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Cells are the basic units of tissues, which form organs and systems in the human body. Traditionally, body cells are divided in to two main types: epithelial and mesenchymal cells. In health, the cells remain in accord with each other. In 1859, Virchow first published cellular theory of disease, bringing in the concept that diseases occur due to abnormalities at the level of cells. Since then, study of abnormalities in structure and function of cells in disease has remained the focus of attention in understanding of diseases. Thus, most forms of diseases begin with cell injury followed by consequent loss of cellular function. Cell injury is defined as a variety of stresses a cell encounters as a result of changes in its internal and external environment. In general, cells of the body have inbuilt mechanism to deal with changes in environment to an extent. The cellular response to stress may vary and depends upon the following variables: i) The type of cell and tissue involved. ii) Extent and type of cell injury. Various forms of cellular responses to cell injury may be as follows (Fig. 3.1): 1. When there is increased functional demand, the cell may adapt to the changes which are expressed morphologically and then revert back to normal after the stress is removed (cellular adaptations, see Fig. 3.39). 2. When the stress is mild to moderate, the injured cell may recover (reversible cell injury), while when the injury is persistent cell death may occur (irreversible cell injury). 3. The residual effects of reversible cell injury may persist in the cell as evidence of cell injury at subcellular level (subcellular changes), or metabolites may accumulate within the cell (intracellular accumulations). In order to learn the fundamentals of disease processes at cellular level, it is essential to have an understanding of the causes and mechanisms of cell injury and cellular

adaptations, which can be best understood in the context of basic knowledge of normal structure and functions of cell outlined below.

THE NORMAL CELL Different types of cells of the body possess features which distinguish one type from another. However, most mammalian cells have a basic

plan of common structure and function, except the red blood cell which is devoid of nucleus and its structure is described separately on page 288.

CELL STRUCTURE Under normal conditions, cells are dynamic structures existing in fluid environment. A cell is enclosed by cell membrane that extends internally to enclose nucleus and various subcellular organelles suspended in cytosol (Fig. 3.2).

Cell Membrane Electron microscopy has shown that cell membrane or plasma membrane has a trilaminar structure having a total thickness of about 7.5 nm and is known as unit membrane. The three layers consist of two electron-dense layers separated by an electronlucent layer. Biochemically, the cell membrane is composed of complex mixture of phospholipids, glycolipids, cholesterol, proteins and carbohydrates. These layers are in a gel-like arrangement and are in a constant state of flux. The outer surface of some types of cells shows a coat of mucopolysaccharide forming a fuzzy layer called glycocalyx. Proteins and glycoproteins of the cell membrane may act as antigens (e.g. blood group antigens), or may form receptors (e.g. for viruses, bacterial products, hormones, immunoglobulins and many enzymes). The cell.

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receptors are probably related to the microtubules and microfilaments of the underlying cytoplasm. The microtubules connect one receptor with the next. The microfilaments are contractile structures so that the receptor may move within the cell membrane. Bundle of microfilaments along with cytoplasm and protein of cell membrane may form projections on the surface of the cell called microvilli. Microvilli are especially numerous on the surface of absorptive and secretory cells (e.g. small intestinal mucosa) increasing their surface area. In brief, the cell membrane performs the following important functions: i) Selective permeability that includes diffusion, membrane pump (sodium pump) and pinocytosis (cell drinking). ii) Bears membrane antigens (e.g. blood group antigens, transplantation antigen). iii) Possesses cell receptors for cell-cell recognition and communication.

Nucleus The nucleus consists of an outer nuclear membrane enclosing nuclear chromatin and nucleoli. NUCLEAR MEMBRANE. The nuclear membrane is the outer envelop consisting of 2 layers of the unit membrane which are separated by a 40-70 nm wide space. The outer layer of the nuclear membrane is studded with

ribosomes and is continuous with endoplasmic reticulum. The two layers of nuclear membrane at places are fused together forming circular nuclear pores which are about 50 nm in diameter. The nuclear membrane is crossed by several factor which regulate the gene expression and repair the DNA damage as soon as it occurs. NUCLEAR CHROMATIN. The main substance of the nucleus is comprised by the nuclear chromatin which is in the form of shorter pieces of thread-like structures called chromosomes of which there are 23 pairs (46 chromosomes)

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together measuring about a metre in length in a human diploid cell. Of these, there are 22 pairs (44 chromosomes) of autosomes and one pair of sex chromosomes, either XX (female) or XY (male). Each chromosome is composed of two chromatids connected at the centromere to form 'X' configuration having variation in location of the centromere. Depending upon the length of chromosomes and centromeric location, 46 chromosomes are categorised into 7 groups from A to G according to Denver classification (adopted at a meeting in Denver, USA). Chromosomes are composed of 3 components, each with distinctive function. These are: deoxyribonucleic acid (DNA) comprising about 20%, ribonucleic acid (RNA) about 10%, and the remaining 70% consists of nuclear proteins that include a number of basic proteins (histones), neutral proteins, and acid proteins. DNA of the cell is largely contained in the nucleus. The only other place in the cell that contains small amount of DNA is mitochondria. Nuclear DNA along with histone nuclear proteins form bead-like structures called nucleosomes which are studded along the coils of DNA. Nuclear DNA carries the genetic information that is passed via RNA into the cytoplasm for manufacture of proteins of similar composition. During cell division, one half of DNA molecule acts as a template for the manufacture of the other half by the enzyme, DNA polymerase, so that the genetic characteristics are transmitted to the next progeny of cells (replication). The DNA molecule as proposed by Watson and Crick in 1953 consists of two complementary polypeptide chains forming a double helical strand which is wound spirally around an axis composed of pentose sugar-phosphoric acid chains. The molecule is spirally twisted in a ladder-like pattern, the steps of which are composed of 4 nucleotide bases: two purines (adenine and guanine, i.e. A and G) and two pyrimidines (cytosine and thymine, i.e. C and T);

however, A pairs specifically with T while G pairs with C (Fig. 3.3). The sequence of these nucleotide base pairs in the chain, determines the information contained in the DNA molecule or constitutes the genetic code. In April 2003, sequencing of human genome was completed which revealed that 23 pairs of chromosomes in the nucleus of each human cell contains approximately 3 billion base pairs, and each chromosome contains an estimated 30,000 genes in the human genome, which carry the instructions for making proteins. In the interphase nucleus (i.e. between mitosis), part of the chromatin that remains relatively inert metabolically and appears deeply basophilic due to condensation of chromosomes is called heterochromatin, while the part of chromatin that is lightly stained (i.e. vesicular) due to dispersed chromatin is called euchromatin. For example, in lymphocytes there is predominance of heterochromatin while the nucleus of a hepatocyte is mostly euchromatin. NUCLEOLUS. The nucleus may contain one or more rounded bodies called nucleoli. Nucleolus is the site of synthesis of ribosomal RNA. Nucleolus is composed of granules and fibrils representing newly synthesised ribosomal RNA.

Cytosol and Organelles The cytosol or the cytoplasm is the gel-like ground substance in which the organelles (meaning little organs) of the cells are suspended. These organelles are the site of major enzymatic activities of the cell which are possibly mediated by enzymes in the cytosol. The major organelles are the cytoskeleton, mitochondria, ribosomes, endoplasmic reticulum, Golgi apparatus, lysosomes, and microbodies or peroxisomes. 1. CYTOSKELETON. Microfilaments, intermediate filaments, and microtubules are responsible for maintaining cellular form and movement and are collectively referred to as cytoskeleton. i) Microfilaments are long filamentous structures having a diameter of 6-8 nm. They are composed of contractile proteins, actin and myosin, and diverse materials like parts of microtubules and ribonucleoprotein fibres. Bundles of microfilaments are especially prominent close to the plasma membrane and form terminal web. Extension of these bundles of microfilaments along with part of plasma membrane on the surface of the cell form microvilli which increase the absorptive surface of the cells. ii) Intermediate filaments are filamentous structures, 10 nm in diameter, and are cytoplasmic constituent of a number of cell types. They are composed of proteins. There are 5 principal types of

intermediate filaments: a) Cytokeratin (found in epithelial cells). b) Desmin (found in skeletal, smooth and cardiac muscle).

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c) Vimentin (found in cells of mesenchymal origin). d) Glial fibrillary acidic protein (present in astrocytes and ependymal cells). e) Neurofilaments (seen in neurons of central and peripheral nervous system). Their main function is to mechanically integrate the cell organelles within the cytoplasm. iii) Microtubules are long hollow tubular structures about 25 nm in diameter. They are composed of protein, tubulin. Cilia and flagella which project from the surface of cell are composed of microtubules enclosed by plasma membrane and are active in locomotion of the cells. Basal bodies present at the base of each cilium or flagellum and centriole located at the mitotic spindle of cells are the two other morphologically similar structures composed of microtubules. 2. MITOCHONDRIA. Mitochondria are oval structures and are more numerous in metabolically active cells. They are enveloped by two layers of membrane—the outer smooth and the inner folded into incomplete septa or sheaf-like ridges called cristae. Chemically and structurally, membranes of mitochondria are similar to cell membrane. The inner membrane, in addition, contains lollipop-shaped globular structures projecting into the matrix present between the layers of membrane. The matrix of the mitochondria contains enzymes required in the Krebs' cycle by which the products of carbohydrate, fat and protein metabolism are oxidised to produce energy which is stored in the form of ATP in the lollipop-like globular structures. Mitochondria are not static structures but undergo changes in their configuration during energised state by alteration in the matrix and intercristal space; the outer membrane is, however, less elastic. Mitochondria perform the important metabolic function of oxidative phosphorylation, and in the process generate free radicals injurious to membranes. They also have role in apoptosis. Mitochondria contain 37 genes out of which 13 encode for synthesising proteins. In addition, mitochondria also have some DNA and ribosomes. 3. RIBOSOMES. Ribosomes are spherical particles which contain 80-85% of the cell's RNA. They may be present in the cytosol as 'free' unattached form, or in 'bound' form when they are attached to membrane of endoplasmic reticulum. They may lie as 'monomeric units' or as 'polyribosomes' when many

monomeric ribosomes are attached to a linear molecule of messenger RNA. Ribosomes synthesise proteins by translation of messenger RNA into peptide sequences followed by packaging of proteins for the endoplasmic reticulum. 4. ENDOPLASMIC RETICULUM. Endoplasmic reticulum is composed of vesicles and intercommunicating canals. It is composed of unit membrane which is continuous with both nuclear membrane and the Golgi apparatus, and possibly with the cell membrane. The main function of endoplasmic reticulum is the manufacture of protein. Morphologically, there are 2 forms of endoplasmic reticulum: rough (or granular) and smooth (or agranular). i) Rough endoplasmic reticulum (RER) is so-called because its outer surface is rough or granular due to attached

ribosomes on it. RER is especially well-developed in cells active in protein synthesis e.g. Russell bodies of plasma cells, Nissl granules of nerve cells. ii) Smooth endoplasmic reticulum (SER) is devoid of ribosomes on its surface. SER and RER are generally continuous with each other. SER contains many enzymes which metabolise drugs, steroids, cholesterol, and carbohydrates and partake in muscle contraction. 5. GOLGI APPARATUS. The Golgi apparatus or Golgi complex is generally located close to the nucleus. Morphologically, it appears as vesicles, sacs or lamellae composed of unit membrane and is continuous with the endoplasmic reticulum. The Golgi apparatus is particularly welldeveloped in exocrine glandular cells. Its main functions are synthesis of carbohydrates and complex proteins and packaging of proteins synthesised in the RER into vesicles. Some of these vesicles may contain lysosomal enzymes and specific granules such as in neutrophils and in beta cells of the pancreatic islets. 6. LYSOSOMES. Lysosomes are rounded to oval membrane-bound organelles containing powerful lysosomal digestive (hydrolytic) enzymes. There are 3 forms of lysosomes: i) Primary lysosomes or storage vacuoles are formed from the various hydrolytic enzymes synthesised by the RER and packaged in the Golgi apparatus. ii) Secondary lysosomes or autophagic vacuoles are formed by fusion of primary lysosomes with the parts of damaged or worn-out cell components. iii) Residual bodies are indigestible materials in the lysosomes, e.g. lipofuscin. 7. CENTRIOLE OR CENTROSOME. Each cell contains a pair of centrioles in the cytoplasm close to nucleus in the area called centrosome. Centrioles are cylindrical structure composed of electron-dense evenly-shaped microtubules. They perform the

function of formation of cilia and flagellae and constitute the mitotic spindle of fibrillary protein during mitosis.

INTERCELLULAR COMMUNICATION All cells in the body constantly exchange information with each other to perform their functions properly. This process is accomplished in the cells by direct cell-to-cell contact (intercellular junctions), and by chemical agents, also called as molecular agents or factors (molecular interactions between cells) as under.

Intercellular Junctions Plasma membranes of epithelial and endothelial cells, though closely apposed physically, are separated from each other by 20 nm wide space. These cells communicate across this space through intercellular junctions or junctional complexes visible under electron microscope and are of 4 types (Fig. 3.4): 1. Occluding junctions (Zonula occludens). These are tight junctions situated just below the luminal margin of adjacent

cells. As a result, the regions of occluding zones are impermeable to macromolecules. The examples of occluding zones are seen in renal tubular epithelial cells, intestinal epithelium, and vascular endothelium in the brain constituting blood-brain barrier. 2. Adhering junctions (Zonula adherens). These are located just below the occluding zones between the adjacent cells and are permeable to tracer particles. These zones are in contact with actin microfilaments e.g. in small cell carcinoma of the lung. 3. Desmosomes (Macula densa). These are tiny adhesion plates present focally between the adjacent epithelial cells, especially numerous in the epidermis. Bundles of intermediate filaments (termed tonofilaments in the case of epidermis) project from the intercellular desmosomes and radiate into the cytoplasm. Hemidesmosomes are a variant of desmosomes, occurring at the basal region of epithelial cells between plasma membrane and the basement membrane. 4. Gap junctions (Nexus). Gap junctions or nexus are the regions on the lateral surfaces of epithelial cells where the gap between the adjoining plasma membranes is reduced from 20 nm to about 2 nm in width. Pits or holes are present in the regions of gap junctions so that these regions are permeable to small tracer particles.

Molecular Interactions between Cells Besides having intercellular junctions, most cells communicate at molecular level as follows: 1. Cell adhesion molecules (CAMs) 2. Cytokines 3. Membrane receptors 1. CELL ADHESION MOLECULES (CAMs). These are chemicals which mediate the interaction between cells (cellcell interaction) as well as between cells and extracellular matrix (cell-ECM interaction). The ECM is the ground substance or matrix of connective tissue which provides environment to the cells and consists of 3 components:

i) fibrillar structural proteins (collagen, elastin); ii) adhesion proteins (fibronectin, laminin, fibrillin, osteonectin, tenacin); and iii) molecules of proteoglycans and glycosaminoglycans (heparan sulphate, chondroitin sulphate, dermatan sulphate, keratan sulphate, hyaluronic acid). CAMs participate in fertilisation, embryogenesis, tissue repair, haemostasis, cell death by apoptosis and in inflammation. CAMs may be detected on the surface of cells as well as free in circulation. There are 5 groups of CAMs: i) Integrins. They have alpha (or CD11*) and beta (CD18) subunits and have a role in cell-ECM interactions and in leucocyte-endothelial cell interaction. ii) Cadherins. These are calcium-dependent adhesion molecules which bind adjacent cells together and prevent invasion of ECM by cancer cells. Various types of cadherins include: E-cadherin (epithelial cell), N-cadherin (nerve cell), M-cadherin (muscle cell), and P-cadherin (placenta). iii) Selectins. Also called as lectins, these CAMs contain lectins or lectin-like protein molecules which bind to glycoproteins and glycolipids on the cell surface. Their major role is in movement of leucocytes and platelets and develop contact with endothelial cells. Selectins are of 3 types: P-selectin (from platelets, also called CD62), E-selectin (from endothelial cells, also named ECAM), and L-selectin (from leucocytes, also called LCAM). iv) Immunoglobulin superfamily. This group consists of a variety of immunoglobulin molecules present on most cells of the body. These partake in cell-to-cell contact through various other CAMs and cytokines. They have a major role in recognition and binding of immunocompetent cells. This group includes ICAM-1,2 (intercellular adhesion molecule, also called CD54), VCAM (vascular cell adhesion molecule, also named CD106), NCAM (neural cell adhesion molecule). v)CD44. The last group of adhesion molecules is a break away from immunoglobulin superfamily. CD44 molecule binds to hyaluronic acid and is expressed on leucocytes. It is involved in leucocyte-endothelial interactions as well as in cell-ECM interactions. 2.

