition and heredity play a role in the development of cancers. In general, the risk of developing cancer in relatives of a known cancer patient is almost three times higher as compared to control subjects. The overall estimates suggest that genetic cancers comprise not greater than 5% of all cancers. Some of the common examples are as under:

i) **Retinoblastoma.** About 40% of retinoblastomas are familial and show an autosomal dominant inheritance. Carriers of such genetic composition have 10000 times higher risk of developing retinoblastoma which is often bilateral. Such patients are predisposed to develop another primary malignant tumour, notably osteogenic sarcoma. Retinoblastoma susceptibility gene, Rb gene, located on chromosome 13 was the first cancer-predisposing gene identified.

ii) **Familial polyposis coli.** This condition has autosomal dominant inheritance. The polypoid adenomas may be seen at birth or in early age. By the age of 50 years, almost 100% cases of familial polyposis coli develop cancer of the colon.

iii) **Multiple endocrine neoplasia (MEN).** A combination of adenomas of pituitary, parathyroid and pancreatic islets (MEN-I) or syndrome of medul-

lary carcinoma thyroid, pheochromocytoma and parathyroid tumour (MEN-II) are encountered in families.

iv) **Neurofibromatosis or von Recklinghausen's disease.** This condition is characterized by multiple neurofibromas and pigmented skin spots (cafe au lait spots). These patients have family history consistent with autosomal dominant inheritance in 50% of patients.

v) **Cancer of the breast.** Female relatives of breast cancer patients have 2 to 3 times higher risk of developing breast cancer.

vi) **DNA-chromosomal instability syndromes.** These are a group of pre-neoplastic conditions having defect in DNA repair mechanism. A classical example is xeroderma pigmentosum, an autosomal recessive disorder, characterized by extreme sensitivity to ultraviolet radiation. The patients may develop various types of skin cancers such as basal cell carcinoma, squamous cell carcinoma and malignant melanoma.

2. **Racial and geographic factors.** Differences in racial incidence of some cancers may be partly attributed to the role of genetic composition but are largely due to influence of the environment and geographic differences affecting the whole population such as climate, soil, water, diet, habits, customs etc. Some of the examples of racial and geographic variations in various cancers are as under:

i) **White Europeans and Americans** develop most commonly malignancies of the lung, breast, and colon. Liver cancer is uncommon in these races. Breast cancer is uncommon in Japanese women but is more common in American women.

ii) **Black Africans**, on the other hand, have more commonly cancers of the skin, penis, cervix and liver.

iii) **Japanese** have five times higher incidence of carcinoma of the stomach than the Americans.

iv) **South-East Asians**, especially of Chinese origin have more commonly nasopharyngeal cancer.

3. **Environmental and cultural factors.** Surprising as it may seem, we are surrounded by an environment of carcinogens which we eat, drink, inhale and touch. Some of the examples are given below:

i) **Cigarette smoking** is the single most important environmental factor implicated in the etiology of cancer of the oral cavity, pharynx, larynx, oesophagus, lungs, pancreas and urinary bladder.

ii) **Alcohol abuse** predisposes to the development of cancer of oropharynx, larynx, oesophagus and liver.

iii) **Alcohol and tobacco together** further accentuate the risk of developing cancer of the upper aerodigestive tract.

iv) **Cancer of the cervix** is linked to a number of factors such as age at first coitus, frequency of coitus, multiplicity of partners, parity etc. Sexual partners of circumcised males have lower incidence of cervical cancer than the partners of uncircumcised males.

v) **Penile cancer** is rare in the Jews and Muslims as they are customarily circumcised. Carcinogenic component of smegma appears to play a role in the etiology of penile cancer.

vi) **Betel nut cancer** of the cheek and tongue is quite common in some parts of India due to habitual practice of keeping the bolus of paan in a particular place in mouth for a long time.

vii) A large number of **industrial and environmental substances** are carcinogenic and are occupational hazard for some populations. These include exposure to substances like arsenic, asbestos, benzene, vinyl chloride, naphthylamine etc.

viii) **Certain constituents of diet** have also been implicated in the causation of cancer. Overweight individuals, deficiency of vitamin A and people consuming diet rich in animal fats and low in fibre content are more at risk of developing certain cancers such as colonic cancer. Diet rich in vitamin E, on the other hand, possibly has some protective influence by its antioxidant action.

4. **Age.** Generally, cancers occur in older individuals past 5th decade of life, though there are variations in age incidence in different forms of cancers. It is not clear whether higher incidence of cancer in advanced age is due to alteration in the cells of the host, longer exposure to the effect of carcinogen, or decreased ability of the host immune response. Some tumours have two peaks of incidence e.g. acute leukaemias occur in children and in older age group. The biologic behaviour of tumours in children does not always correlate with histologic features. Besides acute leukaemias, other tumours in infancy and childhood are: neuroblastoma, nephroblastoma (Wilms' tumour), retinoblastoma, hepatoblastoma, rhabdomyosarcoma, Ewing's sarcoma, teratoma and CNS tumours.

5. **Sex.** Apart from the malignant tumours of organs peculiar to each sex, most tumours are generally more common in men than in women except cancer of the breast, gall bladder, thyroid and hypopharynx. Although there are geographic and racial variations, cancer of the breast is the commonest cancer in women throughout the world while lung cancer is the commonest cancer in men. The differences in incidence of certain cancers in the two sexes may be related to the presence of specific sex hormones.

6. **Premalignant lesions (tumour progression).** Premalignant lesions are a group of conditions which predispose to the subsequent development of cancer. Such conditions are important to recognize so as to prevent the subsequent occurrence of an invasive cancer. Many of these conditions are characterized by morphologic changes in the cells such as increased nuclear-cytoplasmic ratio, pleomorphism of cells and nuclei, increased mitotic activity, poor differentiation, and sometimes accompanied by chronic inflammatory cells.

Some examples of premalignant lesions are given below:

1. **Carcinoma in situ (intraepithelial neoplasia).** When the cytological features of malignancy are present but the malignant cells are confined to epithelium without invasion across the basement membrane, it is called as carcinoma in situ or intraepithelial neoplasia (CIN). The common sites are:

- i) Uterine cervix at the junction of ecto and endocervix,
- ii) Bowen's disease of the skin,
- iii) Actinic or solar keratosis,
- iv) Oral leukoplakia,

v) Intralobular and intraductal carcinoma of the breast. The area involved in carcinoma in situ may be single and small, or multifocal. As regards the behaviour of CIN, it may regress and return to normal or may develop into invasive cancer. In some instances such as in cervical cancer, there is a sequential transformation from squamous metaplasia, to epithelial dysplasia, to carcinoma in situ, and eventually to invasive cancer.

2. **Some benign tumours.** Commonly, benign tumours do not become malignant. However, there are some exceptions e.g.

i) Multiple adenomas of the large intestine have high incidence of developing adenocarcinoma.

ii) Neurofibromatosis (von Recklinghausen's disease) may develop into sarcoma.

3. **Miscellaneous conditions.** Certain inflammatory and hyperplastic conditions are prone to development of cancer, e.g.

i) Patients of long-standing ulcerative colitis are predisposed to develop colorectal cancer.

ii) Cirrhosis of the liver has predisposition to develop hepatocellular carcinoma.

iii) Chronic bronchitis in heavy cigarette smokers may develop cancer of the bronchus.

iv) Chronic irritation from jagged tooth or ill-fitting denture may lead to cancer of the oral cavity.

CARCINOGENESIS

Carcinogenesis means induction of tumours; agents which can induce tumours are called carcinogens. These terms are used for neoplastic proliferation of benign as well as malignant tumours, though the word 'carcino' implies cancer. Other terms used synonymously with carcinogenesis are oncogenesis and tumorogenesis.

Carcinogens are a variety of extrinsic agents which are broadly divided into 4 groups:

A. Chemical carcinogens,

B. Physical carcinogens (mainly radiation),

C. Hormonal carcinogens,

D. Biologic carcinogens (chiefly viruses).

Many of these agents have been shown to induce tumours in experimental animals, while some have evidence of their role in human beings.

A. CHEMICAL CARCINOGENESIS

The first ever evidence of any cause for neoplasia came from the observation of Sir Percival Pott in 1775 that there was higher incidence of cancer of the scrotum in chimney-sweeps in London than in the general population. This invoked wide interest in soot and coal tar as possible carcinogenic agent and the possibility of other occupational cancers. The first successful experimental induction of cancer was produced by two Japanese workers (Yamagiwa and Ichikawa) in 1914 in the rabbit's skin by repeatedly painting with coal tar. Since then the list of chemical carcinogens which

can experimentally induce cancer in animals and have epidemiological evidence in causing human neoplasia, is ever increasing.

Stages in Chemical Carcinogenesis

The induction of cancer by chemical carcinogens occurs after a delay – weeks to months in the case of experimental animals, and often several years in man. Other factors that influence the induction of cancer are the dose and mode of administration of carcinogenic chemical, individual susceptibility and various predisposing factors explained above.

Though the initial event is the conversion of “the target cell” into the “neoplastic cell”, the subsequent changes of cellular proliferation are quite important in the evolution of clinical cancer. The phenomena of cellular transformation by chemical carcinogens is a progressive process involving 2 distinct sequential stages: initiation and promotion of carcinogenesis.

1. Initiation of carcinogenesis

Initiation is the first stage in carcinogenesis induced by chemical carcinogens. The change can be produced by a single dose of the initiating agent for a short time, though larger dose for longer duration is more effective.

The change so induced is sudden, irreversible and permanent. Chemical carcinogens acting as initiators of carcinogenesis can be grouped into 2 categories:

I. Direct-acting carcinogens. These are a few chemical substances (e.g. Alkylating agents, acylating agents) which can induce cellular transformation without undergoing any prior metabolic activation.

II. Indirect-acting carcinogens or pro-carcinogens. These require metabolic conversion within the body so as to become “ultimate” carcinogens having carcinogenicity e.g. polycyclic aromatic hydrocarbons, aromatic amines, azo dyes, naturally-occurring products and others.

In either case, the following steps are involved in transforming “the target cell” into “the initiated cell”:

a) **Metabolic activation.** Vast majority of chemical carcinogens are indirect-acting or procarcinogens requiring metabolic activation, while direct-acting carcinogens do not require this activation. Most carcinogens are activated chiefly by the mixed oxidases of the cytochrome P-450 system located in the microsomal component of the endoplasmic reticulum or in the nucleus. The activity of the enzyme system and potency of the procarcino-

gen are affected by genetic and environmental influences, and stimulated by administration of drugs such as phenobarbital. In some circumstances, the procarcinogen may be detoxified and rendered inactive metabolically.

b) **Reactive electrophiles.** The two types of initiating carcinogens – direct-acting and procarcinogens, behave alike following metabolic activation of the latter. These substances become electron-deficient i.e. reactive electrophiles, which bind to electron-rich portions of other molecules of the cell such as DNA, RNA and other proteins.

c) **Target molecules.** The primary target of electrophiles is DNA, producing mutagenesis. The change in DNA may lead to 'the initiated cell' or some form of cellular enzymes may be able to repair the damage in DNA. The classic example of the latter situation is xeroderma pigmentosum, a precancerous condition, in which there is hereditary defect in DNA repair mechanism of the cell.

d) **The initiated cell.** The unrepaired damage produced in the DNA of the cell becomes permanent only if the altered cell undergoes at least one cycle of proliferation. This results in transferring the change to the next progeny of cells so that the DNA damage becomes permanent and irreversible, which are the characteristics of the initiated cell, vulnerable to the action of promoters of carcinogenesis.

2. Promotion of carcinogenesis

Promotion is the next sequential stage in the chemical carcinogenesis. Promoters of carcinogenesis are substances such as phorbol esters, phenols, hormones, artificial sweeteners and drugs like phenobarbital. They differ from initiators in the following respects:

- i) They do not produce sudden change,
- ii) They require application or administration, as the case may be, for sufficient time and in sufficient dose,
- iii) The change induced may be reversible,
- iv) They do not damage the DNA per se but instead enhance the effect of direct-acting carcinogens or procarcinogens.
- v) Tumour promoters act by activation of growth factor pathways leading to further clonal proliferation and expansion of initiated (mutated) cells. Though tumour promoters themselves are not mutagenic, they can cause DNA breaks in some cells by formation of oxygen-derived free radicals.

CO-CARCINOGENS. When the process of carcinogenesis is continued uninterrupted by replacing one chemical carcinogen with another, or alternatively, when two carcinogens are administered simultaneously to enhance the effect it is called co-carcinogenesis and such substances are called co-carcinogens.

CARCINOGENIC CHEMICALS IN HUMANS

The list of diverse chemical compounds which can produce cancer in experimental animals is a long one but only some of them have sufficient epidemiological evidence in human neoplasia.

Depending upon the mode of action of carcinogenic chemicals, they are divided into 2 broad groups:

1. Initiators of carcinogenesis,
2. Promoters of carcinogenesis

1. INITIATORS OF CARCINOGENESIS

Chemical carcinogens which can initiate the process of neoplastic transformation are further categorized into 2 subgroups – direct-acting carcinogens and indirect-acting carcinogens or procarcinogens.

I. Direct-acting carcinogens. These chemical carcinogens do not require metabolic activation and fall into 2 classes:

a) **Alkylating agents.** This group includes mainly various anti-cancer drugs (e.g. cyclophosphamide, chlorambucil, busulfan, melphalan, nitrosourea etc), 5-propiolactone and epoxides. They are weakly carcinogenic and are implicated in the etiology of the lymphomas and leukaemias in human beings.

b) **Acylating agents.** These are substances like acetyl imidazole which is a direct-acting chemical carcinogen.

II. Indirect-acting carcinogens (pro-carcinogens). These are chemical substances requiring metabolic activation for becoming potent “ultimate” carcinogens. Majority of carcinogenic chemicals fall into this group. These include the following classes:

a) **Polycyclic aromatic hydrocarbons.** They comprise the largest group of common procarcinogens which, after metabolic activation, can induce neoplasia in many tissues in experimental animals and are also implicated in a number of human neoplasms. Main sources of polycyclic aromatic hydrocarbons are tobacco, smoke, fossil fuel (e.g. coal), soot, tar,

mineral oil, smoked animal foods, industrial and atmospheric pollutants. Important chemical compounds included are benzanthracene, benzapyrene and methylcholanthrene. The following examples have evidence to support the etiologic role of these substances:

- Smoking: There is 20 times higher incidence of lung cancer in smokers of 2 packs (40 cigarettes) per day for 20 years.

- Skin cancer: Direct contact of polycyclic aromatic hydrocarbon compounds with skin is associated with higher incidence of skin cancer. For example, the natives of Kashmir carry an earthen pot containing embers, the kangri, under their clothes close to abdomen for purposes of warmth, and skin cancer of the abdominal wall termed kangri cancer is common among them.

- Tobacco and betel nut chewing: Cancer of the oral cavity is more common in people chewing tobacco and betel nuts. The chutta is a cigar that is smoked in South India (in Andhra Pradesh) with the lighted end in the mouth (i.e. reversed smoking) and such individuals have higher incidence of cancer of the mouth.

b) **Aromatic amines and azodyes.** This category includes the following substances implicated in chemical carcinogenesis:

- 3-naphthylamine in the causation of bladder cancer, especially in aniline dye and rubber industry workers.

- Benzidine in the induction of bladder cancer.

- Azo-dyes used for colouring foods (e.g. butter yellow for colouring margarine and butter, scarlet red for colouring cherries etc) in the causation of hepato-cellular carcinoma.

- Acetyl aminofluorene in experimental induction of hepatocellular carcinoma.

c) **Naturally-occurring products.** Some of the important chemical carcinogens derived from plant and microbial sources are aflatoxin B₁, actinomycin D, mitomycin C, safrole and betel nuts. Out of these, aflatoxin B₁ implicated in causing human hepatocellular carcinoma is the most important. It is derived from the fungus, *Aspergillus flavus*, that grows in stored grains and plants.

d) **Miscellaneous.** A variety of other chemical carcinogens having a role in the etiology of human cancer are as under:

- Nitroso-compounds like nitrosamines involved in gastric carcinoma.

- Vinyl chloride monomer derived from PVC (polyvinylchloride) polymer in the causation of haemangio-sarcoma of the liver.
- Asbestos in bronchogenic carcinoma and mesothelioma.
- Arsenical compounds in causing epidermal hyperplasia and basal cell carcinoma.
- Metals like nickel, lead, cobalt chromium etc. in industrial workers causing lung cancer.
- Insecticides and fungicides (e.g. aldrin, dieldrin, chlordane) in carcinogenesis in experimental animals.

2. PROMOTERS OF CARCINOGENESIS

Certain chemical substances lacking the intrinsic carcinogenic potential but helping the initiated cell to proliferate further are called promoters of carcinogenesis. Some of such substances which are either known promoters in experimental animals or are implicated as promoters in human neoplasia are:

- a) Phorbol esters such as TPA,
- b) Phenols,
- c) Hormones, e.g. effect of oestrogen on endometrium or breast,
- d) Drugs, e.g. Phenobarbital,
- e) Artificial sweeteners, e.g. saccharin and cyclamates.

Tests for Chemical Carcinogenicity

There are 2 main methods of testing chemical compound for its carcinogenicity:

1. **EXPERIMENTAL INDUCTION.** The traditional method is to administer the chemical compound under test to a batch of experimental animals like mice or other rodents by an appropriate route e.g. painting on the skin, giving orally or parenterally, or by inhalation.

The chemical is administered repeatedly, the dose varied, and promoting agents are administered subsequently. After many months, the animal is autopsied and results obtained. However, all positive or negative tests cannot be applied to humans since there is sufficient species variation in susceptibility to particular carcinogen. Besides, the test is rather prolonged and expensive.

2. **TESTS FOR MUTAGENICITY (AMES' TEST).** A mutagen is a substance that can permanently alter the genetic composition of a cell. Ames' test

evaluates the ability of a chemical to induce mutation in the mutant strain of *Salmonella typhimurium* that cannot synthesise histidine. Such strains are incubated with the potential carcinogen to which liver homogenate is added to supply enzymes required to convert procarcinogen to ultimate carcinogen. If the chemical under test is mutagenic, it will induce mutation in the mutant strains of *S. typhimurium* in the form of functional histidine gene, which will be reflected by the number of bacterial colonies growing on histidine-free culture medium. Most of the carcinogenic chemicals tested positive in Ames' test are carcinogenic in vivo.

B. PHYSICAL CARCINOGENESIS

Physical agents in carcinogenesis are divided into 2 groups:

1. Radiation both ultraviolet light and ionising radiation, is the most important physical agent.
2. Non-radiation physical agents are the various forms of injury and are less important.

1. Radiation Carcinogenesis

Ultraviolet (UV) light and ionising radiation are the two main forms of radiation carcinogens which can induce cancer in experimental animals and are implicated in causation of some forms of human cancers. A property common between the two forms of radiation carcinogens is the appearance of carcinogenic effects long after exposure, often 10-20- years or even later. Also, radiation carcinogens may act to enhance the effect of another carcinogen i.e. they may act as co-carcinogens. Ultraviolet light and ionising radiation differ in their mode of action as described below:

i) **ULTRAVIOLET LIGHT.** The main source of UV radiation is the sunlight; others are UV lamps and Welder's arcs. UV light penetrates the skin for a few millimetres only so that its effect is limited to epidermis. The efficiency of UV light as carcinogen depends upon the extent of light-absorbing protective melanin pigmentation of the skin. In humans, excessive exposure to UV rays can cause various forms of skin cancers – squamous cell carcinoma, basal cell carcinoma and malignant melanoma. In support of this is the epidemiological evidence of high incidence of these skin cancers in fair-skinned Europeans, albinos who do not tan readily, in people of Queensland in Australia living close to the equator who receive more sunlight, and in farmers and outdoor workers due to the effect of actinic light radiation.

Mechanism. UV radiation may have various effects on the cells. The most important is induction of mutation; others are inhibition of cell division, inactivation of enzymes and sometimes killing of the cells. The most important biochemical effect of UV radiation is the formation of pyrimidine dimers in DNA but such UV-induced DNA damage in normal individuals is repaired. The proof in favour of mutagenic effect of UV radiation comes from recessive hereditary diseases characterized by a defect in DNA repair mechanism and associated with high incidence of cancers. Such examples are as under:

- a) Xeroderma pigmentosum is predisposed to skin cancers at younger age (under 20 years of age).
- b) Ataxia telangiectasia is predisposed to leukaemia.
- c) Bloom's syndrome is predisposed to all types of cancers.

ii) **IONISING RADIATION.** Ionising radiation of all kinds like X-rays, α -, β - and γ -rays, radioactive isotopes, protons and neutrons can cause cancer in animals and in man. Most frequently radiation-induced cancers are all forms of leukaemias (except chronic lymphocytic leukaemia); others are cancers of the thyroid, skin, breast, lung and salivary glands. The risk is increased by higher dose and with high LET (linear energy transfer) such as in neutrons and α -rays than with low LET as in X-rays and γ -rays. The evidence in support of carcinogenic role of ionising radiation is cited in the following examples:

a) Higher incidence of radiation dermatitis and subsequent malignant tumours of the skin was noted in X-ray workers and radiotherapists who did initial pioneering work in these fields before the advent of safety measures.

b) High incidence of osteosarcoma was observed in young American watch-working girls engaged in painting the dials with luminous radium who unknowingly ingested radium while using lips to point their brushes.

c) Miners in radioactive elements have higher incidence of cancers.

d) Japanese atom bomb survivors of the twin cities of Hiroshima and Nagasaki after World War II have increased frequency of malignant tumours, notably acute and chronic myeloid leukaemias, and various solid tumours of breast, colon, thyroid and lung.

e) More recently in 1985, about 5000 people fell victim to hazardous effects of radioactive material due to leakage of nuclear reactor at Chernobyl (in erstwhile USSR, now in Ukraine).

f) Therapeutic X-ray irradiation may result in increased frequency of cancers such as in patients of ankylosing spondylitis, in children with enlarged thymus, and in children irradiated in utero during investigations on the mother.

Mechanism. How ionising radiation induces cancer is not clearly known. Radiation damages the DNA of the cell by one of the 2 possible mechanisms:

a) It may directly alter the cellular DNA.

b) It may dislodge ions from water and other molecules of the cell and result in formation of highly reactive free radicals that may bring about the damage.

Damage to the DNA resulting in mutagenesis is the most important action of ionising radiation. The effect depends upon a number of factors such as type of radiation, dose, dose-rate and various host factors such as age, individual susceptibility, immune competence, hormonal influences and type of cells irradiated.

2. Non-radiation Physical Carcinogenesis

Mechanical injury to the tissues such as from stones in the gallbladder, stones in the urinary tract, and healed scars following burns or trauma, has been suggested as the cause of increased risk of carcinoma in these tissues but the evidence is not convincing. Asbestosis and asbestos-associated tumours of the lung are discussed on page 462; the characteristic tumour being malignant mesothelioma of the pleura. Other examples of physical agents in carcinogenesis are the implants of inert materials such as plastic, glass etc in prostheses or otherwise, and foreign bodies observed to cause tumour development in experimental animals. However, tumorigenesis by these materials in humans is rare.

C. HORMONAL CARCINOGENESIS

Cancer is more likely to develop in organs and tissues which undergo proliferation under the influence of excessive hormonal stimulation. On cessation of hormonal stimulation, such tissues become atrophic. Hormone-sensitive tissues developing tumours are the breast, endometrium, myometrium, vagina, thyroid, liver, prostate and testis. Some examples of hor-

mones influencing carcinogenesis in experimental animals and humans are given below.

1. **OESTROGEN.** Examples of oestrogen-induced cancers are as under:

i) *In experimental animals.* Induction of breast cancer in mice by administration of high dose of oestrogen and reduction of the tumour development following oophorectomy is the most important example. Associated infection with mouse mammary tumour virus (MMTV, Bittner milk factor) has an added influence on the development of breast cancer in mice. Other cancers which can be experimentally induced in mice by oestrogens are squamous cell carcinoma of the cervix, connective tissue tumour of the myometrium, Leydig cell tumour of the testis in male mice, tumour of the kidney in hamsters, and benign as well as malignant tumours of the liver in rats.

ii) *In humans.* Women receiving oestrogen therapy and women with oestrogen-secreting granulosa cell tumour of the ovary have increased risk of developing endometrial carcinoma. Adenocarcinoma of the vagina is seen with increased frequency in adolescent daughters of mothers who had received oestrogen-therapy during pregnancy.