CYTOKINES. Another way the cells may communicate with each other is by release of peptides and other molecules acting as paracrine function. Cytokines are soluble proteins secreted by haemopoietic and non-haemopoietic cells in response to various stimuli. Their main role is in activation of immune system. Presently, about 200 cytokines have been identified which are grouped in 6 categories: i) Interferons (IFN) ii) Interleukins (IL) iii) Tumour necrosis factor group (TNF, cachectin) iv) Transforming growth factor (TGF) v) Colony stimulating factor (CSF) vi) Growth factors (e.g. platelet-derived growth factor PDGF, epidermal growth factor EGF, fibroblast growth factor FGF,

Figure 3.4 Diagrammatic representation of the intercellular junctions.

*CD number (for Cluster of Differentiation) is the nomenclature given to the clone of cells which carry these molecules on their cell surface or in their cytoplasm.

endothelial-derived growth factor EDGF, transforming growth factor TGF). Many of these cytokines have further subtypes as alpha, beta, or are identified by numbers. Cytokines involved in leucocyte-endothelial cell interaction are called chemokines while growth factors and other cytokines are named crinopectins. 3. CELL MEMBRANE RECEPTORS. Cell receptors are molecules consisting of proteins, glycoproteins or lipoproteins and may be located on the outer cell membrane, inside the cell, or may be trans-membranous. These receptor molecules are synthesised by the cell itself depending upon their requirement, and thus there may be upregulation or downregulation of number of receptors. There are 3 main types of receptors: i)Enzyme-linked receptors. These receptors are involved in control of cell growth e.g. tyrosine kinase associated receptors take part in activation of synthesis and secretion of various hormones. ii) Ion channels. The activated receptor for ion exchange such as for sodium, potassium and calcium and certain peptide hormones, determines inward or outward movement of these molecules. iii)G-protein receptors. These are trans-membranous receptors and activate phosphorylating enzymes for metabolic and synthetic functions of cells. The activation of adenosine monophosphate-phosphatase cycle (c-AMP) by the G-proteins (guanosine nucleotide binding regulatory proteins) is the most

important signal system, also known as ‘second messenger’ activation. The activated second messenger (cyclic-AMP) then regulates other intracellular activities.

Heat Shock Proteins and Ubiquitin Two proteins which move molecules within the cell cytoplasm are heat shock proteins (HSP) (also called stress proteins) and ubiquitin (so named due to its universal presence in the cells of the body). HSPs. These are a variety of intracellular carrier proteins present in most cells of the body, especially in renal tubular epithelial cells. They normally perform the role of chaperones (house-keeping) i.e. they direct and guide metabolic molecules to the sites of metabolic activity e.g. protein folding, disaggregation of protein-protein complexes and transport of proteins into various intracellular organelles (protein kinesis). However, in response to stresses of various types (e.g. toxins, drugs, poisons, ischaemia), their level goes up both inside the cell as well as they leak out into the plasma, and hence the name stress proteins. In experimental studies HSPs have been shown to limit tissue necrosis in ischaemic reperfusion injury in myocardial infarcts. In addition, they have also been shown to have a central role in protein aggregation in amyloidosis. Ubiquitin. This is another related stress protein which has ubiquitous presence in human body cells. Like HSPs, ubiquitin too directs intracellular molecules for either degradation or for synthesis. Ubiquitin has been found to be involved in a variety of human degenerative diseases,

especially in the nervous system in aging e.g. activation of genes for protein synthesis in neurodegenerative diseases such as in Alzheimer’s disease, Creutzfeldt-Jakob disease, Parkinson’s disease.

CELL CYCLE Multiplication of the somatic (mitosis) and germ (meiosis) cells is the most complex of all cell functions. Mitosis is controlled by genes which encode for release of specific proteins molecules that promote or inhibit the process of mitosis at different steps. Mitosis-promoting protein molecules are cyclins A, B and E. These cyclins activate cyclindependent kinases (CDKs) which act in conjunction with cyclins. After the mitosis is complete, cyclins and CDKs are degraded and the residues of used molecules are taken up by cytoplasmic caretaker proteins, ubiquitin, to the peroxisome for further degradation. Period

between the mitosis is called interphase. The cell cycle is the phase between two consecutive divisions (Fig. 3.5). There are 4 sequential phases in the cell cycle: G1 (gap 1) phase, S (synthesis) phase, G2 (gap 2) phase, and M (mitotic) phase. G1 (Pre-mitotic gap) phase is the stage when messenger RNAs for the proteins and the proteins themselves required for DNA synthesis (e.g. DNA polymerase) are synthesised. The process is under control of cyclin E and CDKs. S phase involves replication of nuclear DNA. Cyclin A and CDKs control it. G2 (Pre-mitotic gap) phase is the short gap phase in which correctness of DNA synthesised is assessed. This stage is promoted by cyclin B and CDKs. M phase is the stage in which process of mitosis to form two daughter cells is completed. This occurs in 4 sequential stages: prophase, metaphase, anaphase, and telophase (acronym= PMAT). Prophase. Each chromosome divides into 2 chromatids which are held together by centromere. The centriole divides and the two daughter centrioles move towards opposite poles of the nucleus and the nuclear membrane disintegrates. Metaphase. The microtubules become arranged between the two centrioles forming spindle, while the chromosomes line up at the equatorial plate of the spindle. Anaphase. The centromeres divide and each set of separated chromosomes moves towards the opposite poles of the spindle. Cell membrane also begins to divide. Telophase. There is formation of nuclear membrane around each set of chromosomes and reconstitution of the nucleus. The cytoplasm of the two daughter cells completely separates. G0 phase. The daughter cells may continue to remain in the cell cycle and divide further, or may go out of the cell cycle into resting phase, called G0 phase. Stimulation of mitosis can be studied in a number of ways as under: Compensatory stimulation of mitosis by removal of part of an organ. Reparative stimulation of mitosis occurs when a tissue is injured.

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Target organ stimulation of mitosis occurs under the influence of specific hormones which have mitogenic effect on cells of the target organ. ETIOLOGY OF CELL INJURY The cells may be broadly injured by two major ways: A. By genetic causes B. By acquired causes The genetic causes of various diseases are discussed in Chapter 10. The acquired causes of disease comprise vast majority of common

diseases afflicting mankind. Based on underlying agent, the acquired causes of cell injury can be further categorised as under: 1. Hypoxia and ischaemia 2. Physical agents 3. Chemical agents and drugs 4. Microbial agents

5. Immunologic agents 6. Nutritional derangements 7. Aging 8. Psychogenic diseases 9. Iatrogenic factors 10. Idiopathic diseases. In a given situation, more than one of the above etiologic factors may be involved. These are briefly outlined below. 1. HYPOXIA AND ISCHAEMIA. Cells of different tissues essentially require oxygen to generate energy and perform metabolic functions. Deficiency of oxygen or hypoxia results in failure to carry out these activities by the cells. Hypoxia is the most common cause of cell injury. Hypoxia may result from the following: The most common mechanism of hypoxic cell injury is by reduced supply of blood to cells due to interruption i.e. ischaemia.

Figure 3.5 The cell cycle in mitosis. Premitotic phases are the G1, S and G2 phase while M (mitotic) phase is accomplished in 4 sequential stages: prophase, metaphase, anaphase, and telophase. On completion of cell division, two daughter cells are formed which may continue to remain in the cell cycle or go out of it in resting phase (interphase), the G0 phase. (CDK = cyclin dependent kinase).

However, hypoxia may result from other causes as well e.g. disorders of oxygen-carrying RBCs (e.g. anaemia, carbon monoxide poisoning), heart diseases, lung diseases and increased demand of tissues. 2. PHYSICAL AGENTS. Physical agents in causation of disease are as under: mechanical trauma (e.g. road accidents); thermal trauma (e.g. by heat and cold); electricity; radiation (e.g. ultraviolet and ionising); and rapid changes in atmospheric pressure. 3. CHEMICALS AND DRUGS. An ever increasing list of chemical agents and drugs may cause cell injury. Important examples include the following: chemical poisons such as cyanide, arsenic, mercury; strong acids and alkalis; environmental pollutants; insecticides and pesticides; oxygen at high concentrations; hypertonic glucose and salt; social agents such as alcohol and narcotic drugs; and therapeutic administration of drugs. 4. MICROBIAL AGENTS. Injuries by microbes include infections caused by bacteria, rickettsiae, viruses, fungi, protozoa, metazoa, and other parasites. Diseases caused by biologic agents are discussed in Chapter 7. 5. IMMUNOLOGIC

AGENTS. Immunity is a 'doubleedged sword'—it protects the host against various injurious agents but it may also turn lethal and cause cell injury e.g. hypersensitivity reactions; anaphylactic reactions; and autoimmune diseases. Immunologic tissue injury is discussed in Chapter 4. 6. NUTRITIONAL DERANGEMENTS. A deficiency or an excess of nutrients may result in nutritional imbalances. Nutritional deficiency diseases may be due to overall deficiency of nutrients (e.g. starvation), of protein calorie (e.g. marasmus, kwashiorkor), of minerals (e.g. anaemia), or of trace elements. Nutritional excess is a problem of affluent societies resulting in obesity, atherosclerosis, heart disease and hypertension. Nutritional diseases are discussed in Chapter 9. 7.AGING. Cellular aging or senescence leads to impaired ability of the cells to undergo replication and repair, and ultimately lead to cell death culminating in death of the individual. This aspect is dealt at the end of this chapter. 8. PSYCHOGENIC DISEASES. There are no specific biochemical or morphologic changes in common acquired mental diseases due to mental stress, strain, anxiety, overwork and frustration e.g. depression, schizophrenia. However, problems of drug addiction, alcoholism, and smoking result in various organic diseases such as liver

damage, chronic bronchitis, lung cancer, peptic ulcer, hypertension, ischaemic heart disease etc. 9. IATROGENIC CAUSES. Although as per Hippocratic oath, every physician is bound not to do or administer anything that causes harm to the patient, there are some diseases as well as deaths attributed to iatrogenic causes (owing to physician). Examples include occurrence of disease or death due to error in judgment by the physician and untoward effects of administered therapy (drugs, radiation). 10. IDIOPATHIC DISEASES. Idiopathic means "of unknown cause". Finally, although so much is known about the etiology of diseases, there still remain many diseases for which exact cause is undetermined. For example, most common form of hypertension (90%) is idiopathic (or essential) hypertension. Similarly, exact etiology of many cancers is still incompletely known. PATHOGENESIS OF CELL INJURY Injury to the normal cell by one or more of the above listed etiologic agents may result in a state of reversible or irreversible cell injury. The underlying alterations in biochemical systems of cells for reversible and irreversible cell injury by various agents is complex and varied. However, in general, the following principles apply in pathogenesis of most forms

of cell injury by various agents: 1. Type, duration and severity of injurious agent: The extent of cellular injury depends upon type, duration and severity of the stimulus e.g. small dose of chemical toxin or short duration of ischaemia cause reversible cell injury while large dose of the same chemical agent or persistent ischaemia cause cell death. 2. Type, status and adaptability of target cell: The type of cell as regards its susceptibility to injury, its nutritional and metabolic status, and adaptation of the cell to hostile environment determine the extent of cell injury e.g. skeletal muscle can withstand hypoxic injury for long-time while cardiac muscle suffers irreversible cell injury after 20-30 minutes of persistent ischaemia. 3. Underlying intracellular phenomena: Irrespective of other factors, following essential biochemical phenomena underlie all forms of cell injury: i) Mitochondrial damage causing ATP depletion. ii) Cell membrane damage disturbing the metabolic and trans-membrane exchanges. iii). Release of toxic free radicals. 4. Morphologic consequences: All forms of biochemical changes underlying cell injury are expressed in terms of morphologic changes. The ultrastructural changes become apparent earlier than the light microscopic alterations. The morphologic changes of reversible cell injury (e.g. hydropic swelling) appear earlier than morphologic alterations in cell death (e.g. in myocardial infarction). The interruption of blood supply (i.e. ischaemia) and impaired oxygen supply to the tissues (i.e. hypoxia) are most common form of cell injury in human beings. Patho

genesis of hypoxic and ischaemic cell injury is, therefore, described in detail below followed by brief discussion on pathogenesis of chemical and physical (ionising radiation) agents.

PATHOGENESIS OF ISCHAEMIC AND HYPOXIC INJURY Ischaemia and hypoxia are the most common forms of cell injury. Although underlying intracellular mechanisms and ultrastructural changes involved in reversible and irreversible cell injury by hypoxia and ischaemia depending upon extent of hypoxia and type of cells are involved are a continuation of the process, these mechanisms are discussed separately below and illustrated diagrammatically in Figs. 3.