2. **CONTRACEPTIVE HORMONES.** The sequential types of oral contraceptives increase the risk of developing breast cancer. Other tumours showing a slightly increased frequency in women receiving contraceptive pills for long durations are benign tumours of the liver, and a few patients have been reported to have developed hepatocellular carcinoma.

3. **ANABOLIC STEROIDS.** Consumption of anabolic steroids by athletes to increase the muscle mass is not only unethical athletic practice but also increases the risk of developing benign and malignant tumours of the liver.

4. **HORMONE-DEPENDENT TUMOURS.** It has been shown in experimental animals that induction of hyperfunction of adeno-hypophysis is associated with increased risk of developing neoplasia of the target organs following preceding functional hyperplasia. There is tumour regression on removal of the stimulus for excessive hormonal secretion. A few examples of such phenomena are seen in humans:

i) Prostatic cancer usually responds to the administration of oestrogens.

ii) Breast cancer may regress with oophorectomy, hypophysectomy or on administration of male hormones,

iii) Thyroid cancer may slow down in growth with administration of thyroxine that suppresses the secretion of TSH by the pituitary.

D. BIOLOGIC CARCINOGENESIS

The epidemiological studies on different types of cancers indicate the involvement of transmissible biologic agents in their development, chiefly viruses. Other biologic agents implicated in carcinogenesis are parasites (e.g. schistosomiasis) which is associated with high incidence of cancer of the urinary bladder in some parts of the world such as in Egypt) and more recently bacteria (*Helicobacter pylori* infection in the causation of gastric lymphoma and gastric carcinoma). The role of viruses in the causation of cancer is more significant. It has been estimated that about 20% of all cancers worldwide are virus-associated cancers. Therefore, biologic carcinogenesis is largely viral carcinogenesis, described below.

VIRAL CARCINOGENESIS

The association of oncogenic viruses with neoplasia was first observed by an Italian physician Sanarelli in 1889 who noted association between myxomatosis of rabbits with poxvirus. The contagious nature of the common human wart was first established in 1907. Since then, a number of viruses capable of inducing tumours (oncogenic viruses) in experimental animals, and some implicated in man, have been identified.

Oncogenic viruses can be transmitted by one of the 3 routes:

- i) Vertical transmission, when the infection is genetically transmitted from infected parents to offsprings,
- ii) Horizontal transmission, when the infection passes from one to another by direct contact as occurs in most contagious diseases.
- iii) By inoculation as is done in experimental animals.

When target cells are grown in vitro with oncogenic virus, the cultured cells lose contact inhibition and are piled on top of one another rather than growing in regular monolayer which the normal cells do. Such virus-induced altered cells are called “transformed cells” which are capable of growing into a neoplasm. Recent advances in molecular biology of carcinogenesis have greatly enhanced our knowledge of virus-induced neo-plastic processes.

Based on their nucleic acid content, oncogenic viruses fall into 2 broad groups:

1. Those containing deoxyribonucleic acid are called DNA oncogenic viruses.
2. Those containing ribonucleic acid are termed RNA oncogenic viruses.

The two types of oncogenic viruses are described separately below, followed by their mechanisms of action. Most of the work is based on studies in experimental animals and only some have a role in human neoplasia.

DNA Oncogenic Viruses

DNA oncogenic viruses are classified into 5 subgroups, each of which is capable of producing neoplasms in different hosts. These are: Papovaviruses, Herpesviruses, Adenoviruses, Poxviruses and Hepadna viruses.

1. **PAPOVAVIRUSES.** This group consists of the papilloma virus including the human papilloma virus (HPV), polyoma virus and SV-40 (simian vacuolating) virus. These viruses have an etiologic role in a variety of benign and malignant neoplasms in animals and in humans:

- i) **Papilloma viruses.** These viruses were the first to be implicated in the etiology of any human neoplasia. These viruses appear to replicate in the layers of stratified squamous epithelium. About 50 different types of HPV have been identified. Those implicated in the causation of benign squamous papillomas include types 1, 2, 4 and 7, while DNA sequence of types 16 and 18 are found in squamous cell carcinoma. The following examples are cited to demonstrate their role in oncogenesis:

In humans:

- HPV was first detected as etiologic agent in common skin warts or papillomas by Shope in 1933. The condition is infectious.

- HPV is responsible for causing an uncommon condition, epidermodysplasia verruciformis. The condition is characterized by multiple skin warts and a genetic defect in the cell-mediated immunity. About one third of cases develop squamous cell carcinoma in the sun-exposed warts.

- The same virus HPV (types 6 and 11) may cause genital warts or condyloma acuminata.

- Some strains of HPV are responsible for causing multiple juvenile papillomas of the larynx.

- There is strong association between HPV infection with high risk types (HPV types 16 and 18) and development of genital neoplasia, especially cervical cancer.

In animals:

- Benign warty lesions similar to those seen in humans are produced by different members of the papilloma virus family in susceptible animals such as in rabbits by cottontail rabbit papilloma virus, and in cattle by bovine papilloma virus (BPV).

- There is evidence to suggest the association of BPV and cancer of the alimentary tract in cattle.

ii) **Polyoma virus.** Polyoma virus occurs as a natural infection in mice.

- In animals:

Polyoma virus infection is responsible for various kinds of carcinomas and sarcomas in immunodeficient (nude) mice and other rodents.

- In humans:

Polyoma virus infection is not known to produce any human tumour but can cause progressive demyelinating leucoencephalopathy which is a fatal demyelinating disease.

iii) **SV-40 virus.** As the name suggests, simian vacuolating virus occurs in monkeys without causing any harm but can induce sarcoma in hamsters. There is no evidence of involvement of SV-40 infection in causing any human tumours.

2. **HERPESVIRUSES.** Important members of herpesvirus family are Epstein-Barr virus, herpes simplex virus type 2 (HSV-2) and human herpesvirus 8 (HHV8), cytomegalovirus (CMV), Lucke's frog virus and Marek's disease virus. Of these, Lucke's frog virus and Marek's disease virus are implicated in animal tumours only (renal cell carcinoma and T-cell leukaemia-lymphoma respectively) while the oncogenic role of HSV-2 and CMV in human tumours has been refuted. The other two – EBV and HHV are implicated in human tumours as follows. Primary infection of all the herpes viruses in man persists probably for life in a latent stage which can get reactivated later.

EPSTEIN-BARR VIRUS (EBV). EBV infects human B-lymphocytes and stimulates them to proliferate. EBV is implicated in 2 human tumours –

Burkitt's lymphoma and anaplastic nasopharyngeal carcinoma. It is also shown to be the cause of infectious mononucleosis in man.

Burkitt's lymphoma. Burkitt's lymphoma was initially noticed in African children by Burkitt in 1958 but is now known to occur in 2 forms – African endemic form, and sporadic form seen elsewhere in the world. The morphological aspects of the tumour are explained on page 419 while oncogenesis is described here.

There is strong evidence linking Burkitt's lymphoma, a B- lymphocyte neoplasm, with EBV as observed from the following features:

- a) Over 90% of Burkitt's lymphomas are EBV-positive in which each tumour cell carries the virus.
- b) 100% cases of Burkitt's lymphoma show elevated levels of anti- body titers to various EBV antigens.
- c) EBV has strong tropism for B lymphocytes. EBV-infected B cells grown in cultures are immortalized i.e. they continue to develop further along B cell-line to propagate their progeny in the altered form.
- d) Though EBV infection is almost worldwide in all adults and is also known to cause self-limiting infectious mononucleosis, but the fraction of EBV-infected circulating B cells in such individuals is extremely small.
- e) Linkage between Burkitt's lymphoma and EBV infection is very high in African endemic form of the disease and probably in cases of AIDS than in sporadic form of the disease.

However, a few observations, especially regarding sporadic cases of Burkitt's lymphoma, suggest that certain other supportive factors may be contributing. Immunosuppression appears to be one such most significant factor. The evidence in favour is as follows:

- The normal EBV-infected individuals as well as cases developing infectious mononucleosis are able to mount good immune response so that they do not develop Burkitt's lymphoma.
- There is prevalence of Burkitt's lymphoma in cases of AIDS in which there is marked reduction in body's T-cell immune response.
- It is observed that malaria, which confers immuno-suppressive effect on the host, is prevalent in endemic proportions in regions where endemic form of Burkitt's lymphoma is frequent. This supports the linkage of EBV infection and immunosuppression in the etiology of Burkitt's lymphoma.

Anaplastic nasopharyngeal carcinoma. This is the other tumour having close association with EBV infection. The tumour is prevalent in

South-East Asia and in Eskimos. The evidence linking EBV infection with this tumour is as follows:

- a) 100% cases of nasopharyngeal carcinoma carry DNA of EBV.
- b) Individuals with this tumour have high titers of antibodies to various EBV antigens.

However, like in case of Burkitt's lymphoma, there may be some co-factors that account for the unusual geographic distribution.

HUMAN HERPESVIRUS 8 (HHV 8). Recently it has been shown that infection with a new virus, HHV 8 or Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with Kaposi's sarcoma, a vascular neoplasm common in patients of AIDS. HHV 8 has lymphotropism and is also implicated in causation of B cell lymphoma and multicentric variant of Castleman's disease.

3. **ADENOVIRUSES.** The human adenoviruses cause upper respiratory infections and pharyngitis.

- In man, they are not known to be involved in any tumour.
- In hamsters, they may induce sarcomas.

4. **POXVIRUSES.** This group of oncogenic viruses is involved in the etiology of following lesions:

- In rabbits – poxviruses cause myxomatosis.
- In humans – poxviruses cause molluscum contagiosum and may induce squamous cell papilloma.

5. **HEPADNAVIRUSES.** Hepatitis B virus (HBV) is a member of hepadnavirus family. HBV infection in man causes an acute hepatitis and is responsible for a carrier state, which can result in some cases to chronic hepatitis progressing to hepatic cirrhosis, and onto hepatocellular carcinoma. Suffice this to say here that there is strong epidemiological evidence linking HBV infection to development of hepatocellular carcinoma as evidenced by the following:

a) The geographic differences in the incidence of hepatocellular carcinoma closely match the variation in prevalence of HBV infection e.g. high incidence in Far-East and Africa.

b) Epidemiological studies in high incidence regions indicate about 200 times higher risk of developing hepatocellular carcinoma in HBV-infected cases as compared to uninfected population in the same area.

Possible mechanism of hepatocellular carcinoma occurring in those harbouring long association with HBV is chronic destruction of HBV-infected hepatocytes followed by continued hepatocyte proliferation. This process renders the hepatocytes vulnerable to the action of other risk factors such as aflatoxin causing mutation and neoplastic proliferation.

More recent evidence has assigned an oncogenic role to another hepatotropic virus, hepatitis C virus (HCV), an RNA virus unrelated to HBV. HCV is implicated in about half the cases of hepatocellular carcinoma in much the same way as HBV.

RNA ONCOGENIC VIRUSES

RNA oncogenic viruses are retroviruses i.e. they contain the enzyme reverse transcriptase, though all retroviruses are not oncogenic. The enzyme, reverse transcriptase, is required for reverse transcription of viral RNA to synthesise, viral DNA strands i.e. reverse of normal in which DNA is transcribed into messenger RNA. Based on their activity to transform target cells into neoplastic cells, they are divided into 3 subgroups: acute transforming viruses, slow transforming viruses, and human T-cell lymphotropic viruses (HTLV). The former two are implicated in inducing a variety of tumours in animals while HTLV is causative for human T-cell leukaemia and lymphoma.

1. Acute transforming viruses. This group includes retroviruses which transform all the cells infected by them into malignant cells rapidly ("acute"). All the viruses in this group possess one or more viral oncogenes (v-oncs). All the members of acute transforming viruses discovered so far are defective viruses in which the particular v-onc has substituted other essential genetic material such as gag, pol and env. These defective viruses cannot replicate by themselves unless the host cell is infected by another "helper virus". Acute oncogenic viruses have not been detected in any human tumour so far, though they have been identified in tumours in different animals, e.g.

a) Rous sarcoma virus in chickens.

b) Leukaemia-sarcoma viruses of various types such as avian, feline, bovine and primate.

2. Slow transforming viruses. These oncogenic retroviruses cause development of leukaemias and lymphomas in different species of animals (e.g. in mice, cats and bovine) and include the mouse mammary tumour virus (MMTV) that causes breast cancer in the daughter-mice suckled by the

MMTV- infected mother via the causal agent in the mother's milk (Bittner milk factor). These viruses have long incubation period between infection and development of neoplastic transformation (“slow”). Slow transforming viruses cause neoplastic transformation by insertional mutagenesis i.e. viral DNA synthesized by viral RNA via reverse transcriptase is inserted or integrated near the proto-oncogenes of the host cell resulting in enhanced expression of proto-oncogenes as well as causes genetic damage (mutagenesis) to the host cell genome leading to neoplastic transformation.

3. **Human t-cell lymphotropic viruses (HTLV).** HTLV is a form of slow transforming virus but is described separately because of 2 reasons:

- i) This is the only retrovirus implicated in human cancer.
- ii) The mechanism of neoplastic transformation is different from slow transforming as well as from acute transforming viruses.

Four types of HTLVs are recognized – HTLV-I, HTLV-II, HTLV-III and HTLV-IV. It may be mentioned in passing here that the etiologic agent for AIDS, HIV, is also an HTLV (HTLV-III).

A link between HTLV-I infection and adult T-cell leukaemia-lymphoma syndrome (ATLL) has been identified while HTLV-II is implicated in causation of T-cell variant of hairy cell leukaemia. HTLV-I is transmitted through sexual contact, by blood, or to infants during breast feeding. The highlights of this association and mode of neoplastic transformation are as under:

- i) Epidemiological studies by tests for antibodies have shown that HTLV-I infection is endemic in parts of Japan and West Indies where the incidence of ATLL is high. The latent period after HTLV-I infection is, however, very long (20-30 years).

- ii) The initiation of neoplastic process is similar to that for Burkitt's lymphoma except that HTLV-I has tropism for T-lymphocytes (especially CD4 subset of T cells similar to HIV infection in AIDS), while EBV of Burkitt's lymphoma has tropism for B lymphocytes.

- iii) As in Burkitt's lymphoma, immunosuppression plays a supportive role in the neoplastic transformation by HTLV-I infection.

The underlying molecular mechanism of neoplastic transformation by HTLV-I infection differs from acute transforming viruses because it does not contain v-onc, and from other slow transforming viruses because it does not have fixed site of insertion for insertional mutagenesis.

The genome of HTLV-I contains gag, pol, env and long terminal repeat (LTR) regions similar to other retro-viruses but in addition it contains tat gene region as well which stimulates neoplastic cell proliferation of T cells. Initially, this proliferation of T cells is polyclonal which then undergoes new mutations and leads to monoclonal T cell leukaemia-lymphoma. Chronic leukaemia retrovirus, HTLV-II, brings about transformation by 'promoter insertion' that produces onco-genesis by oncogenes and growth factors.

MECHANISMS OF VIRAL ONCOGENESIS

1. **Mode of DNA viral oncogenesis.** Host cells infected by DNA oncogenic viruses may have one of the following 2 results:

i) *Replication.* The virus may replicate in the host cell with consequent lysis of the infected cell and release of virions.

ii) *Integration.* The viral DNA may integrate into the host cell DNA.

The latter event (integration) results in neoplastic transformation of the host cell, while the former (replication) brings about cell death but no neoplastic transformation. A feature essential for host cell transformation is the expression of virus-specific (transforming protein) antigens immediately after infection of the host cell by DNA oncogenic virus.

2. **Mode of RNA viral oncogenesis.** As the name suggests, RNA viruses or retroviruses contain two identical strands of RNA and the enzyme, reverse transcriptase. The steps involved in transformation of host cells by RNA oncogenic viruses are as under:

i) Reverse transcriptase is RNA-dependent DNA synthetase that acts as a template to synthesise a single strand of matching viral DNA i.e. reverse of the normal in which DNA is transcribed into messenger RNA.

ii) The single strand of viral DNA is then copied by DNA dependent DNA synthetase to form another strand of complementary DNA resulting in double-stranded viral DNA or provirus.

iii) The provirus is then integrated into the DNA of the host cell genome and may transform the cell into neoplastic cell.

iv) Retroviruses are replication-competent. The host cells which allow replication of integrated retrovirus are called permissive cells. Non-permissible cells do not permit replication of the integrated retrovirus.

v) Viral replication begins after integration of the pro-virus into host cell genome. Integration results in transcription of proviral genes or pro- genes into messenger RNA which then forms components of the virus par-

ticle – virion core protein from gag gene, reverse transcriptase from pol gene, and envelope glycoprotein from env gene. The three components of virus particle are then assembled at the plasma membrane of the host cell and the virus particles released by budding off from the plasma membrane, thus completing the process of replication.

VIRUSES AND HUMAN CANCER: A SUMMARY

In man, epidemiological as well as circumstantial evidence has been accumulating since the discovery of contagious nature of common human wart (papilloma) in 1907 that cancer may have viral etiology. Presently, about 20% of all human cancers worldwide are believed to have 'virus association'. Aside from experimental evidence, the etiologic role of DNA and RNA viruses in a variety of human neoplasms has already been explained above. Here, a summary of different viruses implicated in human tumours is presented:

1. There are 2 conditions which are actually doubtful as tumours in which definite viral etiology is established. These are:

- i) Human wart (papilloma) caused by human papilloma virus; and
- ii) Molluscum coritagiosum caused by poxvirus.

2. The following 8 human cancers have enough epidemiological and serological evidence that viruses, in addition to other concomitant factors, are implicated in their etiology:

- i) Burkitt's lymphoma by Epstein-Barr virus.
- ii) Nasopharyngeal carcinoma by Epstein-Barr virus.
- iii) Primary hepatocellular carcinoma by hepatitis B virus and hepato- tis C virus.
- iv) Cervical cancer by high risk human papilloma virus types (HPV 16 and 18).
- v) Kaposi's sarcoma by human herpes virus type 8 (HHV 8).
- vi) B cell lymphoma by HHV8.
- vii) Adult T-cell leukaemia and lymphoma by HTLV-I.
- viii) T-cell variant of hairy cell leukaemia by HTLV-II.

MOLECULAR GENETICS OF CANCER

Genetic basis for cancer has long been suspected due to the following observations:

- 1. Hereditary predisposition in some cancers.

2. Chromosomal abnormalities in many forms of cancers.
3. Correlation of syndrome of defective DNA repair mechanisms and occurrence of cancers.
4. Genetic damage (mutagenesis) by the action of various exogenous agents followed by progression to carcinogenesis.

However, in the last decade of 20th Century, there has been vast accumulation of literature to explain the genetic basis of cancer at molecular level.

Broadly speaking, genes and molecular factors involved in the pathogenesis of cancer can be grouped into 4 categories.

1. Oncogenes (i.e. cancer causing genes).
2. Anti-oncogenes (i.e, cancer suppressor genes).
3. Mutator genes (i.e. genes that regulate DNA repair).
4. Telomerase in cancer (i.e. telomere shortening as cancer suppressor mechanisms).

Oncogenes are cancer causing genes. They are derived from proto-oncogenes or cellular oncogenes (abbreviated as c-oncs) which are detected on normal animal and human cells and promote normal growth and differentiation of cells. Historically, c-oncs were first discovered in the genome of animal tumours caused by viruses. In the case of DNA oncogenic viruses in animal tumours (e.g. chicken leukaemia-lymphoma virus, Shope papilloma virus, polyoma virus, SV 40 virus), viral DNA is incorporated into the cellular genome, while retroviruses in animal tumours (e.g. Rous sarcoma virus, Bittner milk factor) are transduced during their passage through cellular DNA. Since these genes were initially discovered as viral genes, proto-oncogenes are also called viral oncogenes abbreviated as v-oncs. Thus proto-oncogenes become oncogenic by retroviral transduction (v-oncs) and change them into cellular oncogenes (c-oncs) in animal tumour induction experiments.

Different types of v-oncs are named by three letter word derived from the oncogenic virus e.g. v-ras for Rat Sarcoma virus, v-fes for Feline Sarcoma virus etc., while their corresponding proto-oncogenes are termed simply as ras and fes respectively.

While in animals, identification of v-onc genes is by induction of tumours by acute and slow transforming retroviruses, predictably human tumours of viral as well as nonviral etiology too would have similar mutations termed human oncogenes. Such human oncogenes are identified by DNA transfection (i.e. gene transfer in vitro into cultured mouse fibroblasts) and by non-random chromosomal abnormalities (e.g. translocations).

Mechanisms of activation of cellular oncogenes. As stated above, there is similarity between normal genes coding for proteins for growth and differentiation on one hand, and oncogenes of viral and tumour origin on the other. How these “normal genes” are activated to become “oncogenes” is explained on the basis of two types of mechanisms:

- change in the structure of gene; and
- change in the regulation of gene expression.

Based on this, examples of activation of human oncogenes in human tumours are as under:

i) Point mutations and deletion. Mutations of v-onc sequences increase the tumorigenic ability of acute transforming retroviruses. The most important example is ras oncogene carried in many human tumours such as bladder cancer, pancreatic adenocarcinoma, cholangiocarcinoma.

ii) Chromosomal translocation. Mechanism of transfer of a portion of one chromosome to another is implicated in the pathogenesis of leukaemias and lymphomas e.g.

- Philadelphia chromosome seen in 95% cases of chronic myelogenous leukaemia in which c-abl proto-oncogene on chromosome 9 is translocated to chromosome 22.

- In 75% cases of Burkitt's lymphoma, translocation of c-myc proto-oncogene from its site on chromosome 8 to a portion on chromosome 14.

iii) Gene amplification. Chromosomal alterations that result in increase in the number of copies of a gene is found in some examples of solid human tumours e.g.

- Neuroblastoma having n-myc HSR region.
- erb-B in breast and ovarian cancer.

Mechanism of action of oncogenes. Oncogenes have oncoproteins which are altered form of their normal counterparts, proto-oncogenes, regulating growth and differentiation. Thus proliferation of cells by oncogenes is discussed in the context of alteration in the normal cell proliferation that includes the following steps:

i) Extracellular growth factor: Binding of growth factor to specific receptor on cell membrane.

ii) Transmembrane cell surface receptors: Activation of growth factor receptor on the cell surface that triggers activation of signal-transducing proteins on the inner layer of plasma membrane.

- iii) Intracellular signal transduction proteins: Transduced signal transmitted from cytosol to the nucleus.
- iv) Transcription proteins: DNA binding nuclear regulatory proteins.
- v) Cell cycle regulatory proteins: Cyclins and cyclin-dependent kinases.
- vi) Apoptotic inhibitors: bcl-2.

Anti-oncogenes (Tumour-suppressor genes)

Tumour suppressor genes or anti-oncogenes, just as proto-oncogenes, are also a pair of normal genes which perform the physiologic function of regulation of cell growth. Mechanism of stimulation of carcinogenesis by tumour-suppressor genes is mutation in both alleles producing deficiency of normal gene product which normally suppresses tumour formation. Evidence for the presence of tumour-suppressor genes comes largely from studies of about 50 human hereditary cancers identified so far. Homozygous deletion or mutation at specific genetic loci are seen in such tumour cells e.g. in retinoblastoma, Wilms` tumour, familial adenomatous polyposis coli (APC), breast cancer etc. Tumour-suppressor genes are also implicated in some spontaneous human cancers.

Two of the most widely studied tumour-suppressor genes are retinoblastoma (Rb) gene and p53 gene products, besides some others.

Oncogene	Associated Neoplasms
c-erb-B2	Breast and ovarian carcinomas
ras	Many carcinomas and leukemias
c-sis	Gliomas
c-abl	Chronic myelogenous leukemia, acute lymphocytic leukemia
c-myc	Lymphomas
BRCA-1	Breast and ovarian carcinomas
APC	Colonic adenocarcinomas
NF-1	Neurofibromas and neurofibrosarcomas
Rb	Retinoblastomas, osteosarcomas, small cell lung carcinomas
p53	Many carcinomas
bcl-2	Chronic lymphocytic leukemia, lymphomas

i) *Rb gene*. Rb gene is the first ever tumour suppressor gene identified. In patients of hereditary retinoblastoma which comprise 40% of all such tumours, all somatic cells have one defective allele of Rb gene located on long arm of chromosome 13, while all the retinoblastoma tumour cells have both alleles of Rb gene as inactive i.e. hereditary retinoblastoma is due to two genetic defects (two hit hypothesis). Besides, children inheriting mutated Rb gene have 200 times greater risk of development of mesenchymal tumour in early adult life, most notably osteosarcoma.

ii) *p53 gene*. p53 gene, the other well studied tumour suppressor gene, is located on small arm of chromosome 17. Protein product of p53 gene is present in all normal tissues. p53 is the most common target for mutation or deletion in human tumours, occurring in more than 50% of all human cancers. Normally, p53 is a negative regulator of cell division. Mutation of p53 permits cells with damaged DNA to progress through the cell cycle.

Deletion of both alleles of p53 is seen in 80% cases of colorectal cancer. Cases of germline mutation in one p53 allele have inherited predisposition to develop cancers in many organs, termed Li-Fraumeni syndrome.

iii) *Other tumour-suppressor genes*. Besides these two common anti-oncogenes, a few other tumour-suppressor genes having mutated germline are as under:

a) APC gene: Implicated in the pathogenesis of familial adenomatous polyposis coli.

b) WT 1 gene: WT 1 gene is seen in hereditary Wilms' tumour.

c) NF 1 gene: Neurofibromatosis type 1 is characterized by altered NF 1 gene.

d) BRCA 1 and BRCA 2 genes: These are breast (BR) cancer (CA) susceptibility genes which are also implicated in ovarian cancer.

Mutator Genes

Normal cells have caretaker genes to take care of integrity of genetic information in response to DNA damage. The mutated version "mutator gene" is characterized by loss of normal surveillance function and render the DNA susceptible to accumulation of mutations and, therefore, progression to cancer.

The examples of mutator genes exist in two hereditary syndromes with cancer:

i) Hereditary non-polyposis colon cancer (Lynch syndrome) is characterized by hereditary predisposition to develop colorectal cancer in cases unassociated with APC.

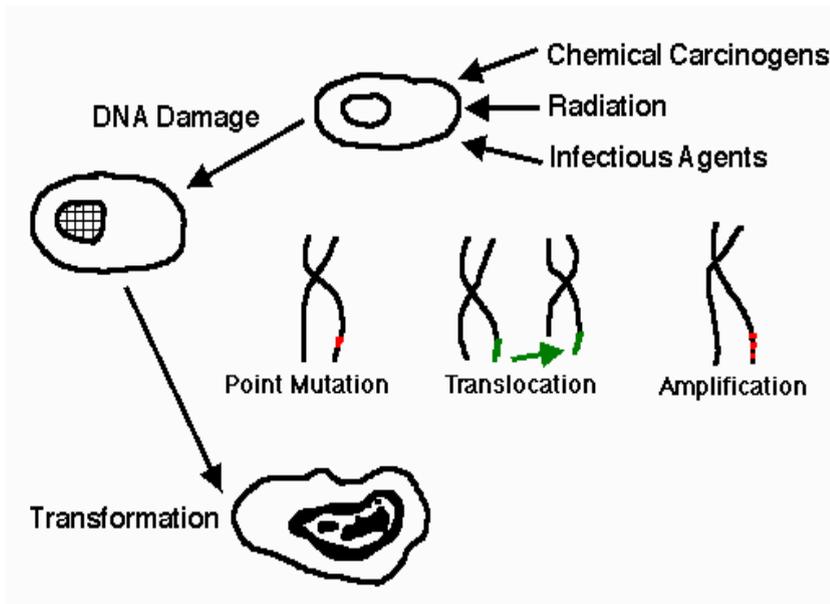
ii) Ataxia telangiectasia has ATM (M for mutated) gene. These patients have multiple cancers besides other features such as cerebellar degeneration, immunologic derangements and oculo-cutaneous manifestations.

Telomerase in Cancer

As discussed in pathology of aging, telomeres are the terminal tips of chromosomes which progressively shorten due to repetitive cell division. Telomerase is the enzyme required for continued recognition of telomere in successive cell divisions. Cancer cells express telomerase with consequent telomerase lengthening and further immortalization of cancer cells.

THEORIES OF CARCINOGENESIS

Different carcinogens may induce neoplasms by different mechanisms, and in many tumours more than one mechanism is involved in carcinogenesis. These mechanisms have already been explained under carcinogenesis by



different carcinogens. The various hypotheses combining features of carcinogenesis by different agents are considered together below.

GENETIC THEORY. Most popular theory that cells become neoplastic due to alterations in the genetic material.

Fig. 29. Scheme of Oncogenesis.

cells transmit their characters to the next progeny of cells.

Evidence in support of genetic theory comes from all types of etiologic agents in carcinogenesis:

i) Many physical (e.g. radiation) and chemical agents causing cancer bring about mutation in the host cells,

ii) Patients of xeroderma pigmentosum, a rare hereditary disorder, are excessively prone to develop skin cancer due to the inherent inability to re-pair DNA damaged by the ultraviolet rays,

iii) In many examples of viral oncogenesis (e.g. in leukaemia, lymphoma, sarcoma) the viral DNA is integrated into the host cell genome producing transformed cells,

iv) Some examples of cancer are associated with specific chromosomal abnormalities e.g. Philadelphia chromosome in chronic myeloid leukaemia,

v) Recent concept of activation of growth-promoting oncogenes in induction of some cancers and inactivation of growth-suppressing anti-oncogenes in a few others (e.g. in retinoblastoma) further supports the genetic theory of carcinogenesis.

2. **THE EPIGENETIC THEORY.** This theory is less well supported than the genetic theory. According to the epigenetic theory, the carcinogenic agents act on activators or suppressors of genes and not on the genes themselves and result in the abnormal expression of genes. Possibly, in most cases the initial mutation by the carcinogen superimposes on the epigenetic phenomena, because examples of tumorigenesis based on the epigenetic theory alone are rare.

3. **THE MULTI-STEP THEORY.** This is the other well-accepted and documented theory. According to this theory, carcinogenesis is a multi-step process. This is substantiated by in vitro changes in experimental animals as well as in vivo changes in human cancers. For example:

i) In chemical carcinogenesis, there are 2 essential features in proper sequence – initiation and promotion,

ii) Most cancers arise after several mutations which have been acquired in proper sequence,

iii) It is possible that many tumours arise from combination of activation of growth-promoting oncogenes and inactivation of growth-suppressing anti-oncogenes,

iv) In some cancers, there is stepwise an initial dysplastic change that may progress onto carcinoma in situ, and then into invasive carcinoma.

4. **IMMUNE SURVEILLANCE THEORY.** This hypothesis suggests that an immune-competent host mounts an attack on developing tumour cells so as

to destroy them while an immune-incompetent host fails to do so. Unproven support in favour of this theory comes from the following examples:

- i) There is high incidence of cancer in immunodeficient individuals e.g. in AIDS.
- ii) Most cancers occur more frequently in old age when the host immune responses are weak.
- iii) In experimental animals, there is rising titer of circulating immune complexes in the initial few weeks of tumour induction when the tumour is growing, followed by decline in the levels.
- iv) Certain tumours accompanied by good host immune response in the form of stromal infiltration by lymphocytes and plasma cells have better prognosis e.g. medullary carcinoma of the breast, seminoma of the testis etc.
- v) Some tumours may disappear spontaneously from the primary site due to good immune attack by the host to reappear as metastasis subsequently, e.g. malignant melanoma.

5. **MONOCLONAL HYPOTHESIS.** Currently, there is strong evidence on studies of human and experimental tumours that most cancers arise from a single clone of transformed cells. This theory is supported by the following examples:

- i) In a case of multiple myeloma (a malignant disorder of plasma cells), there is production of a single type of immunoglobulin or its chain as seen by monoclonal spike in serum electrophoresis.
- ii) In many other haematopoietic malignancies too, cell surface markers can be used to establish their monoclonal origin.
- iii) The best documentation of monoclonal origin of cancer cells comes from the study of glucose-6-phosphatase dehydrogenase (G6PD) in women who are heterozygous for its two isoenzymes, A and B. These isoenzymes are encoded on genes located on X chromosome for the purpose of study. It is observed that all the tumour cells in benign uterine tumours (leiomyoma) contain either A or B genotype of G6PD i.e. the tumour cells are derived from a single progenitor cell.

CLINICAL ASPECTS OF NEOPLASIA

Two major aspects of clinical significance in assessing the course and management of neoplasia are: tumour-host inter-relationship (i.e. the effect of tumour on host and vice versa) and laboratory diagnosis of cancer.

TUMOUR-HOST INTER-RELATIONSHIP

The natural history of a neoplasm depends upon 2 features:

- i) Effect of tumour on host,
- ii) Host response against tumour (Immunology of cancer)

EFFECT OF TUMOUR ON HOST

Malignant tumours produce more ill-effects than the benign tumours.

The effects may be local, or generalized and more widespread.

1. **LOCAL EFFECTS.** Both benign and malignant tumours cause local effects on the host due to their size or location. Malignant tumours due to rapid and invasive growth potential have more serious effects. Some of the local effects of tumours are as under:

i) *Compression.* Many benign tumours pose only a cosmetic problem. Some benign tumours, however, due to their critical location, have more serious consequences e.g. pituitary adenoma may lead to serious endocrinopathy; a small benign tumour in ampulla of Vater may lead to biliary obstruction.

ii) *Mechanical obstruction.* Benign and malignant tumours in the gut may produce intestinal obstruction.

iii) *Tissue destruction.* Malignant tumours, both primary and metastatic, infiltrate and destroy the vital structures.

iv) *Infarction, ulceration, haemorrhage.* Cancers have a greater tendency to undergo infarction, surface ulceration and haemorrhage than the benign tumours. Secondary bacterial infection may supervene. Large tumours in mobile organs (e.g. an ovarian tumour) may undergo torsion and produce infarction and haemorrhage.

2. **CANCER CACHEXIA.** Patients with advanced and disseminated cancers terminally have asthenia (emaciation), and anorexia, together referred to as cancer cachexia. Exact mechanism of cachexia is not clear but it does not occur due to increased nutritional demands of the tumour. Possibly, cachectin or tumour necrosis factor α (TNF- α) derived from macrophages plays a contributory role in cachexia. The various other causes include necrosis, ulceration, haemorrhage, infection, malabsorption, anxiety, pain, insomnia, hyper-metabolism and pyrexia.

3. **FEVER.** Fever of unexplained origin may be presenting feature in some malignancies such as in Hodgkin's disease, adenocarcinoma kidney, osteogenic sarcoma and many other tumours. The exact mechanism of tumour associated fever is not known but probably the tumour cells themselves elaborate pyrogens.

4. **PARANEOPLASTIC SYNDROMES.** Paraneoplastic syndromes (PNS) are a group of conditions developing in patients with advanced cancer which are neither explained by direct and distant spread of the tumour, nor by the usual hormone elaboration by the tissue of origin of the tumour. About 10-15% of the patients with advanced cancer develop one or more of the syndromes included in the PNS. Rarely, PNS may be the earliest manifestation of a latent cancer.

The various clinical syndromes included:

i) **Endocrine syndrome.** Elaboration of hormones or hormone-like substances by cancer cells of non-endocrine origin is called as ectopic hormone production. Some examples are given below:

a) *Hypercalcaemia.* Symptomatic hypercalcaemia unrelated to hyperparathyroidism is the most common syndrome in PNS. It occurs from elaboration of parathormone-like substance by tumours such as squamous cell carcinoma of the lung, carcinoma kidney, breast and adult T-cell leukaemia lymphoma.

b) *Cushing's syndrome* About 10% patients of small cell carcinoma of the lung elaborate ACTH or ACTH-like substance producing Cushing's syndrome. In addition, cases with pancreatic carcinoma and neurogenic tumours may be associated with Cushing's syndrome.

c) *Polycythaemia.* Secretion of erythropoietin by certain tumours such as renal cell carcinoma, hepatocellular carcinoma and cerebellar haemangioma may cause polycythaemia.

d) *Hypoglycaemia.* Elaboration of insulin-like substance by fibrosarcoma, islet cell tumours of pancreas and mesothelioma may cause hypoglycaemia.

ii) **Neuromyopathic syndromes.** About 5% of cancers are associated with progressive destruction of neurons throughout the nervous system without evidence of metastasis in the brain and spinal cord. This is probably mediated by immunologic mechanisms. The changes in the neurons may affect the muscles as well. The changes are: peripheral neuropathy, cortical cerebellar degeneration, myasthenia gravis syndrome, polymyositis.

iii) **Effects on osseous, joints and soft tissue,** e.g. hypertrophic osteoarthropathy and clubbing of fingers in cases of bronchogenic carcinoma by unknown mechanism.

iv) **Haematologic and vascular syndrome,** e.g. venous thrombosis (Trousseau's phenomenon), non-bacterial thrombotic endocarditis, dissemi-

nated intravascular coagulation (DIC), leukemoid reaction and normocytic normochromic anaemia occurring in advanced cancers. Autoimmune haemolytic anaemia may be associated with B-cell tumours.

v) **Gastrointestinal syndromes.** Malabsorption of various dietary components as well as hypoalbuminaemia may be associated with a variety of cancers which do not directly involve small bowel.

vi) **Renal syndromes.** Renal vein thrombosis or systemic amyloidosis may produce nephrotic syndrome in patients with cancer.

vii) **Cutaneous syndromes.** Acanthosis nigricans characterized by the appearance of black warty lesions in the axillae and the groins may appear in the course of adenocarcinoma of gastrointestinal tract. Other cutaneous lesions in PNS include seborrheic dermatitis in advanced malignant tumours and exfoliative dermatitis in lymphomas and Hodgkin's disease.

viii) **Amyloidosis.** Primary amyloid deposits may occur in multiple myeloma whereas renal cell carcinoma and other solid tumours may be associated with secondary systemic amyloidosis.

HOST RESPONSE AGAINST TUMOUR (IMMUNOLOGY OF CANCER)

It has long been thought that host defense mechanism in the form of immunological response exists so as to counter the growth and spread of cancer, albeit, more often partially. The following observations provide basis for this thinking:

1. Certain cancers evoke significant lymphocytic infiltrates composed of immunocompetent cells and such tumours have somewhat better prognosis e.g. medullary carcinoma breast, seminoma testis.

2. Rarely, a cancer may spontaneously regress partially or completely, probably under the influence of host defense mechanism. One such example is rare spontaneous disappearance of malignant melanoma from the primary site only to reappear as metastasis.

3. It is highly unusual to have primary and secondary tumours in the spleen due to its ability to destroy the growth and proliferation of tumour cells.

4. There is increased frequency of cancers in immunodeficient hosts, e.g. in AIDS.

In an attempt to substantiate the above observations and to understand the underlying host defense mechanisms, experimental animal studies involving tumour transplants were carried out. The findings of animal experiments coupled with research on human cancers have led to the concept of immunology of cancer described below under the following headings:

1. Tumour antigens,
2. Immune responses,
3. Prospects of immunotherapy.

1. TUMOUR ANTIGENS. There are two types of tumour antigens:

i) **Tumour-specific antigens (TSA)** are located on tumour cells but are not present on normal cells. They are unique or specific antigens for particular tumour and not shared by normal cells. Therefore, TSAs are targets of attack by tumour-specific cytotoxic T cells. Examples of TSAs include:

- mutant form of ras and p53 proteins, and
- bcr-abl gene.

ii) **Tumour associated antigen (TAA)** are present on tumour cells as well as on some normal cells. TAA are, thus, antigens shared by tumour cells and normal host cells from where the tumour originated. These antigens represent the stage at which the differentiation of tumour cells is arrested. Examples of TAAs are:

- CD-10 antigen on early B lymphocytes expressed in B cell leukaemia, lymphoma (CALLA);
- prostate-specific antigen (PSA) expressed by normal as well as malignant prostatic epithelium.

2. IMMUNE RESPONSES. The nature of host immune response to tumours demonstrated in animal studies and in patients with various types of cancers can be categorized as under:

- i) Cell-mediated mechanism,
- ii) Humoral mechanism,
- iii) Inhibitory (regulatory) mechanism.

i) **Cell-mediated mechanism.** This is the main mechanism of destruction of tumour cells by the host. The following cellular responses can destroy the tumour cells and induce tumour immunity in humans:

a) Specifically sensitized cytotoxic T lymphocytes (CTL) which are directly cytotoxic requiring contact between cytotoxic T lymphocyte and tumour cell.

b) Natural killer (NK) cells destroy tumour cells without sensitization, either directly or by antibody-dependent cellular cytotoxicity (ADCC). They are the first line of defense against tumour cells.

c) Macrophage mediated cytotoxicity by ADCC or by cytotoxic products.

ii) **Humoral mechanism.** Humoral antibodies which are capable of killing free tumour cells in the blood and in the serosal cavities have been suggested to play a role in reducing tumour metastases.

iii) **Inhibitory (Regulatory) mechanism.** Paradoxically, both cell-mediated and humoral mechanisms may have inhibitory influence on host immune response to cancer. Although the exact mechanism is uncertain, following possibilities have been suggested:

a) CD8+ T-suppressor cells may play a regulatory role in humoral and cell-mediated tumour immunity.

b) Humoral blocking factors, possibly antigen-antibody complexes, may either block the antigen sites on the tumour cells or block the receptors on immunocompetent cells.

3. PROSPECTS OF IMMUNOTHERAPY. Despite the existence of anti-tumour immune responses, the cancers still progress and eventually cause death of the host. The immune responses to be effective enough must eliminate the tumour cells more rapidly than their rate of proliferation and hence the role of boosting the immune response or immunotherapy.

i) **Non-specific stimulation** of the host immune response was initially attempted with BCG, *Corynebacterium parvum* and levamisole, but except slight effect in acute lymphoid leukaemia, it failed to have any significant influence in any other tumour.

ii) **Specific stimulation** of the immune system was attempted next by immunising the host with irradiated tumour cells but failed to yield desired results because if the patient's tumour within the body failed to stimulate effective immunity, the implanted cells of the same tumour are unlikely to do so.

iii) **Current status of immunotherapy** is focused on following three main approaches:

a) Cellular immunotherapy consists of infusion of tumour-specific cytotoxic T cells which will increase the population of tumour-infiltrating lymphocytes. The patient's peripheral blood lymphocytes are cultured with interleukin-2 which generates lymphokine-activated killer cells having potent anti-tumour effect.

b) Cytokine therapy is used to build up specific and non-specific host defenses. These include: interleukin-2, interferon- α and γ , tumour necrosis factor- α , and granulocyte-monocyte colony stimulating factor (GM-CSF).

c) Monoclonal antibody therapy is currently being tried as tumour cell toxin in the treatment of leukaemias and lymphomas.

DIAGNOSIS OF CANCER

When the diagnosis of cancer is suspected on clinical examination and on other investigations, it must be confirmed. The most certain and reliable method which has stood the test of time is the histological examination of biopsy, though recently many other methods to arrive at the correct diagnosis or confirm the histological diagnosis are available.

1. Histological Methods

These methods are based on microscopic examination of properly fixed tissue (excised tumour mass or open/ needle biopsy from the mass), supported with complete clinical and investigative data. These methods are most valuable in arriving at the accurate diagnosis. The tissue must be fixed in 10% formalin for light microscopic examination and in glutaraldehyde for electron microscopic studies, while quick-frozen section and hormonal analysis are carried out on fresh unfixed tissues.

The histological diagnosis by either of these methods is made on the basis that cytological features of benign tumours resemble those of normal tissue and that they are unable to invade and metastasise, while malignant tumours are identified by lack of differentiation in cancer cells termed “anaplasia” or “cellular atypia” and may invade as well as metastasise.

2. Cytological Methods

Cytological methods for diagnosis consist of study of cells shed off into body cavities (exfoliative cytology) and study of cells by putting a fine needle introduced under vacuum into the lesion (fine needle aspiration cytology, FNAC).

i) **Exfoliative cytology.** Cytologic smear (Papanicolaou Pap smear) method was initially employed for detecting dysplasia, carcinoma in situ and invasive carcinoma of the uterine cervix. However, its use has now been widely extended to include examination of sputum and bronchial washings; pleural, peritoneal and pericardial effusions; urine, gastric secretions, and CSF. The method is based on microscopic identification of the characteristics of malignant cells which are incohesive and loose and are thus shed off or “exfoliated” into the lumen. However, a “negative diagnosis” does not altogether rule out malignancy due to possibility of sampling error.

ii) **Fine needle aspiration cytology (FNAC).** Currently, cytopathology includes not only study of exfoliated cells but also materials obtained from superficial and deep-seated lesions in the body which do not shed off

cells freely. The latter method consists of study of cells obtained by a fine needle introduced under vacuum into the lesion, so called fine needle aspiration cytology (FNAC). The superficial masses can be aspirated under direct vision while deep-seated masses such as intraabdominal, pelvic organs and retroperitoneum are frequently investigated by ultrasound (US) or computed tomography (CT) guided fine needle aspirations. The smears are fixed in 95% ethanol by wet fixation or may be air-dried unfixed. While Papanicolaou method of staining is routinely employed in most laboratories for wet fixed smears, others prefer H and E due to similarity in staining characteristics in the sections obtained by paraffin embedding. Air-dried smears are stained by May-Grunwald-Giemsa or Leishman stain. FNAC has a diagnostic reliability between 80-97% but it must not be substituted for clinical judgement or compete with an indicated histopathologic biopsy.

3. Histochemistry and Cytochemistry

Histochemistry and cytochemistry are additional diagnostic tools which help the pathologist in identifying the chemical composition of cells, their constituents and their products by special staining methods.

Though immunohistochemical techniques are more useful for tumour diagnosis (see below), histochemical and cytochemical methods are still employed for this purpose.

4. Immunohistochemistry

This is an immunological method of recognizing a cell by one or more of its specific components in the cytoplasm, cell membrane or nucleus. These cell components (called antigens) combine with specific antibodies on the formalin-fixed paraffin sections or cytological smears. The complex of antigen-antibody on slide is made visible for light microscopic identification by either fluorescent dyes ("fluorochromes") or by enzyme system ("chromogens"). The specific antibody against a particular cellular antigen is now-a-days obtained by hybridoma technique for monoclonal antibody production. These monoclonal antibodies, besides being specific against antigen, are highly sensitive in detection of antigenic component, and, therefore, impart objectivity to the subjective tumour diagnosis made by the surgical pathologist.

Though the list of immunohistochemical stains is ever increasing, one important group of such antibody stains is directed against various classes of intermediate filaments which is useful in classification of poorly-differentiated tumours of epithelial or mesenchymal origin.

5. Electron Microscopy

Ultrastructural examination of tumour cells offers selective role in diagnostic pathology. EM examination may be helpful in confirming or substantiating a tumour diagnosis arrived at by light microscopy and immunohistochemistry. A few general features of malignant tumour cells by EM examination are:

- i) Cell junctions – their presence and type.
- ii) Cell surface, e.g. presence of microvilli.
- iii) Cell shape and cytoplasmic extensions.
- iv) Shape of the nucleus and features of nuclear membrane.
- v) Nucleoli – size and density.
- vi) Cytoplasmic organelles – their number is generally reduced.
- vii) Dense bodies in the cytoplasm.
- viii) Any other secretory product in the cytoplasm, e.g. melanosomes in melanoma and membrane-bound granules in endocrine tumours.

6. Tumour Markers (Biochemical Assays)

In order to distinguish from the preceding techniques of tumour diagnosis in which “stains” are imparted on the tumour cells in section or smear, tumour markers are biochemical assays of products elaborated by the tumour cells in blood or other body fluids. It is, therefore, pertinent to keep in mind that many of these products are produced by normal body cells too, and thus the biochemical estimation of the product in blood reflects the total substance and not by the tumour cells alone. These methods, therefore, lack sensitivity as well as specificity and can only be employed for:

- *firstly*, as an adjunct to the pathologic diagnosis arrived at by other methods and not for primary diagnosis of cancer; and
- *secondly*, can be used for prognostic and therapeutic purposes.