REVERSIBLE CELL INJURY. If the ischaemia or hypoxia is of short duration, the effects may be reversible on rapid restoration of circulation e.g. in coronary artery occlusion, myocardial contractility, metabolism and ultrastructure are reversed if

the circulation is quickly restored. The sequential biochemical and ultrastructural changes in reversible cell injury are as under (Fig. 3.7,A): 1. Decreased generation of cellular ATP: Damage by ischaemia versushypoxia from other causes.All living cells require continuous supply of oxygen to produce ATP which is essentially required for a variety of cellular functions (e.g.

membrane transport, protein synthesis, lipid synthesis and phospholipid metabolism). ATP in human cell is derived from 2 sources: firstly, by aerobic respiration or oxidative phosphorylation (which requires oxygen) in the mitochondria, and secondly, cells may switch over to anaerobic glycolytic oxidation to maintain constant supply of ATP (in which ATP is generated from glucose/glycogen in the absence of oxygen). Ischaemia due to interruption in blood supply as well as hypoxia from other causes limit the supply of oxygen to the cells, thus causing decreased ATP generation from ADP: In ischaemia, aerobic respiration as well as glucose availability are both compromised resulting in more severe and faster effects of cell injury. Ischaemic cell injury also causes accumulation of metabolic waste products in the cells. On the other hand, in hypoxia from other causes (RBC disorders, heart disease, lung disease), anaerobic glycolytic ATP generation continues, and thus cell injury is less severe. However, highly specialised cells such as myocardium, proximal tubular cells of the kidney, and neurons of the CNS are dependent solely on aerobic respiration for ATP generation and thus these tissues suffer from ill-effects of ischaemia more severely and rapidly. 2. Intracellular lactic acidosis:Nuclear clumping. Due to low oxygen supply to the cell, aerobic respiration by

Figure 3.6 Sequence of events in the pathogenesis of reversible and irreversible cell injury caused by hypoxia/ischaemia. mitochondria fails first. This is followed by switch to anaerobic glycolytic pathway for the requirement of energy (i.e. ATP). This results in rapid depletion of glycogen and accumulation of lactic acid lowering the intracellular pH. Early fall in intracellular pH (i.e. intracellular lactic acidosis) results in clumping of nuclear chromatin. 3. Damage to plasma membrane pumps: Hydropic swelling and other membrane changes. Lack of ATP interferes in generation of phospholipids from the cellular fatty acids which are required for continuous repair of membranes. This results in damage to membrane pumps operating for regulation of sodium and calcium as under: i)

Failure of sodium-potassium pump.Normally, the energy (ATP)-dependent sodium pump (Na+-K+ ATPase) operating at the plasma membrane allows active transport of sodium out of the cell and diffusion of potassium into the cell. Lowered ATP in the cell and consequent increased ATPase activity interfere with this membrane-regulated process. This results in intracellular accumulation of sodium and diffusion of potassium out of cell. The accumulation of sodium in the cell leads to increase in intracellular water to maintain isoosmotic conditions (i.e. hydropic swelling occurs, discussed later in the chapter). ii) Failure of calcium pump. Membrane damage causes disturbance in the calcium ion exchange across the cell membrane. Excess of calcium moves into the cell (i.e. calcium influx), particularly in the mitochondria, causing its swelling and deposition of phospholipid-rich amorphous densities. Ultrastructural evidence of reversible cell membrane damage is seen in the form of loss of microvilli, intramembranous particles and focal projections of the cytoplasm (blebs). Myelin figures may be seen lying in the cytoplasm or present outside the cell, these are derived from membranes (plasma or organellar) enclosing water and dissociated lipoproteins between the lamellae of injured membranes. 4. Reduced protein synthesis: Dispersed ribosomes. As a result of continued hypoxia, membranes of endoplasmic reticulum and Golgi apparatus swell up. Ribosomes are detached from granular endoplasmic reticulum and polysomes are degraded to monosomes, thus dispersing ribosomes in the cytoplasm and inactivating their function. Similar reduced protein synthesis occurs in Golgi apparatus. Up to this point, withdrawal of acute stress that resulted in reversible cell injury can restore the cell to normal state. IRREVERSIBLE CELL INJURY. Persistence of ischaemia or hypoxia results in irreversible damage to the structure and function of the cell (cell death). The stage at which this point of no return or irreversibility is reached from reversible cell injury is unclear but the sequence of events is a continuation of reversibly injured cell. Two essential phenomena always distinguish irreversible from reversible cell injury(Fig. 3.6): Inability of the cell to reverse mitochondrial dysfunction on reperfusion or reoxygenation. Disturbance in cell membrane function in general, and in plasma membrane in particular. In addition, there is further reduction in ATP, continued depletion of proteins, reduced intracellular pH, and leakage

of lysosomal enzymes into the cytoplasm. These biochemical changes have effects on the ultrastructural components of the cell (Fig. 3.7): 1. Calcium influx: Mitochondrial damage. As a result of continued hypoxia, a large cytosolic influx of calcium ions occurs, especially after reperfusion of irreversibly injured cell. Excess intracellular calcium collects in the mitochondria disabling its function. Morphologically, mitochondrial changes are vacuoles in the mitochondria and deposits of amorphous calcium salts in the mitochondrial matrix. 2. Activated phospholipases: Membrane damage. Damage to membrane function in general, and plasma membrane in particular, is the most important event in irreversible cell injury in ischaemia. As a result of sustained ischaemia, there is increased cytosolic influx of calcium in the cell. Increased calcium activates endogenous phospholipases. These in turn degrade membrane phospholipids progressively which are the main constituent of the lipid bilayer membrane. Besides, there is also decreased replacement-synthesis of membrane phospholipids due to reduced ATP. Other lytic enzyme which is activated is ATPase which causes further depletion of ATP. 3. Intracellular proteases: Cytoskeletal damage. The normal cytoskeleton of the cell (microfilaments, microtubules and intermediate filaments) which anchors the cell membrane is damaged due to degradation by activated intracellular proteases or by physical effect of cell swelling producing irreversible cell membrane injury. 4. Activated endonucleases: Nuclear damage. The nucleoproteins are damaged by the activated lysosomal enzymes such as proteases and endonucleases. Irreversible damage to the nucleus can be in three forms: i) Pyknosis: Condensation and clumping of nucleus which becomes dark basophilic. ii) Karyorrhexis: Nuclear fragmentation in to small bits dispersed in the cytoplasm. iii) Karyolysis: Dissolution of the nucleus. 5. Lysosomal hydrolytic enzymes: Lysosomal damage, cell death and phagocytosis. The lysosomal membranes are damaged and result in escape of lysosomal hydrolytic enzymes. These enzymes are activated due to lack of oxygen in the cell and acidic pH. These hydrolytic enzymes include: hydrolase, RNAase, DNAase, protease, glycosidase, phosphatase, lipase, amylase, cathepsin etc) which on activation bring about enzymatic digestion of cellular components and hence cell death. The dead cell is eventually replaced by masses of phospholipids called myelin figures which are either phagocytosed by macrophages or there may be formation of calcium soaps. Liberated enzymes just mentioned leak across the abnormally permeable

cell membrane into the serum, the estimation of which may be used as clinical parameters of cell death. For example, in myocardial infarction, estimation of elevated serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), isoenzyme of creatine kinase (CK-MB), and more recently cardiac troponins (cTn) are useful guides for death of heart muscle. Some of the common enzyme markers of cell death in different forms of cell death are given in Table 3.1.

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While cell damage from oxygen deprivation by above mechanisms develops slowly, taking several minutes to hours, the cell injury is accentuated after restoration of blood supply and subsequent events termed ischaemic-reperfusion injury and liberation of toxic free radicals, discussed below.

Ischaemia-Reperfusion Injury and Free Radical-Mediated Cell Injury Depending upon the duration of ischaemia/hypoxia, restoration of blood flow may result in the following 3 different consequences:

1. From ischaemia to reversible injury. When the period of ischaemia is of short duration, reperfusion with resupply of oxygen restores the structural and functional state of the injured cell i.e. reversible cell injury. 2. From ischaemia to reperfusion injury. When ischaemia is for longer duration, then rather than restoration of structure and function of the cell, reperfusion paradoxically deteriorates the already injured cell. This is termed ischaemia-reperfusion injury. 3. From ischaemia to irreversible injury. Much longer period of ischaemia may produce irreversible cell injury during ischaemia itself when so much time has elapsed that neither blood flow restoration is helpful nor reperfusion injury can develop. Cell death in such cases is not attributed to formation of activated oxygen species. But instead, on reperfusion there is further marked intracellular excess of sodium and calcium ions due to persistent cell membrane damage. The underlying mechanism of reperfusion injury and free radical mediated injury is complex but following three main components are involved in it:

1. Calcium overload. 2. Generation of reactive oxygen radicals (superoxide, H2O2, hydroxyl radicals). 3. Subsequent inflammatory reaction. These are discussed

below: 1. CALCIUM OVERLOAD. Upon restoration of blood supply, the ischaemic cell is further bathed by the blood fluid that has more calcium ions at a time when the ATP stores of the cell are low. This results in further calcium overload on the already injured cells, triggering lipid peroxidation of the membrane causing further membrane damage. 2. GENERATION OF REACTIVE OXYGEN RADICALS. Although oxygen is the lifeline of all cells and tissues, its molecular forms as reactive oxygen radicals or reactive oxygen species can be most devastating for the cells. In recent times, free radical-mediated cell injury has been extensively studied and a brief account is given below. Mechanism of oxygen free radical generation. Normally, metabolism of the cell involves generation of ATP by oxidative process in which biradical oxygen (O2) combines with hydrogen atom (H) and in the process forms water (H2O). This reaction of O2 to H2O involves 'four electron donation' in four steps involving transfer of one electron at each step. Oxygen free radicals are the intermediate chemical species having an unpaired oxygen in their outer orbit. These are generated within mitochondrial inner membrane where cytochrome oxidase catalyses the O2 to H2O reaction. Three intermediate molecules of partially reduced species of oxygen are generated depending upon the number of electrons transferred(Fig. 3.8): Superoxide oxygen (O'2): one electron Hydrogen peroxide (H2O2): two electrons Hydroxyl radical (OH–): three electrons These are generated from enzymatic and non-enzymatic reaction as under: 1. Superoxide (O'2): Superoxide anion O'2 may be generated by direct auto-oxidation of O2 during mitochondrial electron transport reaction. Alternatively, O'2 is produced enzymatically by xanthine oxidase and cytochrome P450 in the mitochondria or cytosol. O'2 so formed is catabolised to produce H2O2 by superoxide dismutase (SOD).

TABLE 3.1: Common Enzyme Markers of Cell Death. Enzyme Disease

1. Aspartate aminotransferase Diffuse liver cell necrosis e.g. (AST, SGOT) viral hepatitis, alcoholic liver disease Acute myocardial infarction 2. Alanine aminotransferase More specific for diffuse liver (ALT, SGPT) cell damage than AST e.g. viral hepatitis 3. Creatine kinase-MB (CK-MB) Acute myocardial infarction, myocarditis Skeletal muscle injury 4. Lipase More specific for acute pancreatitis 5. Amylase Acute pancreatitis Sialadenitis 6. Lactic dehydrogenase (LDH) Acute

myocardial infarction Myocarditis Skeletal muscle injury 7. Cardiac troponin (CTn) Specific for acute myocardial infarction

2. Hydrogen peroxide (H2O2): H2O2 is reduced to water enzymatically by catalase (in the peroxisomes) and glutathione peroxidase GSH (both in the cytosol and mitochondria). 3. Hydroxyl radical (OH–): OH– radical is formed by 2 ways in biologic processes—by radiolysis of water and by reaction of H2O2 with ferrous (Fe++) ions; the latter process is termed as Fenton reaction. Other oxygen free radicals. In addition to superoxide, H2O2 and hydroxyl radicals generated during of O2 to H2O reaction, a few other more active oxygen free radicals which formed in the body are as follows: i) Release of superoxide free radical in Fenton reaction (see below). ii) Nitric oxide (NO), a chemical mediator generated by various body cells (endothelial cells, neurons, macrophages etc), combines with superoxide and forms peroxynitrate (ONOO) which is a potent free radical. iii) Halide reagent (chlorine or chloride) released in the leucocytes reacts with superoxide and forms hypochlorous acid (HOCl) which is a cytotoxic free radical. iv) Exogenous sources of free radicals include some environmental agents such as tobacco and industrial pollutants. Cytotoxicity of oxygen free radicals. Free radicals are formed in physiologic as well as pathologic processes. Basically, oxygen radicals are unstable and are destroyed spontaneously. The rate of spontaneous destruction is determined by catalytic action of certain enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. The net effect of free radical injury in physiologic and disease states, therefore, depends upon the rate of free radical formation and rate of their elimination. However, if not degraded, then free radicals are highly destructive to the cell since they have electron-free residue and thus bind to all molecules of the cell; this is termed oxidative stress. Out of various free radicals, hydroxyl radical is the most reactive species. Free radicals may produce membrane damage by the following mechanisms (Fig. 3.9): i) Lipid peroxidation. Polyunsaturated fatty acids (PUFA) of membrane are attacked repeatedly and severely by oxygenderived free radicals to yield highly destructive PUFA radicals—lipid hydroperoxy radicals and lipid hypo

peroxides. This reaction is termed lipid peroxidation. The lipid peroxides are decomposed by transition metals such as iron. Lipid peroxidation is propagated to

other sites causing widespread membrane damage and destruction of organelles. ii) Oxidation of proteins. Oxygen-derived free radicals cause cell injury by oxidation of protein macromolecules of the cells, crosslinkages of labile amino acids as well as by fragmentation of polypeptides directly. The end-result is degradation of cytosolic neutral proteases and cell destruction. iii) DNA damage. Free radicals cause breaks in the single strands of the nuclear and mitochondrial DNA. This results in cell injury; it may also cause malignant transformation of cells. iv) Cytoskeletal damage. Reactive oxygen species are also known to interact with cytoskeletal elements and interfere in mitochondrial aerobic phosphorylation and thus cause ATP depletion. Conditions with free radical injury. Currently, oxygenderived free radicals have been known to play an important role in many forms of cell injury: i) Ischaemic reperfusion injury ii) Ionising radiation by causing radiolysis of water iii) Chemical toxicity iv) Chemical carcinogenesis v) Hyperoxia (toxicity due to oxygen therapy) vi) Cellular aging vii) Killing of microbial agents viii) Inflammatory damage ix) Destruction of tumour cells x) Atherosclerosis. Antioxidants. Antioxidants are endogenous or exogenous substances which inactivate the free radicals. These substances include the following: Vitamins E, A and C (ascorbic acid) Sulfhydryl-containing compounds e.g. cysteine and glutathione. Serum proteins e.g. ceruloplasmin and transferrin. 3. SUBSEQUENT INFLAMMATORY EACTION. Ischaemia-reperfusion event is followed by inflammatory reaction. Incoming activated neutrophils utilise oxygen quickly (oxygen burst) and release a lot of oxygen free radicals. Ischaemia is also associated with accumulation of precursors of ATP, namely ADP and pyruvate, which further build-up generation of free radicals.

Pathogenesis of Chemical Injury Chemicals induce cell injury by one of the two mechanisms: by direct cytotoxicity, or by conversion of chemical into reactive metabolites. DIRECT CYTOTOXIC EFFECTS. Some chemicals combine with components of the cell and produce direct cytotoxicity without requiring metabolic activation. The cytotoxic damage is usually greatest to cells which are involved in the metabolism of such chemicals e.g. in mercuric chloride

Figure 3.9 Mechanism of cell death by hydroxyl radical, the most reactive oxygen species.

poisoning, the greatest damage occurs to cells of the alimentary tract where it is absorbed and kidney where it is excreted. Cyanide kills the cell by poisoning mitochondrial cytochrome oxidase thus blocking oxidative phosphorylation. Other examples of directly cytotoxic chemicals include chemotherapeutic agents used in treatment of cancer, toxic heavy metals such as mercury, lead and iron.

CONVERSION TO REACTIVE TOXIC METABOLITES. This mechanism involves metabolic activation to yield ultimate toxin that interacts with the target cells. The target cells in this group of chemicals may not be the same cell that metabolised the toxin. Example of cell injury by conversion of reactive metabolites is toxic liver necrosis caused by carbon tetrachloride (CCl_4), acetaminophen (commonly used analgesic and antipyretic) and bromobenzene. Cell injury by CCl_4 is classic example of an industrial toxin (earlier used in drycleaning industry) that produces cell injury by conversion to a highly toxic free radical, CCl_3, in the body's drug-metabolising P450 enzyme system in the liver cells. Thus, it produces profound liver cell injury by free radical generation. Other mechanism of cell injury includes direct toxic effect on cell membrane and nucleus.

Pathogenesis of Physical Injury Injuries caused by mechanical force are of medicolegal significance. But they may lead to a state of shock. Injuries by changes in atmospheric pressure (e.g. decompression sickness) are detailed in Chapter 5. Radiation injury to human by accidental or therapeutic exposure is of importance in treatment of persons with malignant tumours as well as may have carcinogenic influences (Chapter 8). Killing of cells by ionising radiation is the result of direct formation of hydroxyl radicals from radiolysis of water (Fig. 3.10). These hydroxyl radicals damage the cell memb

rane as well as may interact with DNA of the target cell. In proliferating cells, there is inhibition of DNA replication and eventual cell death by apoptosis (e.g. epithelial cells). In nonproliferating cells there is no effect of inhibition of DNA synthesis and in these cells there is cell membrane damage followed by cell death

by necrosis (e.g. neurons). MORPHOLOGY OF CELL INJURY After having discussed the molecular and biochemical mechanisms of various forms of cell injury, we now turn to light microscopic morphologic changes of reversible and irreversible cell injury. Depending upon the severity of cell injury, degree of damage and residual effects on cells and tissues are variable. In general, morphologic changes in various forms of cell injury can be classified as shown in Table 3.2 and are discussed below.

MORPHOLOGY OF REVERSIBLE CELL INJURY In conventional description of morphologic changes, the term degeneration has been used to denote morphology of reversible cell injury. However, now it is realised that this term does not provide any information on the nature of underlying changes and thus currently more acceptable terms of retrogressive changes or simply reversible cell injury are applied to non-lethal cell injury. Following morphologic forms of reversible cell injury are included under this heading: 1. Hydropic change (cloudy swelling, or vacuolar degeneration) 2. Fatty change 3. Hyaline change 4. Mucoid change

Hydropic Change Hydropic change means accumulation of water within the cytoplasm of the cell. Other synonyms used are cloudy swelling (for gross appearance of the affected organ) and vacuolar degeneration (due to cytoplasmic vacuolation). ETIOLOGY. This is the commonest and earliest form of cell injury from almost all causes. The common causes include

TABLE 3.2: Classification of Morphologic Forms of Cell Injury.

Mechanism of Nomenclature Cell Injury

1. Reversible cell injury Retrogressive changes (older term: degenerations)

2. Irreversible cell injury Cell death—necrosis

3. Programmed cell death Apoptosis

4. Residual effects of Subcellular alterations cell injury

5. Deranged cell metabolism Intracellular accumulation of lipid, protein, carbohydrate

6. After-effects of necrosis Gangrene, pathologic calcification

Figure 3.10 Mechanisms of cell injury by ionising radiation.

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acute and subacute cell injury from various etiologic agents such as bacterial toxins, chemicals, poisons, burns, high fever, intravenous administration of hypertonic glucose or saline etc. PATHOGENESIS. Cloudy swelling results from impaired regulation of sodium and potassium at the level of cell membrane. This results in intracellular accumulation of sodium and escape of potassium. This, in turn, leads to rapid flow of water into the cell to maintain iso-osmotic conditions and hence cellular swelling occurs. In addition, influx of calcium too occurs. Hydropic swelling is an entirely reversible change upon removal of the injurious agent.

MORPHOLOGIC FEATURES. Grossly, the affected organ such as kidney, liver, pancreas, 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 (Fig. 3.11): i) The cells are swollen and the microvasculature compressed. ii) Small clear vacuoles are seen in the cells and hence the term vacuolar degeneration. These vacuoles represent distended cisternae of the endoplasmic reticulum. iii) Small cytoplasmic blebs may be seen. iv) The nucleus may appear pale.

Hyaline Change 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. Though fibrin and amyloid have hyaline appearance, they have distinctive features and staining reactions and can be distinguished from non-specific hyaline material. Hyaline change is associated with heterogeneous pathologic conditions. It may be intracellular or extracellular.

INTRACELLULAR HYALINE. Intracellular hyaline is mainly seen in epithelial cells. A few examples are as follows: 1. Hyaline droplets in the proximal tubular epithelial cells in cases of excessive reabsorption of plasma proteins. 2. Hyaline

degeneration of rectus abdominalis muscle called Zenker's degeneration, occurring in typhoid fever. The muscle loses its fibrillar staining and becomes glassy and hyaline. 3. Mallory's hyaline represents aggregates of intermediate filaments in the hepatocytes 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 (Fig. 3.12).

EXTRACELLULAR HYALINE. Extracellular hyaline is seen in connective tissues. A few examples of extracellular hyaline change are as under: 1. Hyaline degeneration in leiomyomas of the uterus (Fig. 3.13). 2. Hyalinised old scar of fibrocollagenous tissues. 3. Hyaline arteriolosclerosis in renal vessels in hypertension and diabetes mellitus. 4. Hyalinised glomeruli in chronic glomerulonephritis. 5. Corpora amylacea are rounded masses of concentric hyaline laminae seen in the prostate in the elderly, in the brain and in the spinal cord in old age, and in old infarcts of the lung.

Mucoid Change Mucus secreted by mucous glands is a combination of proteins complexed with mucopolysaccharides. Mucin, a glycoprotein, is its chief constituent. Mucin is normally produced by epithelial cells of mucous membranes and mucous glands, as well as by some connective tissues like in the umbilical cord. By convention, connective tissue mucin is termed myxoid (mucus like). Both types of mucin

Figure 3.11 Hydropic change kidney. The tubular epithelial cells are distended with cytoplasmic vacuoles while the interstitial vasculature is compressed. The nuclei of affected tubules are pale.

are stained by alcian blue. However, epithelial mucin stains positively with periodic acid-Schiff (PAS), while connective tissue mucin is PAS negative but is stained positively with colloidal iron.

EPITHELIAL MUCIN. Following are some examples of functional excess of epithelial mucin: 1. Catarrhal inflammation of mucous membrane (e.g. of

respiratory tract, alimentary tract, uterus). 2. Obstruction of duct leading to mucocele in the oral cavity and gallbladder. 3. Cystic fibrosis of the pancreas. 4. Mucin-secreting tumours (e.g. of ovary, stomach, large bowel etc) (Fig. 3.14) .

CONNECTIVE TISSUE MUCIN. A few examples of disturbances of connective tissue mucin are as under: 1. Mucoid or myxoid degeneration in some tumours e.g. myxomas, neurofibromas,fibroadenoma, soft tissue sarcomas etc(Fig. 3.15) . 2. Dissecting aneurysm of the aorta due to Erdheim's medial degeneration and Marfan's syndrome. 3. Myxomatous change in the dermis in myxoedema. 4. Myxoid change in the synovium in ganglion on the wrist.

SUBCELLULAR ALTERATIONS IN CELL INJURY Certain morphologically distinct alterations at subcellular level are noticeable in both acute and chronic forms of cell

Figure 3.12 Intracellular hyaline as Russell's bodies in the plasma cells. The cytoplasm shows pink homogeneous globular material due to accumulated immunoglobulins. Figure 3.13 Extracellular hyaline deposit in leiomyoma uterus. Theinjury. 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 Chédiak-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, neurities and senile plaques in Alzheimer's disease are composed of neurofilaments and paired helical filaments.

2.L YSOSOMAL 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 10, 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. SER CHANGES. 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.

INTRACELLULAR ACCUMULATIONS 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. In addition, deposits of amyloid and urate are discussed separately later. 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. These are discussed in Chapter 10. 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. These pathologic states are discussed below.

FATTY CHANGE (STEATOSIS) 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, skeletal muscle, kidneys (lipoid nephrosis or minimum change disease) and other organs.

Fatty Liver Liver is the commonest 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.

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ETIOLOGY. Fatty change in the liver may result from one of the two types of causes: 1. Conditions with excess fat (hyperlipidameia), exceeding the capacity of the liver to metabolise it. 2. Liver cell damage, when fat cannot be metabolised in it. These causes are listed below: 1. Conditions with excess fat: i) Obesity ii) Diabetes mellitus iii) Congenital hyperlipidaemia 2. Liver cell damage: i) Alcoholic liver disease (most common) ii) Starvation iii) Protein calorie malnutrition iv) Chronic illnesses (e.g. tuberculosis) v) Acute fatty liver in late pregnancy vi) Hypoxia (e.g. anaemia, cardiac failure) vii) Hepatotoxins (e.g. carbon

tetrachloride, chloroform, ether, aflatoxins and other poisons) viii) Drug-induced liver cell injury (e.g. administration of methotrexate, steroids, CCl4, halothane anaesthetic, tetracycline etc) ix) Reye's syndrome PATHOGENESIS. Mechanism of fatty liver depends upon the stage at which the etiologic agent acts in the normal fat transport and metabolism. Hence, pathogenesis of fatty liver is best understood in the light of normal fat metabolism in the liver (Fig. 3.16). Lipids as free acids enter the liver cell from either of the following 2 sources:

From diet as chylomicrons (containing triglycerides and phospholipids) and as free fatty acids; and From adipose tissue as free fatty acids. Normally, besides above two sources, a small part of fatty acids is also synthesised from acetate in the liver cells. Most of free 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. While cholesterol, phospholipids and ketones are used in the body, intracellular triglycerides are converted into lipoproteins, which requires 'lipid acceptor protein'. Lipoproteins are released from the liver cells into circulation as plasma lipoproteins (LDL, VLDL). In fatty liver, intracellular accumulation of triglycerides can occur due to defect at one or more of the following 6 steps in the normal fat metabolism shown in Fig. 3.16: 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 which includes: 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; e.g. 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 teetotaller.

MORPHOLOGIC FEATURES. Grossly, the liver in fatty change 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 (Fig. 3.17). Microscopically, characteristic feature is the presence of numerous lipid vacuoles in the cytoplasm of hepatocytes. Fat in H & E stained section prepared by paraffinembedding technique appear non-staining vauloes because it is dissolved in alcohol (Fig. 3.18): i) The vacuoles are initially small and are present around the nucleus (microvesicular). ii) But with progression of the process, the vacuoles become larger pushing the nucleus to the periphery of the cells (macrovesicular). iii) At times, the hepatocytes laden with large lipid vacuoles may rupture and lipid vacuoles coalesce to form fatty cysts.

Figure 3.16 Lipid metabolism in the pathogenesis of fatty liver. Defects in any of the six numbered steps (corresponding to the description in the text) can produce fatty liver by different etiologic agents.

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iv) Infrequently, lipogranulomas may appear consisting of collections of lymphocytes, macrophages, and some multinucleated giant cells. v) Fat can be demonstrated in fresh unfixed tissue by frozen section followed by fat stains such as Sudan dyes (Sudan III, IV, Sudan black) and oil red O. Alternatively, osmic acid which is a fixative as well as a stain can be used to demonstrate fat in the tissue.

Cholesterol Deposits Intracellular deposits of cholesterol and its esters in macrophages may occur when there is hypercholesterolaemia. This

turns macrophages into foam cells. The examples are as follows: 1. Fibrofatty plaques of atherosclerosis (Chapter 15). 2. Clusters of foam cells in tumour-like masses called xanthomas and xanthelasma.

Stromal Fatty Infiltration This form of lipid accumulation is quite different from fatty change just described. Stromal fatty infiltration is the deposition of mature adipose cells in the stromal connective tissue in contrast to intracellular deposition of fat in the parenchymal cells in fatty change. The condition occurs most often in patients with obesity. The two commonly affected organs are the

heart and the pancreas. Thus, heart can be the site for intramyocardial fatty change as well as epicardial (stromal) fatty infiltration. The presence of mature adipose cells in the stroma generally does not produce any dysfunction. In the case of heart, stromal fatty infiltration is associated with increased adipose tissue in the epicardium.

INTRACELLULAR ACCUMULATION OF PROTEINS 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 synthesised 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 hyalin in the hepatocytes is intracellular accumulation of intermediate filaments of cytokeratin and appear as amorphous pink masses.

Figure 3.17 Fatty liver. Sectioned slice of the liver shows pale yellow parenchyma with rounded borders.

Figure 3.18 Fatty liver. Many of the hepatocytes are distended with large fat vacuoles pushing the nuclei to the periphery (macrovesicles), while others show multiple small vacuoles in the cytoplasm (microvesicles).

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INTRACELLULAR ACCUMULATION OF GLYCOGEN Conditions associated with excessive accumulation of intracellular glycogen are as under: 1. In diabetes mellitus, there is intracellular accumulation of glycogen in different tissues because normal cellular uptake of glucose is impaired. 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. Best's 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. These conditions along with other similar genetic disorders are discussed in Chapter 10. PIGMENTS Pigments are coloured substances present in most living beings including humans. There are 2 broad categories of pigments: endogenous and exogenous (Table 3.3) .

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

Melanin Melanin is the brown-black, non-haemoglobin-derived 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 melanophores, 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 is present, 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: i) 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 raindrop pigmentation of the skin. ii) Focal hyperpigmentation: a) Cäfe-au-lait spots are pigmented patches seen in neurofibromatosis and Albright's syndrome. b) Peutz-Jeghers syndrome is

characterised by focal peri-oral pigmentation. c) Melanosis coli is pigmentation of the mucosa of the colon. d) Melanotic tumours, both benign such as pigmented naevi (Fig. 3.19), 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. f) Dermatopathic lymphadenitis is an example of deposition of melanin pigment in macrophages of the lymph nodes draining skin lesions. iii) Generalised hypopigmentation:Albinism is an extreme degree of generalised 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. iv) 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.

Melanin-like Pigments ALKAPTONURIA. This is a rare autosomal recessive disorder in which there is deficiency of an oxidase enzyme required for break-down of homogentisic acid which then accumulates in the tissues and is excreted in the urine (homogentisic aciduria). The urine of patients of alkaptonuria, if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid. The pigment is

 TABLE 3.3: Pigments of the Body. A. ENDOGENOUS PIGMENTS 1. Melanin 2. Melanin-like pigment a. Alkaptonuria b. Dubin-Johnson syndrome 3. Haemoprotein-derived pigments i) Haemosiderin ii) Acid haematin (Haemozoin) c. Bilirubin d. Porphyrins 4. Lipofuscin (Wear and tear pigment)

B. EXOGENOUS PIGMENTS 1. Inhaled pigments 2. Ingested pigments 3. Injected pigments (Tattooing)

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melanin-like and is deposited both intracellularly and intercellularly and is termed ochronosis, first described by Virchow. Most commonly affected tissues are the cartilages, capsules of joints, ligaments and tendons. DUBIN-JOHNSON SYNDROME. Hepatocytes in patients of Dubin-Johnson syndrome, an autosomal recessive form of hereditary conjugated hyperbilirubinaemia, contain melain-like pigment in the cytoplasm (Chapter 21).

Haemoprotein-derived Pigments Haemoproteins are the most important endogenous pigments derived from haemoglobin, cytochromes and their break-down products. For an understanding of disorders of haemoproteins, it is essential to have knowledge of normal iron metabolism and its transport which is described in Chapter 12. In disordered iron metabolism and transport, haemoprotein-derived pigments accumulate in the body. These pigments are haemosiderin, acid haematin (haemozoin), bilirubin, and porphyrins.

1. HAEMOSIDERIN. Iron is stored in the tissues in 2 forms: Ferritin, which is iron complexed to apoferritin and can be identified by electron microscopy. 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 break-down of senescent red cells takes place. Haemosiderin is ferric iron that can be demonstrated by Perl's stain that produces Prussian blue reaction. In this reaction, colourless potassium ferrocyanide reacts with ferric ions of haemosiderin to form deep blue ferric-ferrocyanide (Fig. 3.20). Excessive storage of haemosiderin occurs in situations when there is increased break-down 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 (Fig. 3.21): a) Localised 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. A few examples are as under : 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. 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(Systemic or Diffuse) haemosiderosis. Systemic overload with iron may result in generalised haemosiderosis. There can be two types of patterns:

Figure 3.19 Compound naevus showing clusters of benign naevus cells in the dermis as well as in lower epidermis. These cells contain coarse, granular, brown-black melanin pigment.

Figure 3.20 Haemosiderin pigment in the cytoplasm of hepatocytes seen as Prussian blue granules.