Tumour markers include: cell surface antigens (or oncofoetal antigens), cytoplasmic proteins, enzymes, hormones and cancer antigens. However, two of the best known examples of oncofoetal antigens secreted by fetal tissues as well as by tumours are alpha-fetoproteins (AFP) and carcinoembryonic antigens (CEA):

- a) **Alpha-fetoprotein (AFP):** This is a glycoprotein synthesized normally by fetal liver cells. Their, levels are elevated in hepatocellular carcinoma and non-seminomatous germ cell tumours of the testis. Certain non-neoplastic conditions also have increased levels of AFP e.g. in hepatitis, cirrhosis, toxic liver injury and pregnancy.

b) **Carcino-embryonic antigen (CEA):** CEA is also a glycoprotein normally synthesized in embryonic tissue of the gut, pancreas and liver. Their levels are high in cancers of the gastrointestinal tract, pancreas and breast. As in AFP, CEA levels are also elevated in certain non-neoplastic conditions e.g. in ulcerative colitis, Crohn's disease, hepatitis and chronic bronchitis.

7. Modern Aids in Tumour Diagnosis

In addition to the methods described above, some more modern diagnostic techniques have emerged for pathologic diagnosis but their availability as well as applicability are limited. Briefly, their role in tumour diagnosis is outlined below.

i) **Flow cytometry.** This is a computerized technique by which the detailed characteristics of individual tumour cells are recognized and quantified and the data can be stored for subsequent comparison too. Since for flow cytometry, single cell suspensions are required to “flow” through the “cytometer”, it can be employed on blood cells and their precursors in bone marrow aspirates and body fluids, and sometimes on fresh-frozen unfixed tissue. The method employs either identification of cell surface antigen (e.g. in classification of leukaemias and lymphomas), or by the DNA content analysis (e.g. aneuploidy in various cancers).

ii) **In situ hybridization.** This is a molecular technique by which nucleic acid sequences (cellular/viral DNA and RNA) can be localized by specifically-labeled nucleic acid probe directly in the intact cell (in situ) rather than by DNA extraction (see below). In situ hybridization may be used for analysis of certain human tumours by the study of oncogenes aside from its use in diagnosis of viral infection.

iii) **Molecular diagnostic techniques.** The group of molecular biologic methods in the tumour diagnostic laboratory are a variety of DNA/RNA-based molecular techniques in which the DNA/RNA are extracted (compared from in situ above) from the cell and then analyzed. These techniques are highly sensitive, specific and rapid and have revolutionized diagnostic pathology in neoplastic as well as non-neoplastic conditions (e.g. in infectious and inherited disorders, and in identity diagnosis). Molecular diagnostic techniques include: DNA analysis by Southern blot, RNA analysis by northern blot, and polymerase chain reaction (PCR). The molecular methods in tumour diagnosis can be applied in haematologic as well as non-haematologic malignancies by:

- analysis of molecular cytogenetic abnormalities;
- mutational analysis;
- antigen receptor gene rearrangement; and
- by study of oncogenic viruses at molecular level.

MESENCHYMAL TUMORS

In ontogenesis, mesenchyma is the origin of connective tissue, blood and lymphatic vessels, adipose tissue, smooth and striated muscles, serous membranes, hemopoietic system, bone and cartilage tissue.

So, mesenchymal tumors develop from: 1) connective (fibrous) tissue, 2) fat tissue, 3) muscular tissue, 4) blood and lymphatic vessels, 5) synovial tissue, 6) mesothelial tissue, 7) bone and cartilage.

They may be benign (name of the tissue + oma) and malignant (name of the tissue + sarcoma). There are also special terms (e.g. desmoid, granular-cell tumor).

Connective (fibrous) tissue tumors

Main *benign connective (fibrous) tissue tumors* are:

1. Fibroma – usually looks as a node of differentiated connective tissue with different direction of the cellular bands:
 - a) dense: fibrous structures prevail over the cellular elements;
 - b) soft: loose connective tissue with great amount of stroma cells (fibroblasts and fibrocytes).

Localization of fibroma is varied: skin, breast, spinal canal, orbit, etc.

Fibroma of the skin may have a pedicle.

2. Desmoid fibroma is a kind of dense fibroma that is characterized by infiltrating growth and relapses. More often it is located in the anterior abdominal wall.

3. Dermatofibroma (histiocytoma) is a small fibrous node with

yellow-brown colour. More often it is located in the skin of the limbs. It consists of capillaries and connective tissue with fibrous structures and fibroblasts, fibrocytes, histiocytes, macrophages. There are giant multinuclear cells containing lipids and hemosiderin among cells. Fibroblasts are arranged in a cart-wheel or storiform pattern.

Malignant connective (fibrous) tissue tumors are characterized by atypical cells.

Macroscopically sarcoma looks like fish flesh. As a rule sarcoma metastases disseminate by haematogenous route.

1. Fibrosarcoma looks like an irregular node. There are 3 types of fibrosarcoma:

a) differentiated fibrosarcoma, termed cellular-fibrous fibrosarcoma, when fibrous component prevails over the cellular component;

b) poorly differentiated fibrosarcoma, termed cellular sarcoma. It produces metastases more frequently;

c) round cell tumors of unknown origin, termed unclassified tumor.

2. Malignant fibrous histiocytoma consists of numerous polymorphic fibroblast-like and histiocyte-like cells, multinucleate bizarre giant cells, and numerous mitotic figures. There is a tendency for the spindle shape cells to be arranged in a characteristic cart-wheel or storiform pattern. It grows slowly though its growth is infiltrating. Metastases are frequent.

Tumors of adipose tissue

Benign

1. Lipoma: a soft yellow node, sometimes in a capsule, composed of lobules of mature adipose cells of different size separated by thin fibrous septa. It may develop in any place where adipose tissue is present.

Intramuscular infiltrating lipoma is an infrequent benign tumor without distinct borders, it infiltrates the striated muscle.

2. Hibernoma is a rare tumor of brown fat. It consists of large round cells with granular or foamy cytoplasm (fat vacuoles).

Malignant

1. Liposarcoma is a rare, large tumor, which is composed mostly of lipoblasts and small amount of lipocytes of different degree of maturity. It grows slowly.

There are several types of liposarcoma:

- well-differentiated liposarcoma;
- myxoid (embryonic);
- round cell liposarcoma;
- pleomorphic liposarcoma.

The prognosis of liposarcoma depends upon the location and histologic type. For instance, round cell and pleomorphic liposarcomas metastasize frequently.

2. Malignant hibernoma is a very rare tumor with cellular polymorphism and lots of giant cells.

Tumors of muscles

Benign

1. Leiomyoma consists of smooth muscle with chaotic location of the muscular bands separated by the stroma with blood vessels and nerves. If stroma prevails this tumor is termed fibromyoma. Secondary changes such as necrosis, hemorrhages, cysts, hyalinosis, petrification are characteristic for leiomyoma.

2. Rhabdomyoma consists of striated muscle fibers, which resemble to embryonic muscular fibres and myoblasts. Cardiac rhabdomyoma appears as a hamartomatous lesion and is accompanied by other developmental defects (large masses of striated muscles). Soft tissue rhabdomyomas are predominantly located in the head and neck, tongue, etc. and composed of large, round to oval cells, having abundant, granular cytoplasm.

3. Granular-cell tumor (Abrikosow's tumor) is a small tumor in a capsule, which consists of large, round or polygonal, uniform, with granular cytoplasm (no lipids) cells. Usually it occurs in the tongue, esophagus, and skin.

Malignant

1. Leiomyosarcoma (malignant leiomyoma) is a tumor with cellular and tissue atypia and high mitotic index.

2. Rhabdomyosarcoma (malignant rhabdomyoma) is characterized by severe pleomorphism, loss of muscular tissue characteristic (it is necessary to use special immune antibodies to verify the histogenesis of tumor).

3. Malignant granular-cell tumor (malignant myoblastoma) resembles malignant rhabdomyoma but the cytoplasm is granular.

Tumors of blood and lymph vessels

Benign tumors of blood vessels

1. Hemangioma is a benign tumor of blood vessels. There are several types of hemangioma:

a) capillary - it develops in the skin, mucous membranes, gastrointestinal tract, liver, more often in children. It looks like cyanotic node comprised of branching blood-filled capillaries with narrow lumen, and thin walls;

b) venous – it consists of vascular bands with smooth muscles in

their wall, resembling veins;

c) cavernous hemangioma is common in the liver, skin, bones, muscles, gastrointestinal tract, brain. Tumor consists of thin-walled cavernous vascular spaces, filled partly or completely with blood. The vascular spaces are lined by flattened endothelial cells. They are separated by scanty connective tissue stroma;

d) benign hemangiopericytoma arises in the skin and intramuscular space of the extremities. The tumor is composed of capillaries surrounded by pericytes outside the vascular basement membrane.

2. Glomus tumor (glomangioma). More often develops in hands and feet (fingers and toes). It consists of vessels with endothelium, surrounded by ferrules of epithelioid (glomus) cells. Tumor is rich in nerves thus it is usually painful.

Malignant tumors of blood vessels.

Angiosarcoma:

a) malignant hemangioendothelioma;

b) malignant hemangiopericytoma - is a highly malignant tumor with high mitotic cells rate and areas of necrosis. It has early metastases.

Benign tumors from lymph vessels.

Lymphangioma - is characterized by growth of lymphatic vessels in different direction with the formation of a node or enlargement of the organ. If lymphangioma develops in the tongue, it is termed macroglossia. If lymphangioma develops in the lip it is termed macrocheilia. Microscopically tumor looks consists of cavities filled with lymph.

Malignant tumors from lymph vessels.

Lymphangiosarcoma. It appears as a result of chronic lymphatic stasis and is composed of lymph clefts with proliferating atypical endothelial cells.

Tumors of synovial tissue

1. Benign synovioma develops in the tendons and tendon sheath. It contains a lot of stroma with hyalinosis, and a little number of vessels. It may have xanthome cells and clefts.

2. Synovial sarcoma (malignant synovioma) develops in the large joints. It has irregular structure. Some tumors have pleomorphic cells and pseudoepithelial gland formations with cysts, the other have fibroblast-like atypical cells and collagen fibers, structures resembling tendons.

Tumors of mesothelial tissue

1. Benign mesothelioma resembles a dense nodule in serous membrane (pleura), its structure is similar to fibroma (fibroid mesothelioma).

2. Malignant mesothelioma (peritoneum, pleura, and pericardium) microscopically looks like atypical large cells with vacuolated cytoplasm. Malignant mesothelioma may have tubular and papillary structures. In this case it is called epithelial mesothelioma.

Bone tumors

Benign

1. Osteoma develops as a rule in spongy and tubular bones, skull included. 2 types of osteoma are known: a) spongy osteoma, b) compact osteoma.

This benign tumor almost exclusively involves the skull and facial bones; the frontal sinus is the most common location. Males are affected more often than females, the tumor can occur at any age. Although osteoma is predominantly a solitary lesion, multiple osteomas can occur in association with intestinal polyposis and soft tissue tumors. The tumor of normal bone originates from the periosteum. There is little evidence of osteoblastic activity.

2. Osteoid osteoma is common in young persons, mostly males. Macroscopically, an osteoid osteoma appears as a small round or oval mass containing a central red-brown, friable area. Microscopically, the tumor appears as a mass of irregular bone trabeculae, fibrous tissue, and vessels. The center of the tumor is rich in osteoblasts, calcification, and multinucleate giant cells.

3. Benign osteoblastoma predominantly affects the vertebrae and long bones of young males in the first three decades of life. Macroscopically, the neoplasm varies in size from a few to several centimeters. Microscopically, osteoblasts proliferate and osteoid production increases. Osteoclasts and multinucleate giant cells may be very numerous, especially in areas of blood extravasation.

Malignant

1. Osteosarcoma (osteogenic sarcoma) consists of osteogenic tissue rich in atypical cells of osteoblastic type with a lot of mitoses, the bone is primitive. 2 types of osteosarcoma are known: a) osteoblastic type (bone formation), b) osteolytic type (bone destruction).

Osteosarcoma is a highly malignant bone tumor characterized by the production of osteoid and bone. Most osteosarcomas arise in the metaphyseal end of long bones (predominantly the femur, humerus, and tibia), but they can involve any bone, including the small bones of the hands, feet and face. Osteosarcoma is the most common primary malignant tumor of a bone (next to multiple myeloma), accounting for approximately 16% of all bone malignancies. The disease predominantly affects young males between age 10 and 20.

Gross appearance. The tumor appears as a large necrotic and hemorrhagic mass. The lesion usually ends in the epiphyseal cartilage and rarely extends into the nearby joint space.

Microscopic appearance. Three types of osteosarcomas have been differentiated according to their predominant histologic patterns: osteoblastic, fibroblastic and chondroblastic. The hallmark of the tumor is the presence of a malignant stroma that contains osteoid and bone. The stroma shows bizarre pleomorphic cells, with hyperchromatic, irregular nuclei and abundant mitoses. Multinucleate giant cells are seen most often near zones of necrosis and calcification. Malignant cartilage may be present as well as a small foci or as a large proportion of the tumor.

2. Giant cell tumor of bone (osteoclastoma) is an uncommon malignant tumor characterized by multinucleate giant cells. It occurs predominantly in women over age 19 and peaks in the third decade of life. The neoplasm is almost always localized in the distal part of the long bones (femur or humerus), and 50% of these tumors occur in the area of the knee. Occasionally, the tumor involves the skull, pelvis, or small bones of the hands and feet.

Macroscopically, the tumor characteristically appears as multiple hemorrhagic cystic cavities that destroy the adjacent bone and are enclosed by a thin shell of new bone formation.

Microscopically, it represents as a vascularized stroma composed of spindle cells. It contains multinucleate giant cells, areas of hemorrhage, inflammation, and hemosiderin deposits. Mitoses are present.

Cartilage tumors

Benign

1. Chondroma derives from hyaline cartilage in the feet, spine, breastbone, and pelvis. If tumor is located in the peripheral area of the bone it is termed exchondroma, if in the center area of the bone - enchondroma.

Ollier's disease (enchondromatosis) is a rare, nonhereditary disorder in which multiple chondromas are present in the metaphysis and diaphysis of various bones.

Maffucci's syndrome is a congenital disease characterized by dyschondroplasia and multiple hemangiomas in the skin and viscera.

This neoplasm is thought to originate from heterotropic cartilaginous cell; nests in the medullary cavities of bones. Macroscopically, the lesion appears as a confluent mass of bluish hyaline cartilage with a lobular configuration. Microscopically, the cartilage appears moderately cellular, with occasional binucleate cells. Mitoses are absent.

2. Osteochondroma is the most common benign tumor of bone affecting patients under age 20-21. The lesions may be single or multiple and predominantly involve the metaphysis of long bones. Macroscopically, the tumor may range in size from 1 to several centimeters and appears as a stalked protuberance, with a lobulated surface jutting from the affected bone. The periosteum of the adjacent bone covers the lesion. Microscopically, the cartilaginous cells appear lined up, mimicking the orientation of cartilaginous cells in a normal epiphysis. Mitoses are absent.

3. Benign chondroblastoma consists of chondroblasts, interstitial substance, marked osteoclast reaction. Chondroblastoma is a rare cartilaginous tumor that almost always involves the epiphyseal portion of the long bones. The tumor predominantly affects males in the second decade of life. Macroscopically, the tumor is round or oval in shape, with areas of cystic degeneration and hemorrhage. Microscopically, proliferation of chondroblasts is intermixed with varying amounts of fibrous stroma and chondroid material. Multinucleate giant cells and calcifications are present. Mitoses are virtually absent.

4. Chondromyxoid fibroma is most commonly located in the metaphysis of long bones but occasionally can involve the epiphysis. It primarily affects males in the first and second decades of life. Macroscopically, the tumor is a well-circumscribed, looks as a solid mass with a cartilaginous appearance. The cortex of the bone is expanded by the tumor, which is limited by the periosteum. Microscopically, a variety of fibrous, myxomatous, and chondroid elements are seen together with multinucleate giant cells and macrophages that contain hemosiderin. When the tumor forms lobules, a condensation of nuclei occurs beneath the rim of the compressed adjacent tissue.

Malignant

Chondrosarcoma is a malignant cartilaginous tumor. The most common locations are the spine, pelvic bones, and upper ends of the femur and humerus. The tumor may arise de novo (primary chondrosarcoma) or originate from a preexisting benign cartilaginous lesion (secondary chondrosarcoma). Chondrosarcomas comprise between 7% and 15% of all bone neoplasms. The tumor occurs in patients between age 30 and 60 and in men three times more often than in women.

Macroscopically, a chondrosarcoma appears as a lobulated white or gray mass that contains mucoid material and foci of calcification.

Microscopically, there are islands of immature or poorly developed cartilage in which anaplastic cells with two or more nuclei are present within the lacunar space.

The neoplasm grows slowly and can remain locally aggressive for years, with a high tendency to recur and implant into soft tissues. Hematogenous dissemination to the lungs, liver, and kidneys takes place over the years, with eventual death of the patient. The 10-year survival rate ranges from 50% to 60%.

TUMORS OF EPITHELIAL TISSUES

Organ non-specific epithelial tumors develop in squamous, transitional or glandular epithelium which doesn't perform any specific function.

Depending on epithelium type benign organ non-specific epithelial tumors are subdivided into papillomas (the tumors of squamous and transitional epithelium) and adenomas (tumors of glandular epithelium).

Papilloma occurs in the skin and mucous membranes, covered with squamous or transitional epithelium; it looks like a ledge or a bush of branching papillae. It is a good example of an exophytic tumor. The base of the tumor consists of connective tissue containing blood vessels. It is a continuation of subepithelial connective tissue covered with epithelium like with a glove.

Adenoma is a benign epithelial tumor from the epithelium of the glands and mucous membranes covered with columnar epithelium. More often they can be found in the breast, thyroid gland, ovaries, prostate gland, uterus, and gastrointestinal tract.

Grossly, adenoma is a soft whitish or pinkish node of different size. According to the histological structure adenoma may be *tubular*, *trabecu-*

lar, papillary and alveolar. In tubular adenoma, there are glandular cavities resembling tubes in the connective tissue with vessels. In alveolar adenoma, numerous foci bedded with cylindrical or cubic epithelium are observed in the connective tissue with vessels. Trabecular adenoma consists of cellular cords, while papillary type of adenoma is composed of papillary structures. Sometimes papillary growth of epithelium, bedding glandular cavities, is observed. In some adenomas glandular cavities are widened and form large cavities, cysts filled with the serous fluid or mucus. These cyst-like adenomas are called cystadenomas. In all these cases, the epithelium is separated from the surrounding tissue by its own membrane. Adenomas may transform into malignant tumor.

Immature or malignant epithelium tumor is called **cancer**. The popularity of this term can be explained by the increase of the cancer incidence in the 20th century comparing with previous centuries.

This fact can be explained by the increase of the life expectancy by 20 years, that is the group of people of «cancer age» enlarged (due to increased possibility to be exposed to carcinogenic factors, accumulation of the total number of precancerous processes and increased chance to develop latent cancer with long duration). Besides, increase of the tumors number can be associated with improvements in diagnosis. But the above does not exclude objective causes of cancer development, especially in the population of the developed countries due to increase of the number of industrial tumors (cancer of lungs, skin, urinary bladder) associated with exposure to chemical carcinogens (at present there are about 300 of them, mainly polycyclic aromatic hydrocarbon, azo- or aminocompounds).

Carcinoma in situ is a frequent variant of cancer which is characterized by severe atypia, active cellular proliferation while infiltrative growth is absent, and basement membrane is not invaded. Noninvasive cancer is a step of cancer growth and it can transform into infiltrative malignant tumor. This tumor is well studied in many organs: cervix, mammary gland, skin, stomach, larynx, etc.

Depending on the epithelium *differentiated carcinomas* histologically are subdivided into following types:

1. *Squamous cell carcinoma* may arise in any part of the skin or mucous membranes lined by squamous epithelium (oral cavity, esophagus, larynx, vagina, uterus cervix, etc.). It can arise on glandular or transitional epithelium too after preceding metaplasia. Grossly, this cancer is more com-

monly an ulcerated poorly-circumscribed growth with elevated and indurated margin, less often, fungating or polypoid verrucous with necrotic and ulcerative lesions. Microscopically, squamous cancer is characterized by irregular downward proliferation of epithelial cells into derma or underlying tissues with the formation of epithelial «nests». Masses of tumor cells show atypical features such as variation in cell size and shape, nuclear hyperchromatism, absence of intercellular bridges, atypical mitoses, individual cell keratinisation or whorled arrangement of malignant squamous cells forming horn pearls.

2. *Transitional cell carcinoma* arises on transitional epithelium of mucous membranes of urethra, urinary bladder, etc. Microscopically, it is characterized by irregular downward proliferation of epithelial cells into underlying tissues. Masses of tumor cells show all atypical features too.

3. *Adenocarcinoma* arises appears on prismatic epithelium of mucous membranes or epithelium of glands. It has a structure similar to adenoma but differs from it in presence of atypia, variation in cell and nucleus size and shape, nuclear hyperchromatism, atypical mitoses and infiltrative growth. There are acinar, tubular and papillary variants of adenocarcinoma.

Undifferentiated carcinomas are classified into:

1. *Mucous (colloid) carcinoma*. The tumor grows like masses having gelatinous appearance due to secretion of large quantities of mucus. Histologically, mucoid carcinoma contains abundant pools of mucin in which are seen a small number of tumor cells.

2. *Signet-ring cell carcinoma carcinoma* is a variant of mucous carcinoma when the mucous accumulates in cytoplasm of tumor cells. Signetcells have abundant mucinous cytoplasm, which is positive for mucicarmine.

3. *Small cell carcinoma*. Tumor cells are small, uniform, and lymphocyte-like with scanty cytoplasm. This carcinoma has rapid growth, frequently undergoes the necrosis, gives early and multiple metastasis.

4. *Fibrous (scirrhous) carcinoma*. If there is excessive connective tissue stroma in the undifferentiated carcinoma, it is referred to as desmoplasia and the tumor is hard or scirrhous. Due to marked desmoplasia cancer cells may be difficult to find.

5. *Medullary carcinoma*. If undifferentiated carcinoma is almost entirely composed of parenchymal cells, it is called medullary. The loose con-

nective tissue stroma is scanty and usually has a prominent lymphoid infiltration.

6. *Solid carcinoma* is characterized by presence of solid layers of malignant epithelial cells separated by connective tissue which show marked cytologic atypia and frequent mitoses.

ORGAN SPECIFIC EPITHELIAL TUMORS. TUMORS OF EXOCRINE GLANDS AND SKIN.

Liver tumors.

Metastatic tumors in the liver are more common than primary ones. Primary hepatic tumours may arise from hepatic cells and bile duct epithelium.

Benign hepatic epithelial tumours include hepatocellular adenoma and bile duct adenoma (cholangioma).

Hepatocellular adenoma usually develops as a single node, but about 10% there are multiple nodes. It is partly or completely encapsulated and lighter in colour than adjacent liver tissue or may be bile-stained. Histologically, the tumor is composed of sheets and cords of hepatocytes. Hepatocellular adenomas lack portal tracts and bile ducts but bile canaliculi containing bile-plugs may be present.

Intrahepatic or extrahepatic *bile duct adenoma* is a rare benign tumour. It may be small, composed of acini lined by biliary epithelium and separated by variable amount of connective tissue. And there are larger cystadenomas having locules lined by biliary epithelium.

Malignant hepatic tumours are hepatocellular (liver cell) carcinoma and cholangiocarcinoma, and frequently mixed pattern is seen.

Hepatocellular carcinoma (hepatoma) may develop as a single yellow-brown, large mass, with central necrosis, haemorrhage and occasional bile-staining (expanding or massive type). Less often, multifocal, multiple masses 3-5 cm in diameter, scattered throughout the liver (multifocal or nodular type) are seen. Rarely, the hepatocellular carcinoma forms diffusely infiltrating tumour mass (infiltrating of differentiation, ranging from well-differentiated to highly anaplastic lesions).

Cholangiocarcinoma is the designation used for carcinoma arising from bile duct epithelium within the liver and from the large hilar ducts. Grossly, the tumour is firm to hard and whitish. Microscopically, the tumour

has glandular structure. The tumour cells resemble biliary epithelium or diffuse type. Microscopically, the tumour cells resemble hepatocytes but vary with the degree but are characterized by atypia and have no bile secretion.

Tumors of kidney.

Both benign and malignant tumours occur in the kidney. They may arise from renal tubules (adenoma, adenocarcinoma) and embryonic tissue (nephroblastoma or Wilms' tumor).