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Parenchymatous deposition of haemosiderin occurs in the parenchymal cells of the liver, pancreas, kidney, and heart. Reticuloendothelial deposition occurs in the liver, spleen, and bone marrow. Generalised or systemic overload of iron may occur due to following causes: i) Increased erythropoietic activity: In various forms of chronic haemolytic anaemia, there is excessive break-down of haemoglobin and hence iron overload. The problem is further compounded by treating the condition with blood transfusions (transfusional haemosiderosis) or by parenteral iron therapy. The deposits of iron in these cases, termed as acquired haemosiderosis, are initially in reticuloendothelial tissues but may secondarily affect other organs. ii) Excessive intestinal absorption of iron: A form of haemosiderosis in which there is excessive intestinal absorption of iron even when the intake is normal, is known as idiopathic or hereditary haemochromatosis. It is an autosomal dominant disease associated with much more deposits of iron than cases of acquired haemosiderosis. It is characterised by 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. iii) Excessive dietary intake of iron: A common example of excessive intake of iron is Bantu's disease in black tribals of

South Africa who conventionally brew their alcohol in cast iron pots that serves as a rich source of additional dietary iron. The excess of iron gets deposited in various organs including the liver causing pigment cirrhosis. 2. ACID HAEMATIN (HAEMOZOIN). Acid haematin or haemozoin is a haemoprotein-derived brown-black pigment containing haem iron in ferric form in acidic medium. But it differs from haemosiderin because it cannot be stained by Prussian blue (Perl's) 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. Besides, the malarial pigment can also be deposited in macrophages and

in the hepatocytes. Another variety of haematin pigment is formalin pigment formed in blood-rich tissues which have been preserved in acidic formalin solution. 3. BILIRUBIN. Bilirubin is the normal non-iron containing pigment present in the bile. It is derived from porphyrin ring of the haem moiety of haemoglobin. Normal level of bilirubin in blood is less than 1 mg/dl. Excess of bilirubin or hyperbilirubinaemia causes an important clinical condition called jaundice. Normal bilirubin metabolism and pathogenesis of jaundice are described in Chapter 21. Hyperbilirubinaemia may be unconjugated or conjugated, and jaundice may appear in one of the following 3 ways: a) Prehepatic or haemolytic, when there is excessive destruction of red cells. b) Posthepatic or obstructive, which results from obstruction to the outflow of conjugated bilirubin. c) Hepatocellular 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. In infants, rise in unconjugated bilirubin may produce toxic brain injury called kernicterus. 4.PORPHYRINS. Porphyrins are normal pigment present in haemoglobin, myoglobin and cytochrome. Porphyria refers to an uncommon disorder of inborn abnormality of porphyrin metabolism. It results from genetic deficiency of one of the enzymes required for the synthesis of haem, resulting in excessive production of porphyrins. Often, the genetic deficiency is precipitated by intake of some drugs. Porphyrias are associated with excretion of intermediate products in the urine—delta-aminolaevulinic acid,

porphobilinogen, uroporphyrin, coproporphyrin, and protoporphyrin. Porphyrias are broadly of 2 types—erythropoietic and hepatic. (a) Erythropoietic porphyrias. These have defective synthesis of haem in the red cell precursors in the bone marrow. These may be further of 2 subtypes: Congenital erythropoietic porphyria, in which the urine is red due to the presence of uroporphyrin and coproporphyrin. The skin of these infants is highly photosensitive. Bones and skin show red brown discolouration. Erythropoietic protoporphyria, in which there is excess of protoporphyrin but no excess of porphyrin in the urine. (b) Hepatic porphyrias. These are more common and have a normal erythroid precursors but 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. Porphyria cutanea tarda is the most common of all porphyrias. Porphyrins collect in the liver and small quantity is excreted in the urine. Skin lesions are similar to those in

Figure 3.21 Effects of haemosiderosis.

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variegate porphyria. Most of the patients have associated haemosiderosis with cirrhosis which may eventually develop into hepatocellular carcinoma. ?? ?? ? Mixed (Variegate) porphyrias. It is rare and combines skin photosensitivity with acute abdominal and neurological manifestations.

Lipofuscin (Wear and Tear Pigment) Lipofuscin or lipochrome is yellowish-brown intracellular lipid pigment (lipo = fat, fuscus = brown). The pigment is often found in atrophied cells of old age and hence the name ‘wear and tear pigment’. It is seen in the myocardial fibres, hepatocytes, Leydig cells of the testes and in neurons in senile dementia. However, the pigment may, at times, accumulate rapidly in different cells in wasting diseases unrelated to aging.

By light microscopy, the pigment is coarse, golden-brown granular and 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’ (Fig. 3.22). The pigment can be stained by fat stains but differs from other lipids in being fluorescent and having acid-fastness.

By electron microscopy, lipofuscin appears as intralysosomal electron-dense granules in perinuclear location. These granules are composed of lipid-protein complexes. Lipofuscin represents the collection of indigestible material in the lysosomes after intracellular lipid peroxidation and is therefore an example of residual bodies. Unlike in normal cells, in aging or debilitating diseases the phospholipid endproducts of membrane damage mediated by oxygen free radicals fail to get eliminated and hence are deposited as lipofuscin pigment.

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

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 (Chapter 17). 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 (Fig. 3.23). 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 colouration 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

Figure 3.22 Brown atrophy of the heart. The lipofuscin pigment granules are seen in the cytoplasm of the myocardial fibres, especially around the nuclei.

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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. MORPHOLOGY OF 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. autolysis, 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.

AUTOLYSIS Autolysis (i.e. 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), but the term is generally used for 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.

NECROSIS Necrosis is defined as a localised area of death of tissue followed by degradation of tissue by hydrolytic enzymes liberated from dead cells; it is invariably accompanied by inflammatory reaction. Necrosis can be caused by various agents such as hypoxia, chemical and physical agents, microbial agents, immunological injury, etc. Two essential changes characterise irreversible cell

injury in necrosis of all types (Fig. 3.24,A): i)Cell digestion by lytic enzymes. Morphologically this change is identified as homogeneous and intensely

Figure 3.23 Anthracosis lung. There is presence of abundant coarse black carbon pigment in the septal walls and around the bronchiole.

Figure 3.24 Necrosis and apoptosis. A, Cell necrosis is identified by homogeneous, eosinophilic cytoplasm and nuclear changes of pyknosis, karyolysis, and karyorrhexis. B, Apoptosis consists of condensation of nuclear chromatin and fragmentation of the cell into membrane-bound apoptotic bodies which are engulfed by macrophages.

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eosinophilic cytoplasm. Occasionally, it may show cytoplasmic vacuolation or dystrophic calcification. ii) Denaturation of proteins. This process is morphologically seen as characteristic nuclear changes in necrotic cell. These nuclear changes may include: condensation of nuclear chromatin (pyknosis) which may either undergo dissolution (karyolysis) or fragmentation into many granular clumps (karyorrhexis) (see Fig. 3.7).

Types of Necrosis Morphologically, there are five types of necrosis: coagulative, liquefaction (colliquative), caseous, fat, and fibrinoid necrosis. 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. 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. But cell digestion and liquefaction fail to occur (c.f. liquefaction necrosis). Eventually, the necrosed focus is infiltrated by

inflammatory cells and the dead cells are phagocytosed leaving granular debris and fragments of cells (Fig. 3.25).

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

Figure 3.25 Coagulative necrosis in infarct kidney. The affected area on right shows cells with intensely eosinophilic cytoplasm of tubular cells but the outlines of tubules are still maintained. The nuclei show granular debris. The interface between viable and non-viable area shows nonspecific chronic inflammation and proliferating vessels.

hydrolytic enzymes. The common examples are infarct brain 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 (Fig. 3.26).

3. CASEOUS NECROSIS. 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 cheese 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 (Fig. 3.27). 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.

4. 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 extraabdominal adipose tissue.

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Fat necrosis hydrolyses neutral fat present in adipose cells into glycerol and free fatty acids. The damaged adipose cells assume cloudy appearance. The leaked out free fatty acids 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 (Fig. 3.28).

5. FIBRINOID NECROSIS. Fibrinoid necrosis 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 diseases, Arthus reaction etc), arterioles in hypertension, peptic ulcer etc.

Microscopically, fibrinoid necrosis is identified by brightly eosinophilic, hyaline-like deposition in the vessel wall. Necrotic focus is surrounded by nuclear debris of neutrophils (leucocytoclasis) (Fig. 3.29). Local haemorrhage may occur due to rupture of the blood vessel.

APOPTOSIS

Apoptosis is a form of ‘coordinated and internally programmed cell death’ having significance in a variety of physiologic and pathologic conditions (apoptosis is a Greek

Figure 3.26 Liquefactive necrosis brain. The necrosed area on right side of the field shows a cystic space containing cell debris, while the surrounding zone shows granulation tissue and gliosis.

Figure 3.27 Caseous necrosis lymph node. There is eosinophilic, amorphous, granular material, while the periphery shows granulomatous inflammation.

word meaning ‘falling off’ or ‘dropping off’). The term was first introduced in 1972 as distinct from necrosis by being a form of cell death which is controlled and regulated by the rate of cell division; when the cell is not needed, pathway of cell death is activated (‘cell suicide’) and is unaccompanied by any inflammation and collateral tissue damage. 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. Organised 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. Progressive depletion of CD4+T cells in the pathogenesis of AIDS. 4. Cell death in viral infections e.g. formation of Councilman bodies in viral hepatitis. 5. 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. 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.

8. Heart diseases e.g. heart failure, acute myocardial infarction (20% necrosis and 80% apoptosis).

MORPHOLOGIC FEATURES. The characteristic morphologic changes in apoptosis seen in histologic and electron microscopic examination are as under (see Fig. 3.24,B): 1. Involvement of single cells or small clusters of cells in the background

of viable cells. 2. The apoptotic cells are round to oval shrunken masses of intensely eosinophilic cytoplasm (mummified cell) containing shrunken or almost-normal organelles (Fig. 3.30).

Figure 3.28 Fat necrosis in acute pancreatitis. There is cloudy appearance of adipocytes, coarse basophilic granular debris while the periphery shows a few mixed inflammatory cells.

Figure 3.30 Apoptotic bodies in the layer of squamous mucosa (shown by arrows). The dead cell seen in singles, is shrunken, the nucleus has clumped chromatin, while the cytoplasms in intensely eosinophilic. There is no inflammation, unlike necrosis.

Figure 3.29 Fibrinoid necrosis in autoimmune vasculitis. The vessel wall shows brightly pink amorphous material and nuclear fragments of necrosed neutrophils.

3. The nuclear chromatin is condensed or fragmented (pyknosis or karyorrehexis). 4. The cell membrane may show convolutions or projections on the surface. 5. There may be formation of membrane-bound nearspherical bodies on or around the cell called apoptotic bodies containing compacted organelles. 6. Characteristically, unlike necrosis, there is no acute inflammatory reaction around apoptosis. 7. 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.

Techniques to identify and count apoptotic cells. In addition to routine H & E stain, apoptotic cells can be identified and counted by following methods: 1. Staining of chromatin condensation (haematoxylin, Feulgen, 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. 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. The contrasting features of apoptosis and necrosis are illustrated in Fig. 3.24 and summarised in Table 3.4. 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. Triggers for signalling programmed cell death act at the cell membrane, either intracellularly or extracellularly. These include the following: i) Withdrawal of signals required for normal cell survival (e.g. absence of certain hormones, growth factors, cytokines). ii) Extracellular signals triggering of programmed cell death (e.g. activation of FAS receptor belonging to TNF-R family). iii) Intracellular stimuli e.g. heat, radiation, hypoxia etc. 2. Process of programmed cell death. After the cell hasbeen initiatedinto self-destruct mode, the programme inbuilt in the cell gets activated as under: i) Activation of caspases. Caspases are a series of proteolyitc or protein-splitting enzymes which act on nuclear proteins and organelles containing protein components. The term ‘caspase’ is derived from: c for cystein protease; asp for aspartic acid; and ase is used for naming an enzyme. Caspases get activated either by coming in contact with some etiologic agent of cell injury agent or by unknown mechanism. ii) Activation of death receptors. Activated caspases set in activation of FAS receptor (CD 95), a cell surface receptor present on cytotoxic (CD 8+) T cells, belonging to the family of tumour necrosis factor receptors (TNF-R). FAS receptor is appropriately called a death receptor because on coming in contact with the specific binding site on the target cell, it activates specific growth controlling genes, BCL-2 and p53. iii) Activation of growth controlling genes (BCL-2 and p53). BCL2 gene is a human counterpart of CED-9 (cell death) gene

Feature Apoptosis Necrosis

1. Definition Programmed and coordinated cell death Cell death along with degradation of tissue by hydrolytic enzymes

2. Causative agents Physiologic and pathologic processes Hypoxia, toxins

3. Morphology i) No Inflammatory reaction i) Inflammatory reaction always present ii) Death of single cells ii) Death of many adjacent cells iii) Cell shrinkage iii) Cell swelling initially iv) Cytoplasmic blebs on membrane iv) Membrane disruption v) Apoptotic bodies v) Damaged organelles vi) Chromatin condensation vi) Nuclear disruption vii) Phagocytosis of apoptotic bodies by macrophages vii) Phagocytosis of cell debris by macrophages

4. Molecular changes i) Lysosomes and other organelles intact i) Lysosomal breakdown with liberation of ii) Genetic activation by proto-oncogenes hydrolytic enzymes and oncosuppressor genes, and cytotoxic ii) Cell death by ATP depletion, membrane T cell-mediated target cell killing damage, free radical injury iii) Initiation of apoptosis by intra- and extracellular stimuli, followed by activation of caspase pathway (FAS-R, BCL-2, p53)

found in programmed cell death of nematode worm Caenorabditis elegans. BCL-2 gene family is located in the outer mitochondrial membrane and includes both activators and inhibitors of apoptosis. Thus, it may regulate the apoptotic process by binding to some related proteins (e.g. to BAX and BAD) for promoting apoptosis, or to BCL-XL for inhibiting apoptosis. The net effect on the mitochondrial membrane is thus based on the pro-apoptotic and anti-apoptotic actions of BCL-2 gene family. Besides BCL-2, the apoptotic pathway is partly also governed by p53 molecule which promotes apoptosis. iv) Cell death. The above mechanisms lead to proteolytic actions on nucleus, chromatin clumping, cytoskeletal damage, disruption of endoplasmic reticulum, mitochondrial damage, and disturbed cell membrane. 3. Phagocytosis. The dead apoptotic cells develop membrane changes which promote their phagocytosis. Phosphatidylserine and thrombospondin molecules which are normally present on the inside of the cell membrane, appear on the outer surface of the cells in apoptosis, which facilitate their identification by adjacent phagocytes and promotes phagocytosis. The phagocytosis is unaccompanied by any other

inflammatory cells. The mechanism of apoptosis is schematically represented in Fig. 3.31.

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 the two are produced, is different. The examples of necrotising inflammation are: gangrenous appendicitis, gangrenous stomatitis (noma, cancrum oris). There are 2 main forms of gangrene—dry and wet, and a variant form of wet gangrene called gas gangrene. In all types of gangrene, 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 of dry gangrene foot 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.

MORPHOLOGIC FEATURES. Grossly, 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 formation 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 (Fig. 3.32).

Histologically, there is necrosis with smudging of the tissue. The line of separation consists of inflammatory granulation tissue (Fig. 3.33).

Wet Gangrene Wet gangrene 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 profound systemic manifestations of septicaemia, and finally death. The spreading wet gangrene generally lacks clear-cut line of demarcation and may spread to peritoneal cavity causing peritonitis.

MORPHOLOGIC FEATURES. Grossly, 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 (Fig. 3.34). 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 (Fig. 3.35).

Contrasting features of two main forms of gangrene are summarised in Table 3.5. GAS GANGRENE. It 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.

MORPHOLOGIC FEATURES. Grossly, the affected area is swollen, oedematous, painful and crepitant due to accumulation of gas bubbles within the tissues.

Figure 3.32 Dry gangrene of the foot. The gangrenous area is dry, shrunken and dark and is separated from the viable tissue by clear line of separation.

Figure 3.33 Dry gangrene of the foot. Microscopy shows coagulative necrosis of the skin, muscle and other soft tissue, and thrombsed vessels.

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.

PATHOLOGIC CALCIFICATION Deposition of calcium salts in tissues other than osteoid or enamel is called pathologic or heterotopic calcification. Two 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. Etiology and pathogenesis of the two are different but morphologically the deposits in both 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 intracellular, 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 in Perl's stain.

Figure 3.34 Wet gangrene of the small bowel. The affected part is soft, swollen and dark. Line of demarcation between gangrenous segment and the viable bowel is not clear-cut.

TABLE 3.5: Contrasting Features of Dry and Wet Gangrene.

Feature Dry Gangrene Wet Gangrene

1. Site Commonly limbs More common in bowel 2. Mechanisms Arterial occlusion More commonly venous obstruction, less often arterial occlusion 3. Macroscopy Organ dry, shrunken and black Part moist, soft, swollen, rotten and dark 4. Putrefaction Limited due to very little blood Marked due to stuffing of organ with blood supply 5. Line of demarcation Present at the junction between No clear line of demarcation healthy and gangrenous part 6. Bacteria Bacteria fail to survive Numerous present 7. Prognosis Generally better due to little septicaemia Generally poor due to profound toxaemia

Etiopathogenesis The two types of pathologic calcification result from distinctly different etiologies and mechanisms. DYSTROPHICCALCIFICATION. 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. 3.36). 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. Gamna-Gandy bodies in chronic venous congestion (CVC) of the spleen is characterised by calcific deposits admixed with haemosiderin on fibrous tissue. 5. Infarcts may sometimes undergo dystrophic calcification. 6. Thrombi, especially in the veins, may produce phleboliths. 7. Haematomas in the vicinity of bones may undergo dystrophic calcification. 8. Dead parasites like in hydatid cyst, Schistosoma eggs, and cysticercosis are some of the examples showing dystrophic calcification. 9. Calcification in breast cancer detected by mammography. 10. 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. Mönckeberg’s sclerosis shows calcification in the tunica

media of muscular arteries in elderly people (Chapter 15) (Fig.3.37) . 4. Stroma of tumours such as uterine fibroids, breast cancer, thyroid adenoma, goitre etc show

calcification. 5. 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. 6. Cysts which have been present for a long time may show calcification of their walls e.g. epidermal and pilar cysts. 7. Calcinosis cutis is a condition of unknown cause in which there are irregular nodular deposits of calcium salts in the skin and subcutaneous tissue. 8. 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.

Pathogenesis of dystrophic calcification. It is not quite clear as to how dystrophic calcification takes place. Since serum calcium levels are within normal limits, the denatured proteins in necrotic or degenerated tissue bind phosphate ions, which react with calcium ions to form precipitates of calcium phosphate. 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 precipitates of calcium phosphate 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 parathyroid 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. Sites of metastatic calcification. Metastatic calcification may occur in any normal tissue of the body but affects the following organs more commonly: 1. Kidneys, especially at the basement membrane of tubular epithelium and in the tubular lumina causing nephrocalcinosis (Fig.3.38). 2. Lungs, especially in the alveolar walls. 3. Stomach, on the acid-secreting fundal glands. 4. Blood vessels, especially on the internal elastic lamina. 5. Cornea is another site affected by metastatic calcification. 6. Synovium of the joint causing pain and dysfunction. Pathogenesis of metastatic calcification. Metasatic calcification at the above-mentioned sites occurs due to excessive binding of inorganic phosphate ions with calcium ions, which are elevated due to underlying metabolic derangement. This leads to formation of precipitates of calcium phosphate at the preferential sites. Metastatic calcification is reversible upon correction of underlying metabolic disorder. The distinguishing features between the two types of pathologic calcification are summarised inTable 3.6. CELLULAR ADAPTATIONS For the sake of survival on exposure to stress, the cells make adjustments with the changes in their environment (i.e. adapt) to the physiologic needs (physiologic adaptation) and to non-lethal pathologic injury (pathologic adaptation). Broadly speaking, such physiologic and pathologic adaptations occur by following processes (Fig. 3.39): Decreasing or increasing their size i.e. atrophy and hypertrophy respectively, or by increasing their number i.e.

Figure 3.37 Dystrophic calcification in degenerated tunica media of muscular artery of uterine myometrium in Mönckeberg's arteriosclerosis.

Figure 3.38 Metastatic calcification in tubular basement membrane in nephrocalcinosis due to hypercalcaemia.

hyperplasia (postfix word -trophy means nourishment; -plasia means growth of new cells). Changing the pathway of phenotypic differentiation of cells i.e. metaplasia and dysplasia (prefix word meta- means transformation; dys- means bad development). 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 may occur in sustained atrophy; dysplasia may progress 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 the following: Altered cell surface receptor binding. Alterations in signal for protein synthesis. Synthesis of new proteins by the target cell such as heatshock proteins (HSPs). Common forms of cellular adaptive responses along with examples of physiologic and pathologic adaptations are briefly discussed below (Fig. 3.39).

Differences between Dystrophic and Metastatic Calcification. Feature Dystrophic Calcification Metastatic Calcification

1. Definition Deposits of calcium salts in dead and Deposits of calcium salts in normal tissues degenerated tissues

2. Calcium metabolism Normal Deranged

3. Serum calcium level Normal Hypercalcaemia

4. Reversibility Generally irreversible Reversible upon correction of metabolic disorder

5. Causes Necrosis (caseous, liquefactive, fat), Hyperparathyroidism (due to adenoma, infarcts, thrombi, haematomas, dead hyperplasia, CRF), bony destructive lesions parasites, old scars, atheromas, (e.g. myeloma, metastatic carcinoma), Mönckeberg's sclerosis, certain prolonged immobilisation, hypervitaminosis D, tumours, cysts, calcinosis cutis milk-alkali syndrome, hypercalcaemia of infancy

6. Pathogenesis Increased binding of phosphates with Increased precipitates of calcium phosphate due to necrotic and degenerative tissue, which hypercalcaemia at certain sites e.g. in lungs, stomach, in turn binds to calcium forming blood vessels and cornea calcium phosphate precipitates

ATROPHY Reduction of the number and size of parenchymal cells of an organ or its parts which was once normal is called atrophy (compared from hypoplasia which is the term used for developmentally small size, and aplasia for extreme

failure of development so that only rudimentary tissue is present). CAUSES. 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 with aging. B. Pathologic atrophy. The causes are as under: 1. Starvation atrophy. 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. 2. 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. 3. 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. 4. Neuropathic atrophy. Interruption in nerve supply leads to wasting of muscles e.g. i) Poliomyelitis ii) Motor neuron disease iii) Nerve section. 5. 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. 6. Pressure atrophy. Prolonged pressure from benign tumours or cyst or aneurysm may cause compression and atrophy of the tissues e.g. i) Erosion of spine by tumour in nerve root. ii) Erosion of skull by meningioma arising from piaarachnoid. iii) Erosion of sternum by aneurysm of arch of aorta. 7. Idiopathic atrophy. There are some examples of atrophy where no obvious cause is present e.g. i) Myopathies. ii) Testicular atrophy.

MORPHOLOGIC FEATURES. 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 are not dead cells. 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 (Fig. 3.40).

These autophagic vacuoles may persist to form ‘residual bodies’ in the cell cytoplasm e.g. lipofuscin pigment granules in brown atrophy (page 43).

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. 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 nondividing cells too, only hypertrophy occurs. A. Physiologic hypertrophy. Enlarged size of the uterus in pregnancy is an excellent example of physiologic hypertrophy as well as hyperplasia. B. Pathologic hypertrophy. Examples of certain diseases associated with hypertrophy are as under: 1. Hypertrophy of cardiac muscle may occur in a number of cardiovascular diseases. A few conditions producing left ventricular hypertrophy are as under: i) Systemic hypertension ii) Aortic valve disease (stenosis and insufficiency) iii) Mitral insufficiency 2. Hypertrophy of smooth muscle e.g. i) Cardiac achalasia (in oesophagus) ii) Pyloric stenosis (in stomach)

Figure 3.40 Testicular atrophy. The seminiferous tubules show hyalinisation, peritubular fibrosis and diminished number and size of spermatogenic elements. There is prominence of Leydig cells in the interstitium.

iii) Intestinal strictures iv) Muscular arteries in hypertension. 3. Hypertrophy of skeletal muscle e.g. hypertrophied muscles in athletes and manual labourers. 4. Compensatory hypertrophy may occur in an organ when the contralateral organ is removed e.g. 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.

MORPHOLOGIC FEATURES. 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 (Fig. 3.41). 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. All body cells do not possess hyperplastic growth potential (Chapter 6). 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 is present. CAUSES. As with other non-neoplastic adaptive disorders of growth, hyperplasia has also been divided into physiologic and pathologic. 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 menstrual 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 nephrectomy 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.

Figure 3.41 Cardiac hypertrophy. The myocardial muscle fibres are thick with abundance of eosinophilic cytoplasm. Nuclei are also enlarged with irregular outlines.

Figure 3.42 Pseudocarcinomatous hyperplasia of the skin. The epidermis shows an increase in the number of layers of the squamous epithelium. The intervening dermal soft tissue shows moderate chronic inflammation.

iii) Formation of skin warts from hyperplasia of epidermis due to human papilloma virus. iv) Pseudocarcinomatous hyperplasia of the skin. v) Intraductal epithelial hyperplasia in the breast in fibrocystic breast disease.

PATHOLOGIC FEATURES. There is enlargement of the affected organ or tissue and increase in the number of cells (Fig. 3.42). This is due to increased rate of DNA synthesis and hence increased mitoses of the cells.

METAPLASIA Metaplasia 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 (Fig. 3.43). Metaplasia is broadly divided into 2 types: epithelial and mesenchymal. A. EPITHELIAL METAPLASIA. This is the more common type. The metaplastic change may be patchy or diffuse and usually results in replacement by stronger but less wellspecialised 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. Depending upon the type epithelium transformed, two types of epithelial metaplasia are seen squamous and columnar: 1. Squamous metaplasia. This is more common. Various types of specialised 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 (Fig. 3.44).

iii) In gallbladder (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) Columnar metaplasia in Barrett's oesophagus, in which there is change of normal squamous epithelium to columnar epithelium (Fig. 3.45).

Figure 3.43 Schematic diagram showing sequential changes in uterine cervix from normal epithelium to development of carcinoma in situ. A, Normal mucus-secreting endocervical epithelium. B, Squamous metaplasia. C, Dysplastic change. D, Carcinoma in situ.

iii) Conversion of pseudostratified ciliated columnar epithelium in chronic bronchitis and bronchiectasis to columnar type. iv) 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 (Mönckeberg'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 (Fig. 3.46). 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 characterised by cellular proliferation and cytologic changes. These changes include: 1. Increased number of layers of epithelial cells 2. Disorderly arrangement of cells from basal layer to the surface layer 3. Loss of basal polarity i.e. nuclei lying away from basement membrane 4. Cellular and nuclear pleomorphism 5. Increased nucleocytoplasmic ratio

6. Nuclear hyperchromatism 7. Increased mitotic activity. The two most common examples of dysplastic changes are the uterine cervix (Fig. 3.47) 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. This concept is further discussed again in details in Chapters 8, 17, and 24. The differences between dysplasia and metaplasia are contrasted in Table 3.7.

CELLULAR AGING Old age is a concept of longevity in human beings. The consequences of aging appear after reproductive age. However, aging is distinct from mortality and disease although aged individuals are more vulnerable to disease. The average age of death of primitive man was barely 20-25 years compared to life-expectancy now which is approaching 80 years, survival being longer in women than men (3:2). About a century ago, the main causes of death were accidents and infections. But now with greater safety and sanitation, the mortality in the middle years has sufficiently declined. However, the maximum human lifespan has remained stable at about 110 years. Higher life expectancy in women is not due to difference in the response of somatic cells of the two sexes but higher mortality rate in men is attributed to violent causes and greater susceptibility to cardiovascular disease, cancer, cirrhosis and respiratory diseases, for which cigarette smoking and alcohol consumption are two most important contributory factors. In general, the life expectancy of an individual depends upon the following factors: 1. Intrinsic genetic process i.e. the genes controlling response to endogenous and exogenous factors initiating apoptosis in senility. 2. Environmental factors e.g. consumption and inhalation of harmful substances, diet, role of antioxidants etc. 3. Lifestyle of the individual such as diseases due to alcoholism (e.g. cirrhosis, hepatocellular carcinoma), smoking (e.g. bronchogenic carcinoma and other respiratory diseases), drug addiction. 4. Age-related diseases e.g. atherosclerosis and ischaemic heart disease, diabetes mellitus, hypertension, osteoporosis, Alzheimer's disease, Parkinson's disease etc.

CELLULAR BASIS With age, structural and functional changes occur in different organs and systems of the human body. Although no definitive biologic basis of

aging is established, most acceptable theory is the functional decline of non-dividing

cells such as neurons and myocytes. The following hypotheses based on investigations explain the cellular basis of aging: 1. Experimental cellular senescence. By in vitro studies of tissue culture, it has been observed that cultured human fibroblasts replicate for up to 50 population doublings and then the culture dies out. It means that in vitro there is reduced functional capacity to proliferate with age. Studies have shown that there is either loss of chromosome 1 or deletion of its long arm (1q). Alternatively it has been observed that with every cell division there is progressive shortening of telomere present at the tips of chromosomes, which in normal cell is repaired by the presence of RNA enzyme, telomerase. However, due to aging, because of inadequate presence of telomerase enzyme, lost telomere is not repaired resulting in interference in viability of cell (Fig. 3.48).

TABLE 3.7: Differences between Metaplasia and Dysplasia. Feature Metaplasia Dysplasia

i) Definition Change of one type of epithelial or mesenchymal Disordered cellular development, may be cell to another type of adult epithelial or mesen- accompanied with hyperplasia or metaplasia chymal cell

ii) Types Epithelial (squamous, columnar) and Epithelial only mesenchymal (osseous, cartilaginous)

iii) Tissues affected Most commonly affects bronchial mucosa, uterine Uterine cervix, bronchial mucosa endocervix; others mesenchymal tissues (cartilage, arteries)

iv) Cellular changes Mature cellular development Disordered cellular development (pleomorphism, nuclear hyperchromasia, mitosis, loss of polarity)

v) Natural history Reversible on withdrawal of stimulus May regress on removal of inciting stimulus, or may progress to higher grades of dysplasia or carcinoma in situ

2. Geneticcontrol in invertebrates. Clock (clk) genes responsible for controlling the rate and time of aging have been identified in lower invertebrates e.g. clk-1 gene mutation in the metazoa, Caenorhabditis elegans, results in prolonging the lifespan of the worm and slowing of some metabolic functions. 3. Diseasesof accelerated aging. Aging under genetic control in human beings is supported by the observation of high concordance in lifespan of identical twins. A heritable condition associated with signs of accelerated aging process is termed progeria and is characterised by baldness, cataracts, and coronary artery disease. Another example is Werner's syndrome, a rare autosomal recessive disease, characterised by similar features of premature aging, atherosclerosis and risk for development of various cancers. 4. Oxidative stress hypothesis (free radical-mediated injury).Currently, it is believed that aging is partly caused by progressive and reversible molecular oxidative damage due to persistent oxidative stress on the human cells. In normal cells, very small amount (3%) of total oxygen consumption by the cell is converted into reactive oxygen species. The rate of generation of reactive oxygen species is directly correlated with metabolic rate of the organisms. With aging, there is low metabolic rate with generation of toxic oxygen radicals, which fail to get eliminated causing their accumulation and hence cell damage. The underlying

mechanism appears to be oxidative damage to mitochondria. The role of antioxidant in retarding the oxidant damage has been reported in some studies.