Adenoma of kidney. Histologically, there are basophilic, clear cell and acidophilic types of adenoma. Clear cell adenoma may form tiny nodules up to 3 cm in diameter. It is encapsulated and white or yellow. Microscopically, it is composed of tubular cords or papillary structures projecting into cystic space. The cells are usually uniform, cuboidal with no mitosis. However, size of the tumor rather than histologic criteria is considered more significant parameter to predict the behavior of the tumor – those larger than 3 cm in diameter are potentially malignant. Other types of adenoma have variable size.

Renal cell carcinoma (synonyms: adenocarcinoma of kidney, hypernephroma, Grawitz tumour) is classified into clear cell, papillary, granular cell, chromophobe, sarcomatoid and collecting duct types. Grossly, renal cell carcinoma is a solitary and unilateral, generally large, golden yellow and circumscribed mass. Papillary tumours may be multifocal. Cut section of the tumor commonly shows large areas of ischaemic necrosis, cystic change and foci of haemorrhages. Another significant characteristic is the frequent presence of tumor thrombus in the renal vein which may extend into the vena cava.

Nephroblastoma or Wilms' tumor is an embryonic tumor derived from primitive renal epithelial and mesenchymal components. It is the most common abdominal malignant tumour of young children, seen most commonly between 1 to 6 years of age with equal sex incidence. Grossly, the tumor is usually quite large, spheroidal, replacing most of the kidney tissue. On cut section, the tumor shows variegated appearance – soft, fish flesh like, grey-white to cream-yellow tumor with foci of necrosis and haemorrhages. Microscopically, nephroblastoma shows mixture of primitive epithelial and mesenchymal elements. Most of the tumor consists of small, round to spindle cells, abortive tubules and poorly-formed glomerular structures are present. Mesenchymal elements such as smooth and skeletal muscle, cartilage and bone, fat cells and fibrous tissue, may be seen.

Breast tumors.

Fibroadenoma and *intraductal papilloma* are the most common benign breast tumour.

Fibroadenoma consists of fibrous and epithelial components. Grossly it is a small, solitary, well-encapsulated, spherical or discoid mass. The cut surface is firm, grey-white, slightly myxoid. Microscopically, there are intracanalicular and pericanalicular patterns.

Intraductal papilloma is a benign papillary tumor occurring most commonly in a lactiferous duct or lactiferous sinus near the breast nipple.

Carcinoma of the breast is an important malignant tumor which occurs as non-invasive (carcinoma in situ) and invasive cancer with its various morphologic varieties. Non-invasive carcinoma may be intraductal and intralobular. There are several types of invasive carcinoma: infiltrating ductal carcinoma, invasive lobular carcinoma, medullary carcinoma, colloid carcinoma and etc.

Non-invasive carcinoma (carcinoma in situ) is characterized histologically by presence of tumour cells within the ducts or lobules without invasion.

Infiltrating ductal carcinoma is the common breast cancer. Macroscopically, it looks as irregular, 1-5 cm in diameter, hard cartilage-like mass. The cut surface of the tumour is gray-white to yellowish with chalky streaks and often extends irregularly into the surrounding fat. There are 3 histological types of this carcinoma:

- anaplastic tumour cells forming solid nests, cords, poorly- formed glandular structures and some intraductal foci;
- infiltration by these patterns of tumour cells into diffuse fibrous stroma and fat;
- invasion into perivascular and perineural spaces as well as lymphatic and vascular invasion.

Infiltrating (invasive) lobular carcinoma. Invasive cancers in being more frequently bilateral; and within the same breast, it may have multicentric origin. Macroscopically, the appearance varies from a well-defined scirrhous mass to a poorly-defined area of induration that may remain undetected by inspection as well as palpation.

Microscopically, there are 2 characteristics:

- 1) Pattern – a characteristic single file (Indian file) linear arrangement of stromal infiltration by the tumour cells with very little tendency to gland

formation is seen. Infiltrating cells may be arranged concentrically around ducts in a target-like pattern;

2) Tumour cytology – individual tumour cells resemble cells of *in situ* lobular carcinoma. They are round and regular with very little pleomorphism and infrequent mitoses. Some tumors may show signet-ring cells distended with cytoplasmic mucus.

Medullary carcinoma has a significantly better prognosis than the usual infiltrating duct carcinoma, probably due to good host immune response in the form of lymphoid infiltrate in the tumour stroma. Macroscopically, the tumour is characterized by a large, well-circumscribed, rounded mass that is typically soft and fleshy brain-like.

Colloid (mucinous) carcinoma contains large amount of extracellular epithelial mucin and acini filled with mucin.

Paget's disease of the nipple. The nipple bears a crusted, scaly eczematoid lesion with a palpable subareolar mass in about half the cases. Macroscopically, the skin of the nipple and areola are crusted, fissured and ulcerated with oozing of serosanguineous fluid from the erosions. Microscopically, the skin lesion is characterized by the presence of Paget's cells in the epidermis. These cells are larger than the epidermal cells. The underlying breast contains invasive or non-invasive duct carcinoma which shows no obvious direct invasion of the skin of nipple.

Skin tumors

Tumours and tumour-like lesions may arise from different components of the skin such as epidermis, epidermal appendages and dermal tissues. Each of these tissues may give rise to benign and malignant tumours. The most important of epidermal appendages tumors are hidradenoma, spiradenoma, trichoepithelioma, and basal cell carcinoma.

Hydroadenoma originates from the intradermal portion of the eccrine sweat duct. Histologically, it consists of solid masses and cords of tumour cells which may have an occasional duct-like structure containing mucin.

Spiradenoma is found as solitary, painful, circumscribed nodule in the dermis. Histologically, it consists of lobules which are surrounded by a thin capsule. The tumor lobules contain 2 types of epithelial cells like in the secretory parts of the eccrine sweat gland.

Trichoepithelioma is a tumor of hair follicle. The tumor may occur as solitary lesion or as multiple inherited lesions, predominantly on the face,

scalp and neck. It is often circumscribed. The most characteristic histologic feature is the presence of multiple horn cysts surrounded by basophilic cells.

Basal cell carcinoma is a locally invasive, slow-growing tumor that rarely metastasizes (0.05% of all cases). Grossly, the most common pattern is a nodulo-ulcerative basal cell carcinoma in which a slow-growing small nodule undergoes central ulceration with pearly, rolled margins. Microscopically, the most characteristic feature is the proliferation of basaloid cells (resembling basal layer of epidermis) having typical peripheral palisaded appearance of the nuclei.

Most frequent malignant appendage tumors are are sweat gland carcinoma and sebaceous carcinoma.

Uterus.

Gestational trophoblastic diseases resulting from malignant overgrowth of trophoblast – invasive mole and choriocarcinoma should be discussed as organ specific tumors of uterus.

Invasive (destructive) mole (chorioadenoma destruens) shows invasion of the molar tissue into the uterine wall which may be a source of haemorrhage. Rarely, molar tissue may invade the blood vessels and reach the lungs. Microscopically, the lesion is benign and identical to classic mole but has potential for haemorrhage.

Choriocarcinoma is a highly malignant and widely metastasizing tumour of trophoblast. Features: 1) absence of stroma, 2) invasion of the blood vessels, lymphatics, and myometrium, 3) widespread haematogenous metastases are early and frequent, found mainly in the lungs. Choriocarcinoma is composed of masses and columns of highly anaplastic and bizarre cytotrophoblast and syncytiotrophoblast cells which are intermixed. The tumor is also characterized by invariable presence of haemorrhages and necrosis.

TUMORS OF ENDOCRINE GLANDS

Ovarian tumors

Ovarian tumors arise from normally-occurring cellular components of the ovary. There are five major groups:

- 1). Tumors of surface epithelium (serous tumors, mucinous tumors, etc.),
- 2). Germ cell tumours (teratomas, dysgerminoma, yolk sac tumor, etc.),
- 3). Tumors of Sex Cord and Stromal Origin (granulosa cell tumour, thecoma, fibroma, etc.),
- 4). Miscellaneous tumors,
- 5). Metastatic tumors.

Benign and malignant serous tumors are large unilocular or multiloculated cysts with intracystic papillary projections. *Serous cystadenoma* is characteristically lined by low columnar epithelium. *Serous cystadenocarcinoma* has multilayered malignant cells which show loss of polarity, presence of solid sheets of anaplastic epithelial cells and definite evidence of stromal invasion.

Mucinous tumours are predominantly cystic tumours which contain mucin. Grossly, mucinous tumours are much larger than serous tumors. *Mucinous cystadenoma* has thin wall and septa dividing the loculi. It is lined by a single layer of columnar nonciliated epithelium. There is very little tendency to papillary proliferation of epithelium. *Mucinous cystadenocarcinoma* usually has thickened areas and is characterized by piling up of malignant epithelium, at places forming solid sheets, papillary formation, adenomatous pattern and infiltration into stroma with or without pools of mucin.

Dysgerminoma is an ovarian variant of the testes seminoma. All dysgerminomas are malignant and are extremely radiosensitive. The tumor cells are arranged in diffuse sheets, islands and cords separated by scanty fibrous stroma which contains lymphocytic infiltrate.

Granulosa cell tumor may occur at all ages. It invades locally but occasionally may have more aggressive and malignant behavior. Most granulosa cell tumours secrete oestrogen. Cut section of tumor is yellowish-brown. Microscopically, the granulosa cells are arranged in a variety of patterns including micro- and macrofollicular, trabecular, bands and diffuse sheets.

Thecoma is usually benign. It occurs more frequently in postmenopausal women. Grossly, thecoma is a solid and firm mass, cut surface is yellowish. Histologically, it consists of spindle-shaped theca cells of the ovary admixed with variable amount of hyalinised collagen.

Testicular tumours

All testicular tumors are subdivided into 3 groups: germ cell tumours (seminoma, embryonal carcinoma, teratoma, yolk sac tumor, etc.), sex cord-stromal tumours (Leydig's cell tumor, Sertoli's cell tumor, etc.) and mixed forms (gonadoblastoma).

Seminoma is the commonest malignant tumour of the testis and corresponds to dysgerminoma in female. The tumor has a peak incidence in the 4th decade of life. Grossly, the involved testis is enlarged up to 10 times its normal size. Cut section shows homogeneous, grey-white lobulated appearance. Tumor cells generally lie in cords, sheets or columns forming lobules.

Leydig's (interstitial) cell tumors are quite uncommon. They may occur at any age but are more frequently at the age from 20 to 50 years. The tumor cells secrete androgen, or both androgen and oestrogen, and rarely corticosteroids. Grossly, the tumor appears as a small, well-demarcated and lobulated nodule. Cut surface is homogeneously yellowish or brown. The tumor is composed of sheets and cords of normal-looking Leydig's cells.

Sertoli's cell tumors (androblastoma) may occur at all ages but more frequently in infants and children. The tumor may elaborate oestrogen or androgen and may account for gynaecomastia in an adult or precocious sexual development of child. The tumour is fairly large, firm, round, and well circumscribed. Cut surface of the tumour is yellowish or yellow-grey.

Gonadoblastoma is an example of combination of both germ cells and sex cord stromal components.

Thyroid tumors

Most tumors of the thyroid gland are of follicular epithelial origin; few arise from parafollicular C-cells.

The most common benign thyroid neoplasm is a *follicular adenoma*. It is characterized by four features so as to distinguish it from a nodule of nodular goitre: 1. solitary nodule; 2. complete encapsulation; 3. clearly distinct architecture inside and outside the capsule; 4. and compression of the thyroid parenchyma outside the capsule. There are following 6 types of growth pattern: 1). Microfollicular adenoma; 2). Normofollicular adenoma; 3). Macrofollicular (colloid) adenoma; 4). Trabecular adenoma; 5). Oxyphilic adenoma; 6). Atypical adenoma.

Thyroid cancer. Carcinoma of the thyroid gland has 4 major morphologic types with distinctly different clinical behavior and variable prevalence. These are papillary, follicular, medullary and undifferentiated (anaplastic) carcinoma.

Papillary carcinoma is the most common type of thyroid carcinoma. Papillae are composed of fibrovascular stalk and covered by single layer of tumour cells which have characteristic nuclear features (optically clear appearance).

Follicular thyroid carcinoma is composed of follicles of various sizes and may show trabecular or solid pattern. Vascular and adjacent structures invasion are significant features.

Medullary thyroid carcinoma derived from parafollicular or C-cells. There are 3 distinctive features of this tumor: familial occurrence, secretion of calcitonin, and amyloid stroma.

Anaplastic carcinoma is one of the most malignant tumors in humans. The tumour is generally composed of 3 types of cells occurring in varying proportions: small cells, spindle cells and giant cells.

Parathyroid tumours. Parathyroid adenoma and carcinoma are the most frequent neoplasms found in parathyroid glands. Most of adenomas are characterized by the excessive secretion of parathyroid hormone causing features of hyperparathyroidism. These tumors are composed of chief-cells arranged in sheets or cords.

Tumours of adrenal glands are uncommon and include distinct adrenocortical tumours and medullary tumors. There are 4 types of *cortical adenoma*: 1) clear cell cortical adenoma (produces aldosterone and results in Conn's syndrome); 2) dark cell cortical adenoma (produces androgens and causes virilism or rarely Cushing's syndrome); 3) mixed cortical adenoma (is characterized by hypercortisolism – Cushing's syndrome); 4) glomerulosa cell adenoma (is characterized by increased secretion of mineralocorticoids). *Cortical carcinoma* characterized by cellular atypia, local invasion, and distant metastasis.

Pheochromocytoma is a benign tumor arising from the chromaffin cells of the adrenal medulla. The most common feature of pheochromocytoma is hypertension, resulting from secretion of catecholamines. About

10% of pheochromocytomas may be malignant having tendency for osseous metastases.

Thymoma is a tumor of epithelial origin. It may arise from cortical and medullar thymus cells. Clinically, it is asymptomatic, but sometimes it causes myasthenia, immunodeficiency and autoimmune diseases. There are 4 types of thymoma: 1) cortical cell thymoma (characterized by invasive growth and cellular atypia); 2) medullar cell thymoma (generally benign); 3) mixed cellular thymoma; 4) granulomatous thymoma (is composed of atypical multinuclear cells with the formation of granulomatous masses).

Pituitary tumors. Tumors of the anterior pituitary are more common than those of the posterior one and hypothalamus. The most common of the anterior pituitary tumors are adenomas. They are conventionally classified according to their H&E staining characteristics of granules into *acidophil*, *basophil* and *chromophobe adenomas*. However, this morphologic classification is considered quite inadequate because of the significant functional characteristics of each type of adenoma including the chromophobe adenoma, which on H&E staining doesn't show visible granules. Modern classification of pituitary adenomas is based on functional features correlated with morphologic features of the older classification. Thus, the types of this classification are the following: 1) lactotroph adenoma; 2) somatotroph adenoma; 3) mixed somatotroph-lactotroph adenoma; 4) corticotroph adenoma; 5) gonadotroph adenoma; 6) thyrotroph adenoma; 7) oncocytoma; 8) plurihormonal adenoma.

Epiphysis. Pinealoma is a benign tumor which is composed of glandular epithelium and neuroglia. The tumor causes metabolic and hormonal disturbances.

Endocrinal tumors of the pancreas (islet cell tumors) are rare as compared with tumors of the exocrine part of pancreas. Islet cell tumors are generally small and may be hormonally inactive or may cause hyperfunction. They may be benign or malignant, single or multiple. They are named according to their histogenesis such as:

1. β -cell tumor – insulinoma. The tumor composed of cords and sheets of well-differentiated β -cells which do not differ from normal cells. The neoplastic cells secrete insulin into the blood stream which remains un-

affected by normal regulatory mechanisms. This results in characteristic attacks of hypoglycaemia and high plasma insulin level;

2. G-cell tumor – gastrinoma, is characterized by hypergastrinaemia. Majority of gastrinomas occur in the wall of the duodenum. They may be benign or malignant. Gastrinomas are associated with the development of Zollinger-Ellison's syndrome which is characterized by multiple peptic ulcers at usual sites such as the stomach and duodenum, or sometimes at unusual sites such as in the oesophagus and jejunum;

3. A-cell tumor – glucagonoma. The tumor causes hyperglycemia and develop diabetes mellitus;

4. D-cell tumor – somatostatinoma is characterized by hypoin-sulinemia, hypoglucagonemia, steatorrhea and achlorhydria;

5. D1 cell tumor – vipoma is characterized by hypopotassemia and dehydration;

6. pancreatic polypeptide PP-secreting tumor;

7. carcinoid tumor.

However, except insulinoma and gastrinoma, all others are extremely rare.

Carcinoid tumor is a generic term applied to tumors originating from endocrine cells of APUD-system and are therefore also called as apudomas. The endocrine cells are distributed throughout the mucosa of the gastrointestinal tract. Depending upon the embryologic derivation of the tissues where the tumour is located, these are classified as foregut (stomach, duodenum and oesophagus), midgut (terminal ileum and appendix), and hind-gut (rectum and colon) carcinoids. Other uncommon locations are the bronchus, trachea, gallbladder, and Meckel's diverticulum. Appendiceal, ileal and stomach carcinoids behave as malignant tumors. Tumor cells are monomorphous, having uniform nuclei and are arranged in a variety of patterns – solid nests, sheets, cords, trabeculae and clusters.

TUMORS OF MELANIN-PRODUCING TISSUE

Melanin-producing cells (melanocytes) are of neurogenous origin. They may develop as well tumor-like formations (nevi) as melanomas.

Nevi are defects of neuroectodermal pigment elements development. They look like brown spots of different sizes, and may be either flat or ele-

vated over the surface or may be like warts. Sometimes their size is enormous (giant pigmented nevus).

There are the following types of nevi: 1) junctional nevus, 2) compound nevus, 3) intradermal, 4) blue nevus, etc.

Junctional nevus. Nests of nevus cells are found on the border of epidermis and derma.

Compound nevus. Nevus cells are located as on the border of dermis and epidermis as in derma.

Intradermal nevus. Nevus cells are located only in derma. Some of them can be found near the border between derma and epidermis. The nevus cells look like compact mass. In mature nevi cells may be multinuclear.

Blue nevus. Macroscopically it looks like bluish or bluish-brown (bluish-gray) spot, of round or oval shape, it doesn't elevate over the skin surface. Microscopic examination reveals spindle-shaped melanocytes which are situated deeply in the derma.

Melanoma

Melanoma is one of the most malignant tumors; it spreads through the lymphatic and hematogenous routes. 70% of melanomas develop in the skin of the face, body and extremities, pigment membrane of the eye, meninges, medullar layer of adrenal glands, and rarely in mucous membranes.

Depending upon the clinical course and prognosis there are of melanomas: 1) Superficial spreading melanoma; 2) Nodular melanoma; 3) Lentigo malignant melanoma; 4) Acral lentiginous melanoma.

Some melanomas don't contain pigment. Tumor cells are pleomorphic, usually there are a lot of mitoses in melanoma. Nodular type of melanoma is characterized by hemorrhages and necroses. In the tumor decomposition a great amount of melanin and promelanin enters the bloodstream, which may be accompanied by melaninemia and melaninuria.

TUMORS OF THE NERVOUS SYSTEM AND BRAIN COATS

Tumors of nervous system develop from different elements of the nervous system: 1) central nervous system (CNS); 2) vegetative nervous system; 3) peripheral nervous system; 4) mesenchymal elements (blood vessels, etc.). According to the degree of maturity, brain tumors may be benign or malignant.

nant. But even being benign, with a slow growth tumor affects vitally important centers and causes their dysfunction.

Neuroectodermal (neuroepithelial) tumors of the brain originating from neuroectoderm derivatives are dysontogenetic, i.e. develop from the cells which are known as precursors of mature CNS elements. Therefore, it may be difficult to determine their histological type. More often their cellular composition corresponds to definite stages of development of neuronal and glial elements.

Brain tumors produce metastases within the skull, which spread through the liquor.

Primary CNS tumours in the brain, or intracranial tumours, include: tumours arising from *constituent cells of the brain* (with the only exception of microglial cells) and from *the supporting tissues*. Childhood brain tumours arise from more primitive cells (e.g. neuroblastoma, medulloblastoma).

WHO classification of intracranial tumours include:

- I. TUMOURS OF NEUROGLIA (GLIOMAS)
 1. Astrocytoma
 2. Oligodendroglioma
 3. Ependymoma
 4. Choroid plexus papilloma
- II. TUMOURS OF NEURONS
 1. Neuroblastoma
 2. Ganglioneuroblastoma
 3. Ganglioneuroma
- III. TUMOURS OF NEURONS AND NEUROGLIA
 - Ganglioglioma
- IV. POORLY-DIFFERENTIATED AND EMBRYONAL TUMOURS
 1. Medulloblastoma
 2. Neuroblastoma
 3. PNET (primitive neuroectodermal tumor)
- V. TUMOURS OF MENINGES
 1. Meningioma
 2. Meningeal sarcoma
- VI. NERVE SHEATH TUMOURS

1. Schwannoma (neurilemmoma)
2. Neurofibroma

3. Malignant nerve sheath tumour

Gliomas

The term glioma is used for all tumours arising from neuroglia, or more precisely, from neuroectodermal epithelial tissue. They include tumours arising from :

- *astrocytes* (astrocytomas and glioblastoma multiforme);
- *oligodendrocytes* (oligodendroglioma);
- *ependyma* (ependymoma);
- *choroid plexus* (choroid plexus papilloma).

Gliomas may be well-differentiated or poorly-differentiated. However, gliomas are never truly well-demarcated or encapsulated and thus all grades of gliomas infiltrate the adjacent brain tissue.

Astrocytoma is the most common type of gliomas. It has a tendency to progress from low grade to higher grades of anaplasia. Low-grade astrocytomas evolve slowly, over several years, whereas higher grades (anaplastic astrocytoma and glioblastoma multiforme) bring about rapid clinical deterioration of the patient.

Morphologically, astrocytomas have been conventionally divided into 3 progressive histologic grades: fibrillary (most common), hemistocytic and protoplasmic. WHO classification of astrocytomas includes 4 grades:

Grade I (low grade) Astrocytoma: Juvenile pilocytic astrocytoma, pleomorphic xanthoastrocytoma;

Grade II Astrocytoma: fibrillary, protoplasmic, gemistocytic astrocytoma;

Grade III
Astrocytoma:
anaplastic
astrocytoma;

Grade IV
Astrocytoma:
glioblastoma
multiforme.

Oligodendroglioma is an uncommon glioma of oligodendroglial

origin and may develop in isolation or may be mixed with other glial cells. X-ray examination and CT scan reveal a well-defined mass with numerous small foci of calcification. The tumour is generally slow-growing.

Ependymoma is a common tumour, derived from the layer of epithelium in the brain ventricles or spinal cord central canal. It occurs mainly in

children and young adults (upto 20 years). Typically, it is localized in the fourth ventricle (posterior fossa tumour). Other locations are the lateral ventricles, third ventricle, and in the case of adults - the spinal cord in the region of lumbar spine. Usually it growth slowly.

Neuronal tumors

Ganglioneuroma is a rare mature tumor. More frequently it is localized near the 3rd ventricle, less often in the hemispheres of the brain. It usually occurs in children and adolescents. The tumor consists of mature ganglionic cells separated with the bands of glial stroma. Macroscopically, ganglioneuroma looks like a circumscribed node. In the medulla oblongata it is diffuse, in the cerebellum it looks like hyperplastic folds.

Cerebellum ganglioma is characterized by proliferation of Purkinjer's cells.

Ganglioneuroblastoma is a malignant variant of ganglioneuroma (malignant gangliocytoma). It is an extremely rare tumor of CNS. It is characterized by cellular polymorphism.

Neuroblastoma is a rare highly malignant brain tumor. It occurs mainly in children. The tumor is formed from large cells with bubble-like nucleus. Mitoses are numerous. The cells grow like syncytium.

Poorly-differentiated and embryonal tumours

CNS tumours composed of primitive undifferentiated cells include medulloblastoma and glioblastoma.

Medulloblastoma is the most common variant of primitive neuroectodermal tumour. It occurs in patients over the age of 20 years. The most common location is the cerebellum in the region of fourth ventricle roof, midline of cerebellum, vermis, or in the cerebellar hemispheres.

Medulloblastoma is a highly malignant tumour and spreads to local as well as to distant sites. It invades locally and by the cerebrospinal fluid to meninges, ventricles and subarachnoid space and has a tendency for widespread metastases to extraneural sites such as to lungs, liver, vertebrae and pelvis.

Tumours of meninges

The most common tumour arising from the pia-arachnoid is meningioma (occurs in 20% cases of intracranial tumours).

Meningiomas arise from the cap cell layer of the arachnoid. Their most common sites are in the front half of the head and include lateral cerebral convexities, midline along the falx cerebri adjacent to the major venous sinuses parasagittally, and olfactory groove. Less frequent sites are: within the cerebral ventricles, foramen magnum, cerebellopontine angle and the spinal cord. They are usually found in 2nd to 6th decades of life, with slight

female preponderance. Most meningiomas are benign and can be removed successfully. Rarely, a malignant meningioma may metastasise, mainly to the lungs.

Grossly, meningioma is well-circumscribed, solid, spherical or hemispherical mass of varying size (1-10 cm in diameter). The tumour is generally firmly attached to the dura and indents the surface of the brain but rarely ever invades it.

Microscopically, meningiomas are divided into 5 subtypes: meningotheliomatous (syncytial), fibrous (fibroblastic), transitional (mixed), angioblastic and anaplastic (malignant).

Nerve sheath tumours

Tumours of the peripheral nerves are commonly benign and include schwannoma (neurilemmoma) and neurofibroma. Both of them arise from Schwann's cells but neurofibroma contains large amount of collagen. Rarely, their malignant counterpart, malignant peripheral nerve sheath tumour, develops particularly in patients with von Recklinghausen's neurofibromatosis.

Microscopically, *schwannoma* is composed of fibrocellular bundles forming whorled pattern. There are areas of dense and compact cellularity alternating with loose acellular areas. Areas of pattern show palisaded nuclei called Verocay's bodies.

Neurofibromas may occur as solitary, fusiform cutaneous tumour of a single nerve, but more often are multiple associated with von Recklinghausen's disease. Microscopically, a neurofibroma is composed of bundles and interlacing fascicles of delicate and elongated spindle-shaped cells having wavy nuclei.

Malignant peripheral nerve sheath tumor is a poorly differentiated spindle cell sarcoma of the peripheral nerves occurring most often in adults.

Tumors of vegetative nervous system originate from ganglionic cells of different degree of maturity (sympathogonias, sympathoblasts, ganglioneurocytes) in sympathetic ganglia as well as from the cells of non-chromophinic paraganglia (glomes) genetically associated with sympathetic nervous system. Such benign tumors as ganglioneuroma, paraganglioma (glome tumor, chemodentoma) belong to this group.

TUMOR DISEASES OF THE BLOOD AND LYMPHOID TISSUE

LEUKAEMIAS

Definition and classification. Leukaemias are a group of disorders characterized by malignant transformation of blood-forming cells. The proliferation of leukaemic cells takes place primarily in the red bone marrow, and in certain forms, in the lymphoid tissue. Ultimately, the abnormal cells appear in the peripheral blood raising the total white cell count to high level. In addition, features of bone marrow failure (e.g., anaemia, thrombocytopenia, neutropenia) and involvement of other organs (e.g., liver, spleen, lymph nodes, meninges, brain, skin etc) occur.

In general, leukaemias are classified according to the cell types predominantly involved, into *myeloid* and *lymphoid*, and on the basis of the disease evolution into *acute* (with the proliferation of blast, nondifferentiated cells) and *chronic* (with the proliferation of differentiated cells, relatively benign course).

Depending on impairment in peripheral blood formula and the number of white blood cells there are following forms of leukaemias:

1. Leukemic that are characterized by a significant increase in the number of WBC, including leukemia cells in peripheral blood (tens or hundreds of thousands, sometimes millions in 1 /mcL of blood). This is the most common form.

2. Subleukemic – the WBC count is slightly elevated - up to 30 000 in 1 mcL of blood.

3. Aleukemic – the number of WBC is within the normal range and tumor cells are not present in the blood. This type is rare, but it usually occurs in the early stages of the disease.

4. Leukopenic – the number of WBC is below normal.

Hiatus leukemicus - A condition observed in acute myelogenous leukemia in which there are numerous myeloblasts and a number of mature neutrophils in the peripheral blood, with few or no intermediate forms. The term "hiatus leukemicus" suggests a jump in cell development from an early stage to a late stage with nothing in between, analogous to changing from the appearance of a 10 year old to that of a 60 year old without any intermediate stages.

Leukaemias account for 4% of all cancer deaths. Generally, acute leukaemias have a rapidly progressing course, whereas chronic leukaemias

tend to have more indolent behaviour. The incidence of both acute and chronic leukaemias is higher in men than in women.

Etiology. The etiology of leukaemia is not known in most patients.

However, a number of factors have been implicated.

1. **GENETIC FACTORS.** There is high concordance rate among identical twins if acute leukaemia develops in the first year of life. Families with excessive incidence of leukaemia have been identified. Acute leukaemia occurs with increased frequency with a variety of congenital disorders such as Down's, Bloom's, Klinefelter's, Fanconi's and Wiskott-Aldrich's syndromes.

2. **ENVIRONMENTAL FACTORS.** Certain environmental factors are known to play a role in the etiology of leukaemia. These include the following:

i) **Ionising radiation**, e.g. in individuals exposed to occupational radiation exposure, patients receiving radiation therapy, and Japanese survivors of the atomic bomb explosions. Radiation exposure is related to the development of chronic myelocytic leukemia (CML), acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) but not to chronic lymphocytic leukemia (CLL) or HCL.

ii) **Chemical carcinogens**, e.g. benzene and other aromatic hydrocarbons are associated with the development of AML.

iii) **Certain drugs**, e.g. treatment with alkylating agents and other chemotherapeutic agents is associated with increased incidence of AML.

3. **INFECTIOUS.** Induction of leukaemias in experimental animals by RNA viruses (retroviruses) has been studied for quite sometime but more recently viral etiology of adult T-cell leukaemia-lymphoma (ATLL) by a human retrovirus called human T-cell leukaemia-lymphoma virus I (HTLV- I) and HTLV II for T cell variant of hairy cell leukaemia has been established.

Pathogenesis (Leukaemogenesis). The leukaemia arises following *malignant transformation of a single clone of cells belonging to myeloid or lymphoid series, followed by proliferation of the transformed clone.* The evolution of leukaemia is multi-step process, and in many cases, acute leukaemia may develop after a pre-existing myelodysplastic or myeloproliferative disorder.

The salient features in the pathogenesis of leukaemias are as under:

- In acute leukaemia, the single most prominent characteristic of the leukaemic cells is a defect in maturation beyond the myeloblast or promyelocyte level in AML, and the lymphoblast level in ALL. However, it may be emphasised here that it is the *maturation defect* in leukaemic blasts rather than rapid proliferation of leukaemic cells responsible for causing acute leukaemia. In fact, the generation time of leukaemic blasts is somewhat prolonged rather than shortened.

- The leukaemic cells proliferate primarily in the bone marrow, circulate in the blood and *infiltrate into other tissues* such as lymph nodes, liver, spleen, skin, viscera and the central nervous system.

- The *mechanism of leukaemic transformation* is poorly understood.

But, the basic defect lies in the DNA, conferring a heritable malignant characteristic to the transformed cell and its progeny. A neoplastic phenotype may be induced by RNA viruses (e.g. HTLV-I) and causes insertional mutagenesis for which oncogenes may play a role as. A number of clonal cytogenetic abnormalities have been reported in association with the various forms of acute and chronic leukaemias. The most consistent chromosomal abnormality among these is Philadelphia (Ph) chromosome seen in 70-90% cases with CML, involving reciprocal translocation of parts of long arm of chromosome 22 to the long arm of chromosome 9 i.e. t (9;22).

- As the leukaemic cells accumulate in the bone marrow, they *suppress normal haematopoietic stem cells*, partly by physically replacing the normal marrow precursors. However, some patients with acute leukaemia have a hypocellular marrow indicating that marrow failure is not simply due to overcrowding by leukaemic cells but may inhibit normal haematopoiesis via cell-mediated or humoral mechanisms. Nevertheless, some normal haematopoietic stem cells do remain in the marrow which are capable of proliferating and restoring normal haematopoiesis after effective anti-leukaemic treatment.

ACUTE LEUKAEMIAS

Acute leukaemias are characterised by predominance of undifferentiated leucocyte precursors or leukaemic blasts. Acute leukaemias may be derived from the myeloid stem cells called *acute myeloblastic leukaemia* (AML), or from the lymphoid stem cells termed *acute lymphoblastic leukaemia* (ALL).

Recently, more definite criteria for diagnosis and classification of acute leukaemias have been laid down by a group of French, American and British haematologists commonly called the *FAB classification*. According to this system, a leukaemia is acute if the bone marrow consists of more than 30% blasts. FAB classification divides AML into 8 subtypes (M0 to M7) and ALL into 3 subtypes (L1 to L3).

Most frequent types of acute leucemias are:

1. Acute nondifferentiated,
2. Acute myeloblastic,
3. Acute lymphoblastic,
4. Acute plasmoblastic,
5. Acute monoblastic,
6. Acute myelomonoblastic,
7. Acute erythromyeloblastic,
8. Acute megakaryoblastic.

More recently, another classification called revised European-American classification of lymphoid neoplasms (REAL classification) has been formulated and includes acute and chronic lymphoid leukaemias and non-Hodgkin's lymphomas since they represent the counterparts neoplastic in the bone marrow and lymphoid tissue.

Clinical Features

AML and ALL share many clinical features. In approximately 25% of patients with AML, a preleukaemic syndrome with anaemia and other cytopenias is usually present for a few months to years prior to the development of overt leukaemia.

Clinical manifestations of acute leukaemias are divided into 2 groups: those *due to bone marrow failure*, and those *due to organ infiltration*.

I. DUE TO BONE MARROW FAILURE. These are some syndromes:

1. *Anaemia* producing pallor, lethargy, dyspnoea.
2. *Bleeding manifestations* due to thrombocytopenia: spontaneous bruises, petechiae, and other bleeding tendencies.
3. *Infections* are quite common and include those of mouth, throat, skin, respiratory, perianal and other sites.

4. *Fever* is generally attributed to infections in acute leukaemia but sometimes no obvious source of infection can be found and may occur in the absence of infection.

II. **DUE TO ORGAN INFILTRATION.** The clinical manifestations of acute leukaemia are more often due to replacement of the marrow and other tissues by leukaemic cells. These features are as under:

1. *Pain and tenderness of bones* (e.g. sternal tenderness) are due to bone infarcts or subperiosteal infiltrates by leukaemic cells. These features are more frequent in children with ALL.

2. *Lymphadenopathy* and enlargement of the *tonsils* are more common in ALL.

3. *Splenomegaly* of moderate grade is common, especially in ALL. Splenic infarction, subcapsular haemorrhages, and rarely, splenic rupture may occur.

4. *Hepatomegaly* is frequently present due to leukaemic infiltration but the infiltrates usually do not interfere with the function of the liver.

5. *Leukaemic infiltration of the kidney* may be present and ordinarily does not interfere with its function unless secondary complications such as haemorrhage or blockage of ureter supervene.

6. *Gum hypertrophy* due to leukaemic infiltration of the gingivae is a frequent finding in myelomonocytic (M4) and monocytic (M5) leukaemias.

7. *Chloromas* are localised tumour-forming masses occurring in the skin or orbit due to local infiltration of the tissues by leukaemic cells. They are greenish in appearance due to the presence of myeloperoxidase.

8. *Meningeal involvement* manifested by raised intra-cranial pressure, headache, nausea and vomiting, blurring of vision and diplopia are seen more frequently in ALL during haematologic remission. Sudden death from massive intracranial haemorrhage as a result of leucostasis may occur.

9. *Other organ infiltrations* include testicular swelling in ALL and mediastinal compression in T cell type ALL.

Laboratory Findings

The diagnosis of acute leukaemia is made by a combination of routine blood picture and bone marrow examination, coupled with cytochemical stains and certain biochemical investigations.

I. **BLOOD PICTURE.** Findings of routine haematologic investigations are as under:

1. **Anaemia.** Anaemia is almost always present in acute leukaemias. It is generally severe, progressive and normo-chromic in type. A moderate reticulocytosis up to 5% and a few nucleated red cells may be present.

2. Thrombocytopenia. The platelet count is usually moderately to severely decreased (below 50,000/ μ l) but occasionally it may be normal. Bleeding tendencies in acute leukaemia are usually correlated with the level of thrombocytopenia but most serious spontaneous haemorrhagic episodes develop in patients with fewer than 20,000/ μ l platelets. Acute promyelocytic leukaemia (M3) may be associated with a serious coagulation abnormality called disseminated intravascular coagulation (DIC).

3. White blood cells. The total WBC count ranges from subnormal-to-markedly elevated values. In 25% of patients, the total WBC count at presentation is reduced to 1,000-4,000 / μ l. More often, however, there is progressive rise in white cell count which may exceed 100,000/ μ l in more advanced disease. The majority of leucocytes in the peripheral blood are blasts and there is often neutropenia due to marrow infiltration by leukaemic cells. Some patients present with pancytopenia and have a few blasts (*sub-leukaemic leukaemia*) or no blasts (*aleukaemic leukaemia*) in the blood. Both these conditions are nowadays included under '*myelodysplastic syndrome*'. In some instances, the *identification of blast cells is greatly aided by the company they keep* i.e. by more mature and easily identifiable leucocytes in the company of blastic cells of myeloid or lymphoid series. It is usual to find some '*smear cells*' in the peripheral blood which represent degenerated leucocytes, particularly in the case of ALL.

II. **An examination of bone marrow** reveals the hypercellularity due to pancytopenia.

The diagnosis of the type of leukaemic cells, according to FAB classification, is generally possible with routine Romanowsky stains but cytochemical stains may be employed as an adjunct to Romanowsky staining for determining the type of leukaemia. The essential criteria for diagnosis of acute leukaemia, as per FAB classification, is the presence of at least 30% blasts in the bone marrow.

Erythropoietic cells are reduced. Dyserythropoiesis, megaloblastic features and ring sideroblasts are commonly present. Megakaryocytes are usually reduced or absent.

Chromosomal analysis of dividing leukaemic cells in the marrow shows karyotypic abnormalities in 75% of cases which may have a relationship to prognosis. Three of the common chromosomal abnormalities are as under:

i) *Aneuploidy* is the most common finding in acute leukaemia. In ALL, aneuploidy is almost always hyper-diploid while hypo- and hyper-diploid cell lines are found with approximately equal frequency in AML. Cases with hyper-diploidy between 50 to 60 chromosomes are associated with good prognosis.

ii) *Ph chromosome, t (9; 22)*, though commonly found in CML, is found in 2-3% of children with ALL and 25-30% cases of adults with ALL. It is associated with poor prognosis in both these forms of acute leukaemia.

iii) Mature form of B-ALL has characteristic *t (8;14) trans-location* which too is associated with worse prognosis.

III. **CYTOCHEMISTRY.** Some of the commonly employed cytochemical stains, as an aid to classify the type of acute leukaemia:

1. **Myeloperoxidase:** Positive in immature myeloid cells containing granules and Auer rods but negative in MO myeloblasts.

2. **Sudan Black:** Positive in immature cells in AML.

3. **Periodic acid-Schiff (PAS):** Positive in immature lymphoid cells and in erythroleukaemia (M6).

4. **Non-specific esterase (NSE):** Positive in monocytic series (M4 and M5).

5. Acid phosphatase: Focal positivity in leukaemic blasts in ALL and diffuse reaction in monocytic cells (M4 and M5).

IV. **OTHER INVESTIGATIONS.** Other laboratory tests which may be of some help in classifying leukaemias are as under:

1. **Cell surface markers.** These are of particular value in classifying various types of ALL. These include the following:

i) *For all types of ALL:* Estimation of terminal deoxynucleotidyl transferase (TdT) by immunofluorescence in 95% cases of ALL.

ii) *For B cell ALL,* positive lymphoid markers are CD19 (pan-B) and CD10 in most cases and CD20 in 50% cases.

iii) *For T cell ALL,* lymphoid markers are CD3 and CD7.

2. **Serum muramidase.** Serum levels of lysozyme (i.e. muramidase) are elevated in myelomonocytic (M4) and monocytic (M5) leukaemias.

3. **Serum uric acid.** Because of rapidly growing number of leukaemic cells, serum uric acid level is frequently increased. The levels are further raised after treatment with cytotoxic drugs because of increased cell breakdown.

CHRONIC LEUKAEMIAS

Chronic leukaemias are those haematologic malignancies in which the predominant leukaemic cells are initially well-differentiated and easily recognisable as regards their cell type.

Chronic leukaemias are divided into 2 main types: chronic myeloid (granulocytic) leukaemia (CML or CGL), and chronic lymphocytic leukaemia (CLL).

In general, chronic leukaemias have a better prognosis than the acute leukaemias. Currently CML has come to be classified alongwith other myeloproliferative syndromes due to common histogenesis from haematopoietic stem cells (described later). However, for the purpose of present discussion, CML is described here alongwith other chronic leukaemias.

CHRONIC MYELOID LEUKAEMIAS (CML)

Chronic myeloid (myelogenous, granulocytic) leukaemia comprises about 20% of all leukaemias and its peak incidence is seen in 3rd and 4th decades of life. A distinctive variant of CML seen in children under 3 years of age is called *juvenile CML*. Both the sexes are affected equally. The exact etiology is not known but an increased incidence of both AML and CML was observed years later in Japanese atomic bomb survivors implicating radiation in their etiology.

Chronic leukaemias of myeloid origin fall into one of two WHO categories. The first, the myeloproliferative neoplasms (MPN), includes chronic myelogenous leukaemia (CML), chronic neutrophilic leukaemia (CNL), chronic eosinophilic leukaemia (CEL) and mast cell leukaemia. These are clonal haematopoietic stem cell disorders with essentially normal morphology of the endstage cell. The type of chronic leukaemia is defined by the lineage of the predominant proliferating cell. The second, the myelodysplastic/myeloproliferative neoplasms, consists of chronic myelomonocytic leukaemia (CMML), juvenile myelomonocytic leukaemia (JMML), and atypical chronic myeloid leukaemia (aCML). These all have dysplastic morphology as well as proliferative features.

Clinical Features

The onset of CML is generally insidious. Some of the common presenting manifestations are as under:

1. Features of *anaemia* such as weakness, pallor, dyspnoea and tachycardia.
2. Symptoms due to *hypermetabolism* such as weight loss, lassitude, anorexia, night sweats.
3. *Splenomegaly* is almost always present and is frequently massive. In some patients, it may be associated with acute pain due to splenic infarction.
4. *Bleeding tendencies* such as easy bruising, epistaxis, menorrhagia and haematomas may occur.
5. Less *commonly*, features such as gout, visual disturbance, neurologic manifestations and priapism are present.
6. *Juvenile* CML is more often associated with lymph node enlargement than splenomegaly. Other features are frequent infections, haemorrhagic manifestations and facial rash.

The diagnosis of CML is generally possible on blood picture alone. However, bone marrow, cytochemical stains and other investigations are of help.

The natural history of CML consists of 2 phases - chronic and blastic.

- *The chronic phase of CML* begins as a myeloproliferative disorder and consists of excessive proliferation of myeloid cells of intermediate grade (i.e. myelocytes and metamyelocytes) and mature segmented neutrophils. Myeloblasts usually do not exceed 10% of cells in the peripheral blood and bone marrow. An increase in the proportion of basophils up to

10% is a characteristic feature of CML. A rising *basophilia* is indicative of impending blastic transformation. An *accelerated phase of CML* is also described in which there is progressively rising leucocytosis associated with thrombocytosis or thrombocytopenia and splenomegaly.

- *The blastic phase or blast crisis in CML* may be myeloid or lymphoid in origin. Myeloid blast crisis in CML is more common and resembles AML. However, unlike AML, Auer rods are never seen in myeloblasts of CML in blast crisis. *Lymphoid blast crisis* in CML having the characteristics of lymphoblasts such as presence of TdT is seen in one-third cases of blastic phase in CML.

Generally, there is hypercellularity of marrow with total or partial replacement of fat spaces by proliferating myeloid cells. The myeloid cells pre- dominate in the bone marrow with increased myeloid-erythroid ratio. Erythro- poiesis is normoblastic but there is reduction in erythropoietic cells. Megakar- yocytes are conspicuous but are usually smaller in size than normal.

Cytogenetic studies on blood and bone marrow cells show the characteristic chromosomal abnormality called Philadelphia (Ph) chromosome formed by reciprocal translocation between part of long arm of chromosome 22 and part of long arm of chromosome 9 $\{(t(9;22))\}$ seen in 70-90% cases with CML.

The most common cause of death (in 80% cases) in CML is blastic transformation.

CHRONIC LYMPHOCYTIC LEUKAEMIAS (CLL)

Chronic lymphocytic leukaemia constitutes about 25% of all leukaemias and is predominantly a disease of the elderly (over 50 years of age) with a male preponderance (male-female ratio 2:1).

CLL is the commonest leukaemia in the Western world and occurs in adults. It is characterized by monomorphic, small, round lymphocytes with clumped chromatin and minimal cytoplasm, together with a small number of prolymphocytes. The cells express B-cell antigens (CD19, weak CD20, and CD22), CD5, CD23, and weak IgM. Expression of ZAP70 and CD38, and genetic abnormalities (e.g. p53 and 11q deletions) assist in determining prognosis.

Classification

B-cell

Chronic lymphocytic leukaemia (CLL)

T-cell

Prolym-
phocytic
c
leukaemia
(PLL)
Hairy
cell
leukaemia
(HCL)
Plasma cell (paraproteinaemic) leukaemia

Large granular lymphocytic leukaemia
T-cell
prolymphocytic
leukaemia
(T-PLL) Adult
T-cell
leukaemia/
lymphoma
Sézary
syndrome

Common Clinical Features

The onset of disease is characteristically insidious. Common presenting manifestations are as under:

1. Features of *anaemia* such as gradually increasing weakness, fatigue and dyspnoea
2. Enlargement of superficial *lymph nodes* is a very common finding. The lymph nodes are usually symmetrically enlarged, discrete and non-tender.
3. *Splenomegaly* and *hepatomegaly* are usual.
4. *Haemorrhagic manifestations* are found in case of CLL with thrombocytopenia.
5. *Infections*, particularly of respiratory tract, are common in CLL.
6. *Less common* are: mediastinal pressure, tonsillar enlargement, disturbed vision, and bone and joint pains.

Laboratory Findings

The diagnosis of CLL can usually be made on the basis of physical findings and blood smear examination.

I. **BLOOD PICTURE.** The findings of routine blood picture are as under:

1. **Anaemia.** Anaemia is usually mild to moderate and normocytic normochromic in type. Mild reticulocytosis may be present. About 20% cases develop a Coombs'-positive autoimmune haemolytic anaemia.

2. **White blood cells.** Typically, there is marked leuco-cytosis but less than that seen in CML (50,000-200,000/ μ l). Usually, more than 90% of leucocytes are mature small lymphocytes. Smear cells (degenerated forms) are present. The absolute neutrophil count is, however, generally within normal range. Granulocytopenia occurs in fairly advanced disease only.

3. **Platelets.** The platelet count is normal or moderately reduced.

II. **BONE MARROW EXAMINATION.** The typical findings are:

1. Increased lymphocyte count (25-95%).
2. Reduced myeloid precursors.
3. Reduced erythroid precursors.

III. **OTHER INVESTIGATIONS.** These are:

1. *Erythrocyte rosette test* with mouse red cells is positive in more than 95% of cases indicating that CLL is a monoclonal B cell neoplasm.
2. *Serum immunoglobulin levels* are generally reduced.
3. *Coombs' test* is positive in 20% cases.

Prognosis is generally better than CML since *blastic transformation of CLL seldom occurs*. Rare T-cell variant of CLL, however, runs a more malignant course. Prognosis generally correlates with the stage of disease as under:

Stage A: characterised by lymphocytosis alone, or with limited lymphadenopathy, has a good prognosis (median survival more than 10 years).

Stage B: having lymphocytosis with associated significant lymphadenopathy and hepatosplenomegaly has intermediate prognosis (median survival about 5 years).

Stage C: having lymphocytosis with associated anaemia and thrombocytopenia has a worse prognosis (median survival of less than 2 years).

B-CELL PROLYMPHOCYTIC LEUKAEMIA

B-cell prolymphocytic leukaemia (B-PLL) is rare in comparison with CLL. It presents in elderly patients, predominantly men, with marked splenomegaly and minimal lymphadenopathy, and has a poor prognosis. The neoplastic cell is larger than in CLL and is round with a centrally placed nucleus, single prominent nucleolus, and basophilic cytoplasm. The cells strongly express surface IgM and B-cell antigens; CD5 is present in one-third and CD23 is negative.