ORGAN CHANGES IN AGING Although all organs start showing deterioration with aging, following organs show evident morphologic and functional changes: 1. Cardiovascular system: Atherosclerosis, arteriosclerosis with calcification, Mönckeberg's medial calcification, brown atrophy of heart, loss of elastic tissue from aorta and major arterial trunks causing their dilatation. 2. Nervous system: Atrophy of gyri and sulci, Alzheimer's disease, Parkinson's disease. 3. Musculoskeletal system: Degenerative bone diseases, frequent fractures due to loss of bone density, age related muscular degeneration. 4. Eyes: Deterioration of vision due to cataract and vascular changes in retina. 5. Hearing: Disability in hearing due to senility is related to otosclerosis. 6. Immune system: Reduced IgG response to antigens, frequent and severe infections. 7. Skin: Laxity of skin due to

loss of elastic tissue. 8. Cancers: As discussed later in Chapter 8, 80% of cancers occur in the age range of 50 and 80 years.

❑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 Chédiak-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, neurities and senile plaques in Alzheimer's disease are composed of neurofilaments and paired helical filaments.

2.L YSOSOMAL 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 10, 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. SER CHANGES. 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.

INTRACELLULAR ACCUMULATIONS 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. In addition, deposits of amyloid and urate are discussed separately later. 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. These are discussed in Chapter 10. 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. These pathologic states are discussed below.

FATTY CHANGE (STEATOSIS) 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, skeletal muscle, kidneys (lipoid nephrosis or minimum change disease) and other organs.

Fatty Liver Liver is the commonest 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.

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ETIOLOGY. Fatty change in the liver may result from one of the two types of causes: 1. Conditions with excess fat (hyperlipidameia), exceeding the capacity of the liver to metabolise it. 2. Liver cell damage, when fat cannot be metabolised in it. These causes are listed below: 1. Conditions with excess fat: i) Obesity ii) Diabetes mellitus iii) Congenital hyperlipidaemia 2. Liver cell damage: i) Alcoholic liver disease (most common) ii) Starvation iii) Protein calorie malnutrition iv) Chronic illnesses (e.g. tuberculosis) v) Acute fatty liver in late pregnancy vi) Hypoxia (e.g. anaemia, cardiac failure) vii) Hepatotoxins (e.g. carbon tetrachloride, chloroform, ether, aflatoxins and

other poisons) viii) Drug-induced liver cell injury (e.g. administration of methotrexate, steroids, CCl4, halothane anaesthetic, tetracycline etc) ix) Reye's syndrome PATHOGENESIS. Mechanism of fatty liver depends upon the stage at which the etiologic agent acts in the normal fat transport and metabolism. Hence, pathogenesis of fatty liver is best understood in the light of normal fat metabolism in the liver (Fig. 3.16). Lipids as free acids enter the liver cell from either of the following 2 sources:

From diet as chylomicrons (containing triglycerides and phospholipids) and as free fatty acids; and From adipose tissue as free fatty acids. Normally, besides above two sources, a small part of fatty acids is also synthesised from acetate in the liver cells. Most of free 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. While cholesterol, phospholipids and ketones are used in the body, intracellular triglycerides are converted into lipoproteins, which requires 'lipid acceptor protein'. Lipoproteins are released from the liver cells into circulation as plasma lipoproteins (LDL, VLDL). In fatty liver, intracellular accumulation of triglycerides can occur due to defect at one or more of the following 6 steps in the normal fat metabolism shown in Fig. 3.16: 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 which includes: 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; e.g. 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 teetotaller.

MORPHOLOGIC FEATURES. Grossly, the liver in fatty change 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 (Fig. 3.17). Microscopically, characteristic feature is the presence of numerous lipid vacuoles in the cytoplasm of hepatocytes. Fat in H & E stained section prepared by paraffinembedding technique appear non-staining vauloes because it is dissolved in alcohol (Fig. 3.18): i) The vacuoles are initially small and are present around the nucleus (microvesicular). ii) But with progression of the process, the vacuoles become larger pushing the nucleus to the periphery of the cells (macrovesicular). iii) At times, the hepatocytes laden with large lipid vacuoles may rupture and lipid vacuoles coalesce to form fatty cysts.

iv) Infrequently, lipogranulomas may appear consisting of collections of lymphocytes, macrophages, and some multinucleated giant cells. v) Fat can be demonstrated in fresh unfixed tissue by frozen section followed by fat stains such as Sudan dyes (Sudan III, IV, Sudan black) and oil red O. Alternatively, osmic acid which is a fixative as well as a stain can be used to demonstrate fat in the tissue.

Cholesterol Deposits Intracellular deposits of cholesterol and its esters in macrophages may occur when there is hypercholesterolaemia. This

turns macrophages into foam cells. The examples are as follows: 1. Fibrofatty plaques of atherosclerosis (Chapter 15). 2. Clusters of foam cells in tumour-like masses called xanthomas and xanthelasma.

Stromal Fatty Infiltration This form of lipid accumulation is quite different from fatty change just described. Stromal fatty infiltration is the deposition

of mature adipose cells in the stromal connective tissue in contrast to intracellular deposition of fat in the parenchymal cells in fatty change. The condition occurs most often in patients with obesity. The two commonly affected organs are the heart and the pancreas. Thus, heart can be the site for intramyocardial fatty change as well as epicardial (stromal) fatty infiltration. The presence of mature adipose cells in the stroma generally does not produce any dysfunction. In the case of heart, stromal fatty infiltration is associated with increased adipose tissue in the epicardium.

INTRACELLULAR ACCUMULATION OF PROTEINS 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 synthesised 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 hyalin in the hepatocytes is intracellular accumulation of intermediate filaments of cytokeratin and appear as amorphous pink masses.

Figure 3.17 Fatty liver. Sectioned slice of the liver shows pale yellow parenchyma with rounded borders.

Figure 3.18 Fatty liver. Many of the hepatocytes are distended with large fat vacuoles pushing the nuclei to the periphery (macrovesicles), while others show multiple small vacuoles in the cytoplasm (microvesicles).

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INTRACELLULAR ACCUMULATION OF GLYCOGEN Conditions associated with excessive accumulation of intracellular glycogen are as under: 1. In diabetes mellitus, there is intracellular accumulation of glycogen in different tissues because normal cellular uptake of glucose is impaired. 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. Best's 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. These conditions along with other similar genetic disorders are discussed in Chapter 10. PIGMENTS Pigments are coloured substances present in most living beings including humans. There are 2 broad categories of pigments: endogenous and exogenous (Table 3.3) .

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

Melanin Melanin is the brown-black, non-haemoglobin-derived 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 melanophores, 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 is present, 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: i) 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 raindrop pigmentation of the skin. ii) Focal hyperpigmentation: a) Cäfe-au-lait spots are pigmented patches seen in neurofibromatosis and Albright's syndrome. b) Peutz-Jeghers syndrome is characterised by focal peri-oral pigmentation. c) Melanosis coli is pigmentation of the mucosa of the colon. d) Melanotic tumours, both benign such as pigmented naevi (Fig. 3.19), 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. f) Dermatopathic lymphadenitis is an example of deposition of melanin pigment in macrophages of the lymph nodes draining skin lesions. iii) Generalised hypopigmentation:Albinism is an extreme degree of generalised 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. iv) 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.

Melanin-like Pigments ALKAPTONURIA. This is a rare autosomal recessive disorder in which there is deficiency of an oxidase enzyme required for break-down of homogentisic acid which then accumulates in the tissues and is excreted in the urine (homogentisic aciduria). The urine of patients of alkaptonuria, if allowed to stand for some hours in air, turns black due to oxidation of homogentisic acid. The pigment is

 TABLE 3.3: Pigments of the Body. A. ENDOGENOUS PIGMENTS 1. Melanin 2. Melanin-like pigment a. Alkaptonuria b. Dubin-Johnson syndrome 3. Haemoprotein-derived pigments i) Haemosiderin ii) Acid haematin (Haemozoin) c. Bilirubin d. Porphyrins 4. Lipofuscin (Wear and tear pigment)

B. EXOGENOUS PIGMENTS 1. Inhaled pigments 2. Ingested pigments 3. Injected pigments (Tattooing)

melanin-like and is deposited both intracellularly and intercellularly and is termed ochronosis, first described by Virchow. Most commonly affected tissues are the cartilages, capsules of joints, ligaments and tendons. DUBIN-JOHNSON SYNDROME. Hepatocytes in patients of Dubin-Johnson syndrome, an autosomal recessive form of hereditary conjugated hyperbilirubinaemia, contain melain-like pigment in the cytoplasm (Chapter 21).

Haemoprotein-derived Pigments Haemoproteins are the most important endogenous pigments derived from haemoglobin, cytochromes and their break-down products. For an understanding of disorders of haemoproteins, it is essential to have knowledge of normal iron metabolism and its transport which is described in Chapter 12. In disordered iron metabolism and transport, haemoprotein-derived pigments accumulate in the body. These pigments are haemosiderin, acid haematin (haemozoin), bilirubin, and porphyrins.

1. HAEMOSIDERIN. Iron is stored in the tissues in 2 forms: Ferritin, which is iron complexed to apoferritin and can be identified by electron microscopy. 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 break-down of senescent red cells takes place. Haemosiderin is ferric iron that can be demonstrated by Perl's stain that produces Prussian blue reaction. In this reaction, colourless potassium ferrocyanide reacts with ferric ions of haemosiderin to form deep blue ferric-ferrocyanide (Fig. 3.20). Excessive storage of haemosiderin occurs in situations when there is increased break-down 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 (Fig. 3.21): a) Localised 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. A few examples are as under : 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. 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(Systemic or Diffuse) haemosiderosis. Systemic overload with iron may result in generalised haemosiderosis. There can be two types of patterns:

Parenchymatous deposition of haemosiderin occurs in the parenchymal cells of the liver, pancreas, kidney, and heart. Reticuloendothelial deposition occurs in the liver, spleen, and bone marrow. Generalised or systemic

overload of iron may occur due to following causes: i) Increased erythropoietic activity: In various forms of chronic haemolytic anaemia, there is excessive break-down of haemoglobin and hence iron overload. The problem is further compounded by treating the condition with blood transfusions (transfusional haemosiderosis) or by parenteral iron therapy. The deposits of iron in these cases, termed as acquired haemosiderosis, are initially in reticuloendothelial tissues but may secondarily affect other organs. ii) Excessive intestinal absorption of iron: A form of haemosiderosis in which there is excessive intestinal absorption of iron even when the intake is normal, is known as idiopathic or hereditary haemochromatosis. It is an autosomal dominant disease associated with much more deposits of iron than cases of acquired haemosiderosis. It is characterised by 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. iii) Excessive dietary intake of iron: A common example of excessive intake of iron is Bantu's disease in black tribals of South Africa who conventionally brew their alcohol in cast iron pots that serves as a rich source of additional dietary iron. The excess of iron gets deposited in various organs including the liver causing pigment cirrhosis. 2. ACID HAEMATIN (HAEMOZOIN). Acid haematin or haemozoin is a haemoprotein-derived brown-black pigment containing haem iron in ferric form in acidic medium. But it differs from haemosiderin because it cannot be stained by Prussian blue (Perl's) 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. Besides, the malarial pigment can also be deposited in macrophages and

in the hepatocytes. Another variety of haematin pigment is formalin pigment formed in blood-rich tissues which have been preserved in acidic formalin solution. 3. BILIRUBIN. Bilirubin is the normal non-iron containing

pigment present in the bile. It is derived from porphyrin ring of the haem moiety of haemoglobin. Normal level of bilirubin in blood is less than 1 mg/dl. Excess of bilirubin or hyperbilirubinaemia causes an important clinical condition called jaundice. Normal bilirubin metabolism and pathogenesis of jaundice are described in Chapter 21. Hyperbilirubinaemia may be unconjugated or conjugated, and jaundice may appear in one of the following 3 ways: a) Prehepatic or haemolytic, when there is excessive destruction of red cells. b) Posthepatic or obstructive, which results from obstruction to the outflow of conjugated bilirubin. c) Hepatocellular 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. In infants, rise in unconjugated bilirubin may produce toxic brain injury called kernicterus. 4.PORPHYRINS. Porphyrins are normal pigment present in haemoglobin, myoglobin and cytochrome. Porphyria refers to an uncommon disorder of inborn abnormality of porphyrin metabolism. It results from genetic deficiency of one of the enzymes required for the synthesis of haem, resulting in excessive production of porphyrins. Often, the genetic deficiency is precipitated by intake of some drugs. Porphyrias are associated with excretion of intermediate products in the urine—delta-aminolaevulinic acid, porphobilinogen, uroporphyrin, coproporphyrin, and protoporphyrin. Porphyrias are broadly of 2 types—erythropoietic and hepatic. (a) Erythropoietic porphyrias. These have defective synthesis of haem in the red cell precursors in the bone marrow. These may be further of 2 subtypes: Congenital erythropoietic porphyria, in which the urine is red due to the presence of uroporphyrin and coproporphyrin. The skin of these infants is highly photosensitive. Bones and skin show red brown discolouration. Erythropoietic protoporphyria, in which there is excess of protoporphyrin but no excess of porphyrin in the urine. (b) Hepatic porphyrias. These are

more common and have a normal erythroid precursors but 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. Porphyria cutanea tarda is the most common of all porphyrias. Porphyrins collect in the liver and small quantity is excreted in the urine. Skin lesions are similar to those invariegate porphyria. Most of the patients have associated haemosiderosis with cirrhosis which may eventually develop into hepatocellular carcinoma. □□ □□ □ Mixed (Variegate) porphyrias. It is rare and combines skin photosensitivity with acute abdominal and neurological manifestations.

Lipofuscin (Wear and Tear Pigment) Lipofuscin or lipochrome is yellowish-brown intracellular lipid pigment (lipo = fat, fuscus = brown). The pigment is often found in atrophied cells of old age and hence the name 'wear and tear pigment'. It is seen in the myocardial fibres, hepatocytes, Leydig cells of the testes and in neurons in senile dementia. However, the pigment may, at times, accumulate rapidly in different cells in wasting diseases unrelated to aging.

By light microscopy, the pigment is coarse, golden-brown granular and 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' (Fig. 3.22). The pigment can be stained by fat stains but differs from other lipids in being fluorescent and having acid-fastness.

By electron microscopy, lipofuscin appears as intralysosomal electron-dense granules in perinuclear location. These granules are composed of lipid-protein complexes. Lipofuscin represents the collection of indigestible material in the lysosomes after intracellular lipid peroxidation and is therefore an example of residual bodies. Unlike in normal cells, in aging or

debilitating diseases the phospholipid endproducts of membrane damage mediated by oxygen free radicals fail to get eliminated and hence are deposited as lipofuscin pigment.

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

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 (Chapter 17). 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 (Fig. 3.23). 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 colouration 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 pigmentis 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. MORPHOLOGY OF 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. autolysis, 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.

AUTOLYSIS Autolysis (i.e. 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), but the term is generally used for 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.

NECROSIS Necrosis is defined as a localised area of death of tissue followed by degradation of tissue by hydrolytic enzymes liberated from dead cells; it is invariably accompanied by inflammatory reaction. Necrosis can be caused by various agents such as hypoxia, chemical and physical agents, microbial agents, immunological injury, etc. Two essential changes characterise irreversible cell injury in necrosis of all types (Fig. 3.24,A): i)Cell digestion by lytic enzymes. Morphologically this change is identified as homogeneous and intenselyeosinophilic cytoplasm. Occasionally, it may show cytoplasmic vacuolation or dystrophic calcification. ii) Denaturation of

proteins. This process is morphologically seen as characteristic nuclear changes in necrotic cell. These nuclear changes may include: condensation of nuclear chromatin (pyknosis) which may either undergo dissolution (karyolysis) or fragmentation into many granular clumps (karyorrhexis).