HAIRY CELL LEUKAEMIA

Hairy cell leukaemia (HCL) is a rare leukaemia that occurs primarily in middle-aged men, is indolent and potentially curable. It is typically associated with pancytopenia, splenomegaly, and fibrotic marrow. The leukaemic cells are of intermediate size due to abundant irregular grey cytoplasm with 'hairy' projections. The nuclei are eccentrically located and have a reniform shape. They have a characteristic phenotype with strong B-cell antigens, and express CD11c, CD103, CD123 and CD25 antigens. They have cytochemical tartrate-resistant acid phosphatase positivity.

PARAPROTEINAEMIC LEUKEMIAS

The paraproteinemic leukemias are characterised by abnormal proliferation of immunoglobulin-producing cells and result in accumulation of monoclonal immunoglobulin in serum and urine. The group as a whole is known by various synonyms such as *plasma cell dyscrasias*, *paraproteinaemias*, *dysproteinaemias* and *monoclonal gammopathies*. The group comprises the following four disease entities:

1. Multiple myeloma
2. Waldenstrom's macroglobulinaemia
3. Heavy chain disease
4. Primary amyloidosis.

The feature common to all plasma cell disorders is the neoplastic proliferation of cells derived from B-lymphocyte lineage. Normally B lymphocytes have surface immunoglobulin molecules of both M and G heavy chains. Under normal circumstances, the B-cells are stimulated by exposure to surface immunoglobulin-specific antigen and mature to form IgG-producing plasma cells. However, in the plasma cell disorders, the control over this process is lost and results in abnormal production of immunoglobulin that appears in the blood and urine. These disorders differ from B-cell lymphomas in having monoclonal synthesis, of immunoglobulins and lack of prominent lymphadenopathy. In addition to the rise in complete immunoglobulins, some plasma cell disorders synthesise excess of light chains (kappa or lambda), or heavy chains of a single class (alpha, gamma, or mu). Bence Jones proteins are free light chains present in blood and excreted in urine of some cases with plasma cell disorders. After these brief general comments, we can now turn to the discussion of the specific plasma cell disorders.

There is rise in the total serum protein concentration due to *paraproteinaemia*. Paraproteins are abnormal immunoglobulins or their parts circulating in plasma and excreted in urine. The most common paraprotein is *IgG* seen in two-third cases of myeloma, *IgA* in one-third, and rarely there may be *IgM* or *IgD* paraproteins, or various combinations. Though the commonest cause of paraproteinaemias is multiple myeloma, certain other conditions may also produce serum paraproteins such as:

- Waldenstrom's macroglobulinaemia;
- Benign monoclonal gammopathy;
- B-cell lymphomas;

- CLL;
- Light chain disease;
- Heavy chain disease; and
- Cryoglobulinaemia.

As a result of paraproteinaemia, normal serum immunoglobulins (IgG, IgA and IgM) and albumin are depressed. The paraprotein usually appears as a single narrow homogeneous *M-band* on the serum electrophoresis, most commonly in the region of γ -globulin. About two-third cases of myeloma excrete Bence Jones (light chain) proteins in the urine, consisting of either kappa or lambda light chains, alongwith presence of Bence Jones paraproteins in the serum.

MULTIPLE MYELOMA

Multiple myeloma is a multifocal malignant proliferation of plasma cells derived from a single clone of cells (i.e. monoclonal). The terms multiple myeloma and myeloma are used interchangeably. The tumour, its products (M component), and the host response result in the most important and most common syndrome in the group of plasma cell disorders that produces osseous as well as extraosseous manifestations. Multiple myeloma primarily affects the elderly (peak incidence in 5th-6th decades) and increases in incidence with age. It is rare under the age of 40 and is slightly more common in males.

Etiopathogenesis. Myeloma is a monoclonal proliferation of B-cells. But the exact pathogenesis how it occurs in certain individuals only is not known. Two hypotheses have been proposed:

1. **Antigenic stimulation:** According to this hypothesis, chronic antigenic stimulation in a predisposed individual results in growth deregulation and eventual monoclonal proliferation of B-cell clones.

2. **Predisposition to myeloma:** There is some evidence to suggest that chromosomal translocations and genetic predisposition to myeloma play roles in monoclonal proliferation of B-cell lineage.

The current concept combines the features of both these hypotheses i.e. *induction of myeloma requires prolonged exposure to foreign antigens in predisposed individuals.*

PATHOLOGIC CHANGES. Myeloma affects principally the bone marrow though in the course of the disease other organs are also involved. There-

fore, the pathologic findings are described below under two headings—*osseous (bone marrow) lesions* and *extraosseous lesions*.

I. OSSEOUS (BONE MARROW) LESIONS. In more than 95% of cases, multiple myeloma begins in the bone marrow. In the majority of cases, the disease involves multiple bones. By the time the diagnosis is made, most of the bone marrow is involved. Most commonly affected bones are those with red marrow i.e. skull, spine, ribs and pelvis, but later long bones of the limbs are also involved. The lesions of myeloma secrete osteoclast-stimulating factor resulting in rarefaction of the bones. The lesions begin in the medullary cavity, erode the cancellous bone and ultimately cause destruction of the bony cortex. Radiographically, these lesions appear as punched out, rounded, 1-2 cm sized defects in the affected bone.

Grossly, the normal bone marrow is replaced by soft, gelatinous, reddish-grey tumours. The affected bone usually shows focal or diffuse osteoporosis.

Microscopically, the diagnosis of multiple myeloma can be usually established by examining bone marrow aspiration from an area of bony rarefaction. However, if the bone marrow aspiration yields dry tap or negative results, biopsy of radiologically abnormal or tender site is usually diagnostic. The following features characterise a case of myeloma:

i) **Cellularity:** There is usually hypercellularity of the bone marrow.

ii) **Myeloma cells:** Myeloma cells constitute 15-30% of the marrow cellularity. These cells may form clumps or sheets, or may be scattered among the normal haematopoietic cells. Myeloma cells may vary in size from small, differentiated cells resembling normal plasma cells to large, immature and undifferentiated cells. Binucleate and multinucleate cells are sometimes present. The nucleus of myeloma cell is commonly eccentric similar to plasma cells but usually lacks the cart-wheel chromatin pattern seen in classical plasma cells. Nucleoli are frequently present. The cytoplasm of these cells is abundant and basophilic with perinuclear halo, vacuolisation and contains Russell bodies consisting of hyaline globules composed of synthesised immunoglobulin

In addition to multiple myeloma, *plasmacytosis* in the bone marrow can occur in some other disorders. These are: aplastic anaemia, rheumatoid arthritis, SLE, cirrhosis of liver, metastatic cancer and chronic inflammation. However, in all these conditions the plasma cells are mature and they do not exceed 10% of the total marrow cells.

II. **EXTRAOSSEOUS LESIONS.** Late in the course of disease, lesions at several extraosseous sites become evident. Some of the commonly involved sites are as under:

1. **Blood.** Approximately 50% of patients with multiple myeloma have a few atypical plasma cells in the blood. Other changes in the blood in myeloma are the presence of anaemia (usually normocytic normochromic type), marked red cell rouleaux formation due to hyperviscosity of blood, and an elevated ESR.

2. **Myeloma kidney.** Renal involvement in myeloma called myeloma nephrosis occurs in many cases. The main mechanism of myeloma kidney is by filtration of light chain proteins (Bence Jones proteins) which are precipitated in the distal convoluted tubules in combination with Tamm-Horsfall proteins as tubular casts. The casts may be surrounded by some multinucleate giant-cells and a few inflammatory cells.

3. **Myeloma neuropathy.** Infiltration of the nerve trunk roots by tumour cells produces nonspecific polyneuropathy. Pathologic fractures, particularly of the vertebrae may occur causing neurologic complications.

4. **Systemic amyloidosis.** Systemic primary generalised amyloidosis (AL amyloid) may occur in 10% cases of multiple myeloma and involve multiple organs and systems.

5. **Liver, spleen involvement.** Involvement of the liver and spleen by myeloma cells sufficient to cause hepatomegaly, and splenomegaly occurs in a small percentage of cases.

Clinical Features

The clinical manifestations of myeloma result from the effects of infiltration of the bones and other organs by neoplastic plasma cells and from immunoglobulin synthesis. The principal clinical features are as under :

1. *Bone pain* is the most common symptom. The pain usually involves the back and ribs. Pathological fractures may occur causing persistent localised pain. Bone pain results from the proliferation of tumour cells in the marrow.

2. *Susceptibility to infections* is the next most common clinical feature. Particularly common are bacterial infections such as pneumonias and pyelonephritis. The increased susceptibility to infection is related mainly to decreased production and increased destruction of normal antibodies, and partly to granulocyte dysfunction and neutropenia.

3. *Renal failure* occurs in about 25% of patients, while renal pathology occurs in 50% of cases. Causes of renal failure in myeloma are hypercalcaemia, glomerular deposits of amyloid, hyperuricaemia and infiltration of the kidney by myeloma cells.

4. *Anaemia* occurs in majority of patients of myeloma and is related to marrow replacement by the tumour cells and inhibition of haematopoiesis.

5. *Bleeding tendencies* may appear in some patients due to thrombocytopenia, deranged platelet function and interaction of the M component with coagulation factors.

6. *Neurologic symptoms* occur in a minority of patients.

7. *Hyperviscosity syndrome* may produce headache, fatigue, visual disturbances and haemorrhages.

Diagnosis

The diagnosis of myeloma is made by classic *triad* of features:

1. marrow plasmacytosis of more than 10%;
2. radiologic evidence of lytic bony lesions; and
3. demonstration of serum and/or urine M component.

Two variants of myeloma which do not fulfil the criteria of classical triad are *solitary bone plasmacytoma* and *extramedullary plasmacytoma*. Both these are associated with M component in about a third of cases and occur in young individuals. Solitary bone plasmacytoma is a lytic bony lesion without marrow plasmacytosis. Extramedullary plasmacytoma involves most commonly the submucosal lymphoid tissue of nasopharynx or paranasal sinuses. Both variants have better prognosis than the classic multiple myeloma. *Plasma cell granuloma*, on the other hand, is an inflammatory condition having admixture of other inflammatory cells with mature plasma cells.

Prognosis

Treatment of multiple myeloma consists of systemic chemotherapy in the form of alkylating agents and symptomatic supportive care. The median survival is 2 years after the diagnosis is made. The terminal phase is marked by the development of pancytopenia, severe anaemia and sepsis.

WALDENSTROM'S MACROGLOBULINAEMIA

Waldenstrom's macroglobulinaemia is an uncommon malignant proliferation of monoclonal B lymphocytes which secrete IgM paraproteins called macroglobulins as they have high molecular weight. The condition is more common in men over 50 years of age and behaves clinically like a slowly progressive lymphoma.

Etiopathogenesis. Waldenstrom's macroglobulinaemia is a monoclonal proliferation of B-cells and is accompanied by IgM paraproteinaemia, but the exact etiology is not known. A possible relationship of IgM macroglobulin with myelin-associated glycoprotein which is lost in degenerating diseases has been suggested. The clinical evidence in favour is the appearance of peripheral neuropathy before the occurrence of macroglobulinaemia in some patients.

Pathologic changes. Pathologically, the disease can be regarded as the hybrid between myeloma and small lymphocytic lymphoma.

• *Like myeloma*, the disease involves the bone marrow, but unlike myeloma it usually does not cause extensive bony lesions or hypercalcaemia. The bone marrow shows pleomorphic infiltration by lymphocytes, plasma cells, lymphocytoid plasma cells, mast cells and histiocytes. Like in myeloma, serum M component is present. • *Unlike myeloma and more like small lymphocytic lymphoma*, enlargement of lymph nodes, spleen and liver due to infiltration by similar type of cells is present more frequently.

Clinical features. The clinical features of the disease are due to both infiltration by the disease and paraproteins in the blood.

1. *Hyperviscosity syndrome* is the major clinical manifestation. It results in visual disturbances, weakness, fatiguability, weight loss and nervous system symptoms. Raynaud's phenomenon may occur.

2. *Moderate organomegaly* in the form of lymphadenopathy, hepatomegaly and splenomegaly are frequently seen.

3. *Anaemia* due to bone marrow failure may be present.

4. *Bleeding tendencies* may occur due to interaction of macroglobulins with platelets and coagulation factors.

Diagnosis Unlike myeloma, there are no characteristic radiologic findings. The diagnosis rests on laboratory data. These are:

1. Pleomorphic bone marrow infiltration.

2. Raised total serum protein concentration.

3. Raised serum monoclonal M component which is due to IgM paraprotein.

4. Elevated ESR.

5. Normocytic normochromic anaemia.

Prognosis. The management of the patients is similar to that of myeloma. Patients respond to chemotherapy with a median survival of 3-5 years.

HEAVY CHAIN DISEASES

Heavy chain diseases are rare malignant proliferations of B-cells accompanied by monoclonal excess of one of the heavy chains. Depending upon the type of excessive heavy chain, three types of heavy chain diseases are distinguished: γ , α and μ heavy chain diseases.

GAMMA HEAVY CHAIN DISEASE: Also called Franklin's disease, it is characterised by excess of mostly γ_1 -paraprotein, both in the serum and urine and is demonstrated as M component. Clinically, the condition may develop at any age and present with lymphadenopathy, splenomegaly, hepatomegaly, involvement of pharyngeal lymphoid tissue (Waldeyer's ring) and fever.

Patients have rapidly downhill course due to severe and fatal infection.

ALPHA HEAVY CHAIN DISEASE: This is the commonest of heavy chain diseases characterised by α -heavy chains in the plasma which are difficult- to demonstrate in electrophoresis due to rapid polymerisation. The patients present with bowel symptoms such as chronic diarrhoea, malabsorption and weight loss and may have enlargement of abdominal lymph nodes. Chemotherapy may induce long-term remissions.

MUHEAVY CHAIN DISEASE: This is the rarest heavy chain disease. The neoplastic B-cells produce μ heavy chains as well as κ light chains; the latter appear in the urine while the former do not. Another feature that distinguishes this type of heavy chain disease from the others is the presence of vacuoles in the malignant B lymphocytes. The course and prognosis are like those of leukaemia or lymphoma.

HAIRY CELL LEUKAEMIA

Hairy cell leukaemia (HCL) is an unusual and uncommon form of chronic leukaemia in which there is presence of abnormal mononuclear

cells with hairy cytoplasmic projections in the bone marrow, peripheral

blood and spleen. These cells are best recognised under phase contrast microscopy but may also be visible in routine blood smears. These leukaemic 'hairy cells' have characteristically positive cytochemical staining for *tartrate-resistant acid phosphatase*. The controversy on the origin of hairy cells whether these cells represent neoplastic T-cells, B-cells or monocytes, is settled with the molecular analysis of these cells which assigns them *B-cell origin* expressing CD19 and CD20 antigen.

Hairy cell leukaemia occurs in the older males. It is characterised clinically by the manifestations due to infiltration of reticuloendothelial organs (bone marrow, liver and spleen) and, hence, its previous name as *leukaemic reticuloendotheliosis*. Laboratory diagnosis is made by the presence of pancytopenia due to marrow failure and splenic sequestration, and identification of characteristic hairy cells positive for tartrate-resistant acid phosphatase.

The disease often runs a chronic course requiring supportive care. The mean survival is 4-5 years. Splenectomy is considered beneficial in many cases.

LYMPHOMAS

Lymphomas are malignant tumours of lymphoreticular origin i.e. from lymphocytes and histiocytes and their precursor cells. Clinically and pathologically, lymphomas are quite heterogeneous. However, two distinct clinicopathologic groups are routinely distinguished:

I. *Hodgkin's lymphoma* or *Hodgkin's disease (HD)* characterised by pathognomonic presence of Reed-Sternberg cells. This group comprises about 25% of all cases of malignant lymphoma.

II. *Non-Hodgkin's lymphomas (NHL)* are more common and comprise the rest of cases.

Both these groups have further histopathologic subtypes and are considered separately below.

HODGKIN'S DISEASE

Hodgkin's disease (HD) primarily arises within the lymph nodes and involves the extranodal sites secondarily. The incidence of the disease has

bimodal peaks - one in young adults between the age of 15 and 35 years and the other peak after 5th decade of life. The HD is more prevalent in young adult males than females. The classical diagnostic feature is the presence of *Reed-Sternberg (RS) cell*.

Etiopathogenesis. The nature and origin of RS cells, which are the real neoplastic cells in HD, have been a matter of considerable debate. One main reason for this difficulty in their characterisation is that in HD, unlike most other malignancies, the number of neoplastic cells (i.e. RS cells) is very small (less than 5%) which are interspersed in the predominant reactive cells. At different times, various haematopoietic cell lineages have been implicated in the origin of RS cells. These include: B-cells, T-cells, macrophages and follicular reticulum cells. But presently, there is general consensus on monoclonal lymphoid cell origin of RS cell from B-cells in most subtypes of Hodgkin's disease. RS cells in all types of Hodgkin's diseases, except in lymphocyte predominance type, express immunoreactivity for CD15 and CD30. RS cells in lymphocyte predominance type, however, are negative for both CD15 and CD30, but positive for CD20. However, it is still not clear what stimulates these lymphoid cells to undergo transformation. The following possible hypotheses are suggested:

1. *Infectious etiology*, possibly infection with an oncogenic virus such as Epstein-Barr virus, is implicated by some. Recently a few other viruses have been implicated e.g. human T lymphotropic viruses (HTLV-I and HTLV-II), HIV and herpesvirus-6.

2. *Genetic etiology* is suggested by others on the basis of observation of occurrence of HD in families and with certain HLA type. HD is 99 times more common in identical twin of an affected case compared with general population, implicating genetic origin strongly.

3. Presence of *reactive inflammatory cells* in the HD is due to secretion of cytokines (e.g. interleukin-5) from the RS cells.

Thus, the mystery surrounding the etiology of HD is not yet resolved.

Classification. The diagnosis of HD requires accurate microscopic diagnosis by biopsy, usually from lymph node, and occasionally from other tissues. There are following 4 subtypes: of HD:

1. Lymphocyte-predominance type.
2. Nodular-sclerosis type.
3. Mixed-cellularity type.
4. Lymphocyte-depletion type.

Central to the diagnosis of HD is the essential identification of *Reed-Sternberg cell* though this is not the sole criteria (see below).

Reed-Sternberg Cell. The diagnosis of Hodgkin's disease rests on identification of RS cells, though uncommonly similar cells, can occur in infectious mononucleosis and other forms of lymphomas. Therefore, additional cellular and architectural features of the biopsy must be given due consideration for making the histologic diagnosis.

There are several morphologic variants of RS cells which characterise different histologic subtypes of HD:

1. **Classic RS cell** is a large cell which has characteristically a bi-lobed nucleus appearing as mirror image of each other but occasionally the nucleus may be multi-lobed. Each lobe of the nucleus contains a prominent, eosinophilic, inclusion-like nucleolus with a clear halo around it, giving an owl-eye appearance. The cytoplasm of cell is abundant and amphophilic.

2. **Lacunar type RS cell** is characteristically found in nodular sclerotic variety of HD.

3. **Polyploid type RS cells** are seen in lymphocyte predominance type of HD.

4. **Pleomorphic RS cells** are a feature of lymphocyte depletion type.

It may be mentioned here that in general the number of RS cells is inversely proportional to the number of lymphocytes in a particular histologic subtype of HD.

RS cells are invariably accompanied by variable number of *atypical Hodgkin cells* which are believed to be precursor RS cells but are not considered diagnostic of HD. Hodgkin cells are large mononuclear cells but as regards their nuclear, nucleolar and cytoplasmic features, they resemble RS cells.

PATHOLOGIC CHANGES: *Grossly*, the affected lymph nodes initially remain discrete but later are matted together. The sectioned surface is homogeneous and fishflesh-like in *lymphocyte predominance type*, nodular due to scarring in *nodular sclerosis type*, and abundance of necrosis in *mixed cellularity* and *lymphocyte depletion types*. Hodgkin's disease of liver, spleen and other organs forms spherical masses similar to metastatic carcinoma.

Microscopically, the criteria for diagnosis of 4 histologic subtypes are as under:

1. **Lymphocyte-predominance type.** The lymphocyte-predominance type of HD is characterised by proliferation of small lymphocytes admixed with a varying number of histiocytes forming nodular

or diffuse pattern.

i) *Nodular form* is characterised by replacement of nodal architecture by numerous large neoplastic nodules.

ii) *Diffuse form* does not have discernible nodules but instead there is diffuse proliferation of cells.

Sometimes, both nodular and diffuse patterns may be seen in the involved lymph node.

For making the diagnosis definite demonstration of RS cells is essential which are few in number, requiring a thorough search. In addition to typical RS cells, *polyploid variant* having polyploid, and twisted nucleus (popcorn-like) may be found in some cases. This type of HD usually does not show other cells like plasma cells, eosinophils and neutrophils, nor are necrosis or fibrosis seen.

2. **Nodular-sclerosis type.** Nodular sclerosis is the most frequent type of HD, seen more commonly in women than in men. It is characterised by two essential features:

i) *Bands of collagen:* Variable amount of fibrous tissue is characteristically present in the involved lymph nodes. Occasionally, the entire lymph node may be replaced by dense hyalinised collagen.

ii) *Lacunar type RS cells:* Characteristic lacunar type of RS cells with distinctive pericellular halo are present. These cells appear lacunar due to the shrinkage of cytoplasm in formalin-fixed tissue. The pericellular halo is not seen if the tissue is fixed in inker's fluid.

In addition to these 2 characteristics, the nodules between the fibrous septa consist predominantly of lymphocytes and macrophages, sometimes with foci of necrosis.

3. **Mixed-cellularity type.** This form of HD generally replaces the entire affected lymph nodes by heterogeneous mixture of various types of apparently normal cells. These include proliferating lymphocytes, histiocytes, eosinophils, neutrophils and plasma cells. Some amount of fibrosis and focal areas of necrosis are generally present. Typical RS cells are frequent.

4. **Lymphocyte-depletion type.** In this type of HD, the lymph node is depleted of lymphocytes. There are two variants of lymphocyte-depletion HD:

i) *Diffuse fibrotic variant* is hypocellular and the entire lymph node is replaced by diffuse fibrosis, appearing as homogeneous, fibrillar hyaline material. The area of hyalinosis contains some lymphocytes, atypical histiocytes (Hodgkin cells), and numerous typical and atypical (pleomorphic) RS cells.

ii) *Reticular variant* is much more cellular and consists of large number of atypical pleomorphic histiocytes, scanty lymphocytes and a few typical RS cells.

Clinical Features. Hodgkin's disease is particularly frequent among young and middle-aged adults. All histologic subtypes of HD, except the nodular sclerosis variety, are more common in males. The disease usually begins with superficial lymph node enlargement and subsequently spreads to other lymphoid and non-lymphoid structures.

1. Most commonly, patients present with painless, movable and firm *lymphadenopathy*. The cervical and mediastinal lymph nodes are involved most frequently. Other lymph node groups like axillary, inguinal and abdominal are involved sometimes.

2. Approximately half the patients develop *splenomegaly* during the course of the disease. *Liver enlargement* too may occur.

3. *Constitutional symptoms* (type B symptoms) are present in 25-40% of patients. The most common is low-grade fever with night sweats and weight loss. Other symptoms include fatigue, malaise, weakness and pruritus.

Haematologic abnormalities:

1. A moderate, normocytic and normochromic anaemia is often present.

2. *Serum iron and TIBC* are low but marrow iron stores are normal or increased.

3. *Marrow infiltration* by the disease may produce marrow failure with leucoerythroblastic reaction.

4. *Routine blood counts* reveal moderate leukaemoid reaction. Cases with pruritus frequently show peripheral eosinophilia. Advanced disease is associated with absolute lymphopenia.

5. *Platelet count* is normal or increased.

6. *ESR* is invariably elevated.

Immunologic abnormalities:

1. There is progressive fall in immunocompetent T-cells with *defective cellular immunity*. There is reversal of CD4: CDS ratio and anergy to routine skin tests.

2. *Humoral antibody production* is normal in untreated patients until late in the disease.

Staging

Following biopsy and histopathologic classification of HD, the extent of involvement of the disease (i.e. staging) is studied so as to select the

proper treatment and assess the prognosis. *The Ann Arbor staging classification* takes into account both clinical and pathologic stage of the disease.