Types of Necrosis Morphologically, there are five types of necrosis: coagulative, liquefaction (colliquative), caseous, fat, and fibrinoid necrosis. 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. 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. But cell digestion and liquefaction fail to occur (c.f. liquefaction necrosis). Eventually, the necrosed focus is infiltrated by inflammatory cells and the dead cells are phagocytosed leaving granular debris and fragments of cells (Fig. 3.25).

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

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 (Fig. 3.26).

3. CASEOUS NECROSIS. 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 cheese 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 (Fig. 3.27). 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.

4. 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 extraabdominal adipose tissue.

Fat necrosis hydrolyses neutral fat present in adipose cells into glycerol and free fatty acids. The damaged adipose cells assume cloudy appearance. The leaked out free fatty acids 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 5. FIBRINOID NECROSIS. Fibrinoid necrosis 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 diseases, Arthus reaction etc), arterioles in hypertension, peptic ulcer etc.

Microscopically, fibrinoid necrosis is identified by brightly eosinophilic, hyaline-like deposition in the vessel wall. Necrotic focus is surrounded by nuclear debris of neutrophils (leucocytoclasis) (Fig. 3.29). Local haemorrhage may occur due to rupture of the blood vessel.

APOPTOSIS

Apoptosis is a form of 'coordinated and internally programmed cell death' having significance in a variety of physiologic and pathologic conditions (apoptosis is a Greek

Figure 3.26 Liquefactive necrosis brain. The necrosed area on right side of the field shows a cystic space containing cell debris, while the surrounding zone shows granulation tissue and gliosis.

Figure 3.27 Caseous necrosis lymph node. There is eosinophilic, amorphous, granular material, while the periphery shows granulomatous inflammation.

word meaning 'falling off' or 'dropping off'). The term was first introduced in 1972 as distinct from necrosis by being a form of cell death which is controlled and regulated by the rate of cell division; when the cell is not needed, pathway of cell death is activated ('cell suicide') and is unaccompanied by any inflammation and collateral tissue damage. 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. Organised 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. Progressive depletion of CD4+T cells in the pathogenesis of AIDS. 4. Cell death in viral infections e.g. formation of Councilman bodies in viral hepatitis. 5. 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. 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.

8. Heart diseases e.g. heart failure, acute myocardial infarction (20% necrosis and 80% apoptosis).

MORPHOLOGIC FEATURES. The characteristic morphologic changes in apoptosis seen in histologic and electron microscopic examination are as under 1. Involvement of single cells or small clusters of cells in the background of viable cells. 2. The apoptotic cells are round to oval shrunken masses of intensely eosinophilic cytoplasm (mummified cell) containing shrunken or almost-normal organelles (Fig. 3.30).

3. The nuclear chromatin is condensed or fragmented (pyknosis or karyorrehexis). 4. The cell membrane may show convolutions or projections on the surface. 5. There may be formation of membrane-bound nearspherical bodies on or around the cell called apoptotic bodies containing compacted organelles. 6. Characteristically, unlike necrosis, there is no acute inflammatory reaction around apoptosis. 7. 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.

Techniques to identify and count apoptotic cells. In addition to routine H & E stain, apoptotic cells can be identified and counted by following methods: 1. Staining of chromatin condensation (haematoxylin, Feulgen, 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. 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. The contrasting features of apoptosis and necrosis are illustrated in Fig. 3.24 and summarised in Table 3.4. 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. Triggers for signalling programmed cell death act at the cell membrane, either intracellularly or extracellularly. These include the following: i) Withdrawal of signals required for normal cell survival (e.g. absence of certain hormones, growth factors, cytokines). ii) Extracellular signals triggering of programmed cell death (e.g. activation of FAS receptor belonging to TNF-R family). iii) Intracellular stimuli e.g. heat, radiation, hypoxia etc. 2. Process of programmed cell death. After the cell hasbeen initiatedinto self-destruct

mode, the programme inbuilt in the cell gets activated as under: i) Activation of caspases. Caspases are a series of proteolyitc or protein-splitting enzymes which act on nuclear proteins and organelles containing protein components. The term 'caspase' is derived from: c for cystein protease; asp for aspartic acid; and ase is used for naming an enzyme. Caspases get activated either by coming in contact with some etiologic agent of cell injury agent or by unknown mechanism. ii) Activation of death receptors. Activated caspases set in activation of FAS receptor (CD 95), a cell surface receptor present on cytotoxic (CD 8+) T cells, belonging to the family of tumour necrosis factor receptors (TNF-R). FAS receptor is appropriately called a death receptor because on coming in contact with the specific binding site on the target cell, it activates specific growth controlling genes, BCL-2 and p53. iii) Activation of growth controlling genes (BCL-2 and p53). BCL2 gene is a human counterpart of CED-9 (cell death) gene

Feature Apoptosis Necrosis

1. Definition Programmed and coordinated cell death Cell death along with degradation of tissue by hydrolytic enzymes

2. Causative agents Physiologic and pathologic processes Hypoxia, toxins

3. Morphology i) No Inflammatory reaction i) Inflammatory reaction always present ii) Death of single cells ii) Death of many adjacent cells iii) Cell shrinkage iii) Cell swelling initially iv) Cytoplasmic blebs on membrane iv) Membrane disruption v) Apoptotic bodies v) Damaged organelles vi) Chromatin condensation vi) Nuclear disruption vii) Phagocytosis of apoptotic bodies by macrophages vii) Phagocytosis of cell debris by macrophages

4. Molecular changes i) Lysosomes and other organelles intact i) Lysosomal breakdown with liberation of ii) Genetic activation by proto-oncogenes hydrolytic enzymes and oncosuppressor genes, and cytotoxic ii) Cell death

by ATP depletion, membrane T cell-mediated target cell killing damage, free radical injury iii) Initiation of apoptosis by intra- and extracellular stimuli, followed by activation of caspase pathway (FAS-R, BCL-2, p53)

found in programmed cell death of nematode worm Caenorabditis elegans. BCL-2 gene family is located in the outer mitochondrial membrane and includes both activators and inhibitors of apoptosis. Thus, it may regulate the apoptotic process by binding to some related proteins (e.g. to BAX and BAD) for promoting apoptosis, or to BCL-XL for inhibiting apoptosis. The net effect on the mitochondrial membrane is thus based on the pro-apoptotic and anti-apoptotic actions of BCL-2 gene family. Besides BCL-2, the apoptotic pathway is partly also governed by p53 molecule which promotes apoptosis. iv) Cell death. The above mechanisms lead to proteolytic actions on nucleus, chromatin clumping, cytoskeletal damage, disruption of endoplasmic reticulum, mitochondrial damage, and disturbed cell membrane. 3. Phagocytosis. The dead apoptotic cells develop membrane changes which promote their phagocytosis. Phosphatidylserine and thrombospondin molecules which are normally present on the inside of the cell membrane, appear on the outer surface of the cells in apoptosis, which facilitate their identification by adjacent phagocytes and promotes phagocytosis. The phagocytosis is unaccompanied by any other inflammatory cells. The mechanism of apoptosis is schematically represented in Fig. 3.31.

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 the two are produced, is different. The examples of necrotising inflammation are:

gangrenous appendicitis, gangrenous stomatitis (noma, cancrum oris). There are 2 main forms of gangrene—dry and wet, and a variant form of wet gangrene called gas gangrene. In all types of gangrene, 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 of dry gangrene foot 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.

MORPHOLOGIC FEATURES. Grossly, 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 (H2S) produced by bacteria resulting in formation 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. The line of separation consists of inflammatory granulation tissue.

Wet Gangrene Wet gangrene 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 ofvenous, 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 profound systemic manifestations of septicaemia, and finally death. The spreading wet gangrene generally lacks clear-cut line of demarcation and may spread to peritoneal cavity causing peritonitis.

MORPHOLOGIC FEATURES. Grossly, 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.

Contrasting features of two main forms of gangrene are summarised in Table 3.5. GAS GANGRENE. It 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.

PATHOLOGIC CALCIFICATION Deposition of calcium salts in tissues other than osteoid or enamel is called pathologic or heterotopic calcification. Two 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. Etiology and pathogenesis of the two are different but morphologically the deposits in both 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 intracellular, 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 in Perl's stain.

Feature Dry Gangrene Wet Gangrene

1. Site Commonly limbs More common in bowel 2. Mechanisms Arterial occlusion More commonly venous obstruction, less often arterial occlusion 3. Macroscopy Organ dry, shrunken and black Part moist, soft, swollen, rotten and dark 4. Putrefaction Limited due to very little blood Marked due to stuffing of organ with blood supply 5. Line of demarcation Present at the junction between No clear line of demarcation healthy and gangrenous part 6. Bacteria Bacteria fail to survive Numerous present 7. Prognosis Generally better due to little septicaemia Generally poor due to profound toxaemia

Etiopathogenesis The two types of pathologic calcification result from distinctly different etiologies and mechanisms. DYSTROPHICCALCIFICATION. 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. 3.36). 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. Gamna-Gandy bodies in chronic venous congestion (CVC) of the spleen is characterised by calcific deposits admixed with haemosiderin on fibrous tissue. 5. Infarcts may sometimes undergo dystrophic calcification. 6. Thrombi, especially in the veins, may produce phleboliths. 7. Haematomas in the vicinity of bones may undergo dystrophic calcification. 8. Dead parasites like in hydatid cyst, Schistosoma eggs, and cysticercosis are some of the examples showing dystrophic calcification. 9. Calcification in breast cancer detected by mammography. 10. 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. Mönckeberg's sclerosis shows calcification in the tunica

media of muscular arteries in elderly people (Chapter 15) (Fig.3.37) . 4. Stroma of tumours such as uterine fibroids, breast cancer, thyroid adenoma, goitre etc show calcification. 5. 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. 6. Cysts which have been present for a long time may show calcification of their walls e.g. epidermal and pilar cysts. 7. Calcinosis cutis is a condition of unknown cause in which there are irregular nodular deposits of calcium salts in the skin and subcutaneous tissue. 8. 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.

Pathogenesis of dystrophic calcification. It is not quite clear as to how dystrophic calcification takes place. Since serum calcium levels are within

normal limits, the denatured proteins in necrotic or degenerated tissue bind phosphate ions, which react with calcium ions to form precipitates of calcium phosphate. 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 precipitates of calcium phosphate 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 parathyroid 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. Sites of metastatic calcification. Metastatic calcification may occur in any normal tissue of the body but affects the following organs more commonly: 1. Kidneys, especially at the basement membrane of tubular epithelium and in the tubular lumina causing nephrocalcinosis (Fig.3.38). 2. Lungs, especially in the alveolar walls.

3. Stomach, on the acid-secreting fundal glands. 4. Blood vessels, especially on the internal elastic lamina. 5. Cornea is another site affected by metastatic calcification. 6. Synovium of the joint causing pain and dysfunction. Pathogenesis of metastatic calcification. Metasatic calcification at the above-mentioned sites occurs due to excessive binding of inorganic phosphate ions with calcium ions, which are elevated due to underlying metabolic derangement. This leads to formation of precipitates of calcium phosphate at the preferential sites. Metastatic calcification is reversible upon correction of underlying metabolic disorder. The distinguishing features between the two types of pathologic calcification are summarised inTable 3.6. CELLULAR ADAPTATIONS For the sake of survival on exposure to stress, the cells make adjustments with the changes in their environment (i.e. adapt) to the physiologic needs (physiologic adaptation) and to non-lethal pathologic injury (pathologic adaptation). Broadly speaking, such physiologic and pathologic adaptations occur by following processes (Fig. 3.39): Decreasing or increasing their size i.e. atrophy and hypertrophy respectively, or by increasing their number i.e.

hyperplasia (postfix word -trophy means nourishment; -plasia means growth of new cells). Changing the pathway of phenotypic differentiation of cells i.e. metaplasia and dysplasia (prefix word meta- means transformation; dys- means bad development). 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 may occur in sustained atrophy; dysplasia may progress 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 the following: Altered cell surface receptor binding. Alterations in signal for protein synthesis. Synthesis of new proteins by the target cell such as heatshock proteins (HSPs). Common

forms of cellular adaptive responses along with examples of physiologic and pathologic adaptations are briefly discussed below (Fig. 3.39).

□ □ TABLE 3.6: Differences between Dystrophic and Metastatic Calcification. Feature Dystrophic Calcification Metastatic Calcification

1. Definition Deposits of calcium salts in dead and Deposits of calcium salts in normal tissues degenerated tissues

2. Calcium metabolism Normal Deranged

3. Serum calcium level Normal Hypercalcaemia

4. Reversibility Generally irreversible Reversible upon correction of metabolic disorder

5. Causes Necrosis (caseous, liquefactive, fat), Hyperparathyroidism (due to adenoma, infarcts, thrombi, haematomas, dead hyperplasia, CRF), bony destructive lesions parasites, old scars, atheromas, (e.g. myeloma, metastatic carcinoma), Mönckeberg's sclerosis, certain prolonged immobilisation, hypervitaminosis D, tumours, cysts, calcinosis cutis milk-alkali syndrome, hypercalcaemia of infancy

6. Pathogenesis Increased binding of phosphates with Increased precipitates of calcium phosphate due to necrotic and degenerative tissue, which hypercalcaemia at certain sites e.g. in lungs, stomach, in turn binds to calcium forming blood vessels and cornea calcium phosphate precipitates

ATROPHY Reduction of the number and size of parenchymal cells of an organ or its parts which was once normal is called atrophy (compared from hypoplasia which is the term used for developmentally small size, and aplasia for extreme failure of development so that only rudimentary tissue is present). CAUSES. 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 with aging. B. Pathologic atrophy. The causes are as under: 1. Starvation atrophy. 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. 2. 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. 3. 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. 4. Neuropathic atrophy. Interruption in nerve supply leads to wasting of muscles e.g. i) Poliomyelitis ii) Motor neuron disease iii) Nerve section. 5. 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. 6. Pressure atrophy. Prolonged pressure from benign tumours or cyst or aneurysm may cause compression and atrophy of the tissues e.g. i) Erosion of spine by tumour in nerve root. ii) Erosion of skull by meningioma arising from piaarachnoid. iii) Erosion of sternum by aneurysm of arch of aorta. 7. Idiopathic atrophy. There are some examples of atrophy where no obvious cause is present e.g. i) Myopathies. ii) Testicular atrophy.

MORPHOLOGIC FEATURES. 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 are not dead cells. 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 (Fig. 3.40). These autophagic vacuoles may persist to form 'residual bodies' in the cell cytoplasm e.g. lipofuscin pigment granules in brown atrophy (page 43).

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. 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 nondividing cells too, only hypertrophy occurs. A. Physiologic hypertrophy. Enlarged size of the uterus in pregnancy is an excellent example of physiologic hypertrophy as well as hyperplasia. B. Pathologic hypertrophy. Examples of certain diseases associated with hypertrophy are as under: 1. Hypertrophy of cardiac muscle may occur in a number of cardiovascular diseases. A few conditions producing left ventricular hypertrophy are as under: i) Systemic hypertension ii) Aortic valve disease (stenosis and insufficiency) iii) Mitral insufficiency 2. Hypertrophy of smooth muscle e.g. i) Cardiac achalasia (in oesophagus) ii) Pyloric stenosis