The suffix *A* or *B* are added to the above stages depending upon whether the three constitutional symptoms (fever, night sweats and unexplained weight loss exceeding 10% of normal) are absent (A) or present (B). The suffix *E* or *S* are used for extranodal involvement and splenomegaly respectively.

For complete staging, a number of other *essential diagnostic studies* are recommended. These are as under:

1. Detailed physical examination including sites of nodal involvement and splenomegaly.
2. Chest radiograph to exclude mediastinal, pleural and lung parenchymal involvement.
3. CT scan of abdomen and pelvis.
4. Documentation of constitutional symptoms (B symptoms).
5. Laboratory evaluation of complete blood counts, liver and kidney function tests.
6. Bilateral bone marrow biopsy.
7. Finally, histopathologic documentation of the type of Hodgkin's disease.

More invasive investigations include lymphangiography of lower extremities and staging laparotomy. Staging laparotomy includes biopsy of selected lymph nodes in the retroperitoneum, splenectomy and wedge biopsy of the liver.

Prognosis

With use of aggressive radiotherapy and chemotherapy, the outlook for Hodgkin's disease has improved significantly. Although several factors affect the prognosis, two important considerations in evaluating its outcome are the *extent of involvement by the disease (i.e. staging)* and the *histologic subtype*.

- With appropriate treatment, the overall 5 year survival rate for *stage I and II A* is as high as about 100%, while the advanced stage of the disease may have upto 50% 5-year survival rate.

- Patients with *lymphocyte-predominance type* of HD tend to have localised form of the disease and have excellent prognosis.

- The *nodular sclerosis* variety too has very good prognosis but those patients with larger mediastinal mass respond poorly to both chemotherapy and radiotherapy.

- The *mixed cellularity type* occupies intermediate clinical position between the lymphocyte predominance and the lymphocyte-depletion type, but patients with disseminated disease and systemic manifestations do poorly.

- The *lymphocyte-depletion type* is usually disseminated at the time of diagnosis and is associated with constitutional symptoms. These patients usually have the most aggressive form of the disease.

NON-HODGKIN'S LYMPHOMAS

Non-Hodgkin's lymphomas (NHL) are the malignant neoplasms of the immune system of the body and are more common than Hodgkin's lymphoma. The biologic and clinical behaviour of NHL are quite distinct from HD and thus the two are quite different diseases. NHL is most frequent in young adults (20-40 years). Its incidence is showing an upward trend due to increasing incidence of AIDS.

Majority of NHL arise in lymph nodes (65%) while the remaining 35% take origin in extranodal lymphoid tissues. However, all forms of NHL have potential to spread to other lymph nodes, liver, spleen and bone marrow. The involvement of bone marrow may be followed by spill over of NHL into the peripheral blood, and vice versa when lymphoid leukaemia originating in the bone marrow may spread to lymph nodes, creating an overlapping picture of lymphoid leukaemia and NHL. In such situations, it may become difficult to determine which appeared first in the affected patient.

Etiopathogenesis

Malignant lymphomas are considered as the clonal proliferation of immune cells. About 65% of NHL are of B-lymphocyte origin, 35% of T-lymphocytes, and less than 2% are histiocytic derivatives. The agents involved in inducing neoplastic transformation of these cells are poorly understood, but the following etiologic factors have been shown to have strong association with the development of NHL.

1. **Viral association.** There is evidence to suggest that Epstein-Barr virus (EBV) is involved in endemic variety of Burkitt's lymphoma, while human-T-cell leukaemia virus type I (HTLV-I) has strong association with adult T-cell lymphoma.

2. **Genetic association.** A number of cytogenetic abnormalities have been detected in cases of NHL, most important of which are chromosomal translocations.

3. **Immunodeficiency diseases.** Various inherited and acquired immunodeficiency diseases including AIDS are associated with subsequent development of lymphomatous transformation.

4. **Immunosuppression.** Iatrogenic immunosuppression induced by chemotherapy or radiation is associated with induction of NHL.

5. **Autoimmune disease association.** A few autoimmune diseases such as Sjogren's syndrome, non-tropical sprue, rheumatoid arthritis and SLE are associated with higher incidence of NHL.

Classification

Unlike Hodgkin's disease in which Rye classification is the only universally accepted classification since 1966, the pathologic classification of NHL has been difficult for both pathologists and clinicians. Several classifications have been described at different times adding further confusion. The widely used classification systems of NHL (in chronologic order) are: *Rappaport* (1966), *Lukes-Collins* (1974), *Working Formulations for clinical usage* (1982), and *R.E.A.L.* (1994).

R.E.A.L. CLASSIFICATION. International Lymphoma Study Group in 1994 has proposed another classification called revised European-American classification of lymphoid neoplasms abbreviated as R.E.A.L. classification. This classification is based on the hypothesis that all forms of lymphoid malignancies (NHLs as well as lymphoblastic leukaemias) represent malignant counterparts of normal population of immune cells (B-cells, T-cells and histiocytes) present in the lymph node and bone marrow. It is believed that lymphoid malignancies arise due to arrest at the various differentiation stages of B and T-cells since tumours of histiocytic origin are quite uncommon. Accordingly, it is considered essential to understand and correlate the differentiation stages of B and T-cells with various lymphoid malignancies. R.E.A.L. classification divides all lymphoid malignancies into two broad groups, each having further subtypes:

- *Leukaemias and lymphomas of B-cell origin:* B-cell derivation comprises 80% cases of lymphoid leukaemias and 90% cases of NHLs. Based upon these phenotypic and genotypic features, B-cell neoplasms are of pre-B and mature B-cell origin. Based on their biologic behaviour, B-cell

malignancies are further subclassified into indolent and aggressive. All these tumours express Pan-B (CD19) antigen besides other markers.

• *Leukaemias and lymphomas of T-cell origin:* T-cell malignancies comprise the remainder 20% cases of lymphoid leukaemia and 10% cases of NHLs. T-cell malignancies reflect the stages of T-cell ontogeny. Like B-cell malignancies, T-cell derivatives too are further categorised into indolent and aggressive T-cell malignancies. The most widely expressed T-cell antigens are CD2 and CD7.

PATHOLOGIC CHANGES. The histologic diagnosis of NHL can only be reliably made on examination of lymph node biopsy. However, salient features of morphologic subtypes are considered below.

Grossly, the affected groups of lymph nodes are enlarged in majority of cases of NHL. Any lymph node group may be involved but most commonly affected are the cervical, supraclavicular and axillary groups. Less commonly at the time of diagnosis, there may be enlargement of lymphoid tissue elsewhere such as in the tonsils, spleen, stomach, bone etc. Initially, the lymph nodes are discrete and separate from one another but later the lymph nodes form a large matted mass due to infiltration into the surrounding connective tissue. Extranodal involvements produce either a discrete tumour or diffuse enlargement of the affected organ. The sectioned surface of the involved lymph nodes or extranodal organ involved appears grey-white and fishflesh-like. Thus, the gross appearance of Hodgkin's and non-Hodgkin's lymphoma is much the same.

Histologically, the features of various prognostic groups in the Working Formulation for Clinical Usage are given below. The synonyms in brackets in each subgroup of NHL described below correspond to the Rappaport and Lukes-Collins classification respectively.

1. **LOW-GRADE NHL.** This group includes the following three subgroups:

A. **Small lymphocytic lymphoma SLL.** (*Diffuse, well-differentiated lymphocytic; B-small lymphocytic and plasmacytoid lymphoma*). This low-grade lymphoma of B-cell origin constitutes about 4% of all NHLs. The lymph node is replaced by diffuse proliferation of well-differentiated, mature, small and uniform lymphocytes without any cytologic atypia or significant mitoses.

SLL involves bone marrow frequently and produces CLL-like picture. If this occurs, then it may not be possible to distinguish between CLL

and SLL on the basis of lymph node biopsy. Both conditions occur more commonly in middle and older age groups. SLL may be associated with occurrence of an IgM monoclonal gammopathy called Waldenstrom's macroglobulinaemia.

B. Follicular, predominantly small cleaved cell lymphoma (*Nodular, poorly-differentiated lymphocytic; Follicular centre cell, small cleaved lymphoma*). Follicular lymphomas comprise approximately 50% of all NHLs. Small cleaved cell variety is the most common subtype. Morphologically, as the name suggests, this form of follicular lymphoma is characterised by follicular or nodular pattern of growth in which are present predominantly small, normal lymphocytes having irregular, cleaved (indented) nuclei and a few large cleaved or non-cleaved cells. Mitoses are infrequent.

The small cleaved follicular lymphoma occurs in older individuals, most frequently presenting with painless peripheral lymphadenopathy which is usually waxing and waning type. Bone marrow infiltration is typically paratrabecular. Peripheral blood involvement as occurs in SLL is uncommon in this variety. In contrast to diffuse lymphomas, extranodal involvement is also infrequent. Conversion to higher grades of lymphoma may, however, occur. Median survival for patients with this group of NHL is 7-9 years.

C. Follicular, mixed small and large cleaved cell lymphoma (*Nodular mixed; Follicular centre cell, small and large cleaved lymphoma*). This type of follicular lymphoma has features of both follicular small cleaved lymphoma and follicular large cell lymphoma.

It presents commonly with large abdominal masses. Bone marrow infiltration is infrequent. This form of NHL has a less favourable prognosis than the former.

II. INTERMEDIATE GRADE NHL. This prognostic group includes four subtypes: a follicular lymphoma, and three diffuse lymphomas.

D. Follicular, predominantly large cell lymphoma (*Nodular histiocytic; Follicular centre cell, large cleaved or non-cleaved lymphoma*). This is an uncommon follicular lymphoma in which the follicular morphology is preserved, but the type of cells in the follicle are large with cleaved or non-cleaved nuclei and numerous mitoses. In contrast to other follicular lymphomas, this form involves the bone marrow and liver less frequently. Usually, the disease presents with large masses and behaves like a diffuse large cell lymphoma.

E. **Diffuse, small cleaved cell lymphoma** (*Diffuse poorly-differentiated lymphocytic; Follicular centre cell, small cleaved diffuse lymphoma*). This variety is the diffuse counterpart of follicular small cleaved cell lymphoma i.e. it is composed of small cleaved cells spread in a diffuse pattern.

Infiltrations of the bone marrow, liver and spleen are frequent at the time of presentation. The survival of this subgroup is much shorter than in the patients with low-grade lymphomas.

F. **Diffuse, mixed small and large cell lymphoma** (*Diffuse mixed lymphocytic-histiocytic; Follicular centre cell, small or large cleaved or non-cleaved lymphoma*). This subgroup of diffuse NHL is composed of small and large cells which may be cleaved or noncleaved and contains numerous mitoses. Diffuse mixed lymphoma is more common in women and is generally regarded as a waste-basket diagnosis. Majority of cases behave like diffuse large cell lymphoma.

G. **Diffuse large cell lymphoma** (*Diffuse histiocytic; Follicular centre cell, large cleaved or non-cleaved lymphoma*). Morphologically, this subtype contains large cleaved or noncleaved cells in a diffuse pattern.

The patients may have nodal or extranodal disease in the form of large masses. The tumour is highly invasive and may disseminate to other organs, especially to the CNS. The patients with diffuse large cell lymphoma have a poor prognosis.

III. **HIGH-GRADE NHL.** High-grade group of NHL consists of three subgroups:

H. **Large cell immunoblastic lymphoma** (*Diffuse histiocytic; Immunoblastic B or T-cell lymphoma*). This variant is characterised by replacement of the lymph node by distinctive large tumour cells (4 to 5 times the size of small lymphocytes) and having plasma-cytoid features. The nucleus is round to oval with vesicular nuclear chromatin and 1-2 prominent nucleoli. The cytoplasm is abundant and deeply amphophilic or basophilic and pyroninophilic because of proliferation of the rough endoplasmic reticulum in the cell. These tumour cells are most commonly B-cells, sometimes T-cells, but the distinction is possible by immunophenotyping only.

This disease occurs in adults over 50 years of age and has frequent association with prior immunologic disorders such as coeliac disease, Hashimoto's thyroiditis, Sjogren's syndrome, immunosuppressive therapy and in AIDS. Constitutional symptoms (B symptoms) are frequent in advanced

disease. Extranodal involvement and invasion of the bone marrow and CNS are common.

I. Lymphoblastic lymphoma (*Diffuse lymphoblastic; Convuluted T-cell lymphoma*). In this type, the tumour cells resemble lymphoblasts of ALL. They are generally uniform in size with scanty cytoplasm and have numerous mitoses.

Lymphoblastic lymphoma occurs more commonly in children and is more frequent in males. At presentation, there is either peripheral adenopathy in the neck and axilla, or an anterior mediastinal mass. Bone marrow infiltration is present in more than half the cases and develop subsequently into leukaemia that is indistinguishable from T-cell ALL. These patients have very high incidence of CNS involvement.

J. Small non-cleaved cell lymphoma (*Diffuse undifferentiated Burkitt's and Non-Burkitt's; Follicular centre cell, small non-cleaved cell lymphoma*). This subgroup includes African endemic form of Burkitt's lymphoma and non-endemic form of related tumour seen outside Africa.

- **Burkitt's lymphoma** was first described in African children, predominantly presenting as jaw tumour that spreads to extranodal sites such as the bone marrow and meninges. The relationship of this tumour with oncogenic virus, Epstein-Barr virus (EBV). Morphologically, the involved lymph nodes and other tissues are replaced by closely packed, uniform looking tumour cell of the size of macrophages. These cells have a narrow rim of amphophilic or basophilic cytoplasm, round to oval nuclei, prominent nucleoli and high mitotic activity. The tumour cells have moderate amount of basophilic vacuolated cytoplasm. Scattered among the tumour cells are non-neoplastic benign macrophages often surrounded by a clear space, producing what is called a 'starry sky' pattern.'

- **Non-endemic Burkitt's lymphoma** is a related tumour in which the tumour cells are similar to those of Burkitt's lymphoma but are more pleomorphic and may sometimes be multinucleated. Non-Burkitt's small cell lymphoma is seen outside Africa and is sporadic and more aggressive than true Burkitt's lymphoma,

IV. MISCELLANEOUS LYMPHOMAS. Besides the three prognostic groups of NHL described above, the Working Formulations include the following histologic subtypes:

1. **Adult T-cell leukaemia/lymphoma.** This is an uncommon T-cell malignancy but has gained much prominence due to association with human

T-cell lymphotropic virus-1 (HTLV-1). This entity is observed in Japan, the Caribbean and parts of the US but is rare in rest of the world. The patients have usually widespread lymphadenopathy with leukaemia, hepatosplenomegaly and involvement of skin and leptomeninges. The involved lymph nodes have proliferation of large atypical T-cells, most prominent in the paracortical zone. The blood also shows large pleomorphic T-cell leukaemia. This disease has a fulminant course.

2. **Cutaneous T-cell lymphoma.** Its major variants include mycosis fungoides and Sezary's syndrome, both of which are T-cell derived tumours. Advanced cases have infiltration of the bone marrow and leukaemia.

3. **Histiocytic lymphoma.** Histiocytic lymphoma or histiocytic medullary reticulosis is a rare type of lymphoma affecting older people and involving lymph nodes and spleen. The patients may have pancytopenia and constitutional symptoms.

Clinical Features

Features pertaining to specific types of NHL have been given along with their morphologic description above. Some general clinical features common to most cases of NHL are as under:

1. **Superficial lymphadenopathy.** At presentation, there is painless, asymmetric enlargement of one or more groups of peripheral lymph nodes.

2. **Constitutional symptoms.** Fever, night sweats and more than 10% weight loss comprise the constitutional symptoms (type B symptoms) similar to those found in Hodgkin's disease.

3. **Oropharyngeal involvement.** Involvement of Waldeyer's ring is present in 5-10% of patients.

4. **Abdominal disease.** Enlargement of liver, spleen and mesenteric and retroperitoneal lymph nodes are found in some cases. Lymphomatous involvement of the GIT is the most common extranodal involvement after the bone marrow.

5. **Other organs.** Involvement of the skin or brain occurs in certain subtypes of NHL.

Other Laboratory Findings

In addition to clinical and histopathological findings, certain abnormal haematologic and immunologic findings are found in NHL.

Haematologic abnormalities:

1. Anaemia of normocytic normochromic type.
2. Advanced disease with marrow infiltration may show neutropenia, thrombocytopenia and leucoerythro-blastic reaction.
3. Lymphosarcoma cell leukaemia (leukaemic conversion of NHL) occurs in some patients.
4. Marrow involvement by NHL is seen in 20% of cases.
5. Hyperuricaemia and hypercalcaemia occur late in the disease.

Immunologic abnormalities:

Immunologic studies reveal that majority of NHLs are of B lymphocyte origin. There may be associated monoclonal excess of immunoglobulins, usually IgG or IgM. Plasmacytoid lymphoma may produce excess of a heavy chain or a light chain.

Staging

The Ann Arbor staging system developed for Hodgkin's disease is used for staging NHL too. This staging system depends upon the number and location of nodal and extranodal sites involved, and presence or absence of constitutional (B) symptoms. But the concept of staging is much less helpful in NHL than in Hodgkin's disease because the prognosis is not correlated with the anatomic site of involvement of the disease.

Prognosis

Low-grade lymphomas usually progress slowly and local radiotherapy brings about remission lasting for up to 10 years in 75% cases. More extensive disease is treated with combination chemotherapy and local radiotherapy. Children with lymphoblastic lymphoma and Burkitt's lymphoma respond extremely well to chemotherapy. Meningeal involvement is treated with irradiation of the brain. Patients with histiocytic lymphoma and T-cell leukaemia/lymphoma have an extremely poor prognosis.

LYMPH NODE METASTATIC TUMOURS

The regional lymph nodes draining the site of a primary malignant tumour are commonly enlarged. This enlargement may be due to benign *reactive hyperplasia* or *metastatic tumour deposits*.

1. **Benign reactive hyperplasia**, is due to immunologic reaction by the lymph node in response to tumour-associated antigens. It may be ex-

pressed as sinus histiocytosis, follicular hyperplasia, plasmacytosis and occasionally may show non-caseating granulomas.

2. **Metastatic deposits in regional lymph nodes** occurs most commonly from carcinomas and malignant melanoma. Sarcomas generally disseminate by the haematogenous route but uncommonly may metastasise to the regional lymph nodes. Metastatic tumour cells from the primary malignant tumour are drained via lymphatics into the subcapsular sinuses initially but subsequently the lymph node stroma is also invaded. The pushing margins of advancing metastatic tumour in stroma of lymph node is characteristically well demarcated. Areas of necrosis are frequent in metastatic carcinomas. The morphologic features of primary malignant tumour are recapitulated in metastatic tumour in lymph nodes.

LANGERHANS' CELL HISTIOCYTOSIS

Langerhans' cell histiocytosis is a group of malignant proliferations of histiocytes and macrophages. Earlier, this group was referred to as histiocytosis-X and included three conditions: eosinophilic granuloma, Hand-Schuller-Christian disease and Letterer-Siwe syndrome. Now, it is known that:

- *Firstly*, histiocytosis-X are not proliferations of unknown origin (X-for unknown) but proliferating cells are in fact Langerhans' cells of marrow origin. Langerhans' cells are normally present mainly in the epidermis but also in some other organs.

- *Secondly*, the three conditions included under histiocytosis-X are actually different expression of the same basic disorder. This has been made possible by demonstration of common antigens on these cells (positive for S-100 protein, CD1, CD 74 and HLA-DR) as well as by ultrastructural features of Langerhans' cell or Birbeck granules in the cytoplasm.

The three disorders included in the group are briefly considered below.

EOSINOPHILIC GRANULOMA

Unifocal eosinophilic granuloma is more common (60%) than the multifocal variety which is often a component of Hand-Schuller-Christian disease. Most of the patients are children and young adults, predominantly males. The condition commonly presents as a solitary osteolytic lesion in

the femur, skull, vertebrae, ribs and pelvis. The diagnosis requires biopsy of the lytic bone lesion.

Microscopically, the lesion consists largely of closely-packed aggregates of macrophages admixed with variable number of eosinophils. The macrophages contain droplets of fat or a few granules of brown pigment indicative of phagocytic activity. A few multinucleate macrophages may also be seen. The cytoplasm of a few macrophages may contain rod-shaped inclusions called *histiocytosis-X bodies*.

Clinically, unifocal eosinophilic granuloma is a benign disorder. The bony lesion remains asymptomatic until the erosion of the bone causes pain or fracture. Spontaneous fibrosis or healing may occur in some cases, while others may require curettage or radiotherapy.

HAND-SCHULLER-CHRISTIAN DISEASE

A triad of features consisting of *multifocal bony defects*, *diabetes insipidus* and *exophthalmos* is termed Hand-Schuller-Christian disease. The disease develops in children under 5 years of age. The multifocal lytic bony lesions may develop at any site. Orbital lesion causes exophthalmos, while involvement of the hypothalamus causes diabetes insipidus. Multiple spherical lesions in the lungs are frequently present. Half the patients have involvement of the liver, spleen and lymph nodes.

Microscopically, the lesions are indistinguishable from those of unifocal eosinophilic granuloma.

Clinically, the affected children frequently have fever, skin lesions, recurrent pneumonitis and other infections. Though the condition is benign, it is more disabling than the unifocal eosinophilic granuloma. The lesions may resolve spontaneously or may require chemotherapy or radiation.

LETTERER-SIWE DISEASE

Letterer-Siwe disease is an acute clinical syndrome of unknown etiology occurring in infants and children under three years of age. The disease is characterised by hepatosplenomegaly, lymphadenopathy, thrombocytopenia, anaemia and leucopenia. There is generalised hyperplasia of tissue macrophages in various organs.

Microscopically, the involved organs contain aggregates of macrophages which are pleomorphic and show nuclear atypia. The cytoplasm of these cells contains vacuoles and rod-shaped *histiocytosis-X bodies*.

Clinically, the child has acute symptoms of fever, skin rash, loss of weight, anaemia, bleeding disorders and enlargement of lymph nodes, liver and spleen. Cystic bony lesions may be apparent in the skull, pelvis and long bones. Intense chemotherapy helps to control Letterer-Siwe disease but intercurrent infections result in fatal outcome in many cases. The condition is currently regarded as an unusual form of malignant lymphoma.

REFERENS:

1. Colour Atlas of Anatomical Pathology / Cooke R.A., Steward B. - Churchill Livingstone, 2004.- 300p.
2. Majno G, Joris I: Apoptosis, oncosis and necrosis: an overview of cell death. Am. Pathol. 146: 3, 1995.
3. Martin P: Wound healing - aiming for perfect skin regeneration. Science 276: 75, 1997.
4. Mazur MT, Kurman RJ: Diagnosis of Endometrial Biopsies and Curettings. New York, Springer-Verlag, 1995.
5. Pamies RJ. Crawford DR: Tumour markers, an update. Med. Clin. North. Am. 80:185, 1996.
6. Pathology / Eds. E. Rubin, J.L. Farber - Philadelphia: Lippincott-Raven Publ., 1998.
7. Pathology Illustrated / Govan A.D.T., Macflane P.S., Callander R. - Edinburgh: Churchill Livingstone, 1995.
8. Practical guide in pathological anatomy / I.V. Samsonova, O.V. Lesnichaya - Vitebsk, 2015.
9. Risau W: Mechanism of angiogenesis. Nature 380: 671, 1997.
10. Robbins & Cotran Pathologic Basis of Disease (Robbins Pathology) 9th Edition. 2014.- 1408p.
11. Rosai J: Ackerman's Surgical Pathology (2 vols), 11th ed. Elsevier 2018.
12. Rose R: The pathogenesis of atherosclerosis, a perspective for 1990s. Nature 362: 801, 1993.
13. Rosendaal FR: Risk factors for venous thrombosis: prevalence, risk and interaction. Semin Hematol. 34: 171, 1997.
14. Tarin D: Prognostic markers and mechanisms of metastasis. In Recent Advances in Histopathology, number 17. Anthony PP et al (Eds). London, Churchill Livingstone, pp 15, 1997.
15. Textbook of Pathology / H. Mochan - India, New Dehi: Jaypee Brothers, Med. Publ., 2005.
16. Wheater's Basic Histopathology. A Colour Atlas and Text / Burkitt H.G., Stevens A.J.S.L., Young B. - Edinburgh: Churchill Livingstone, 1996.

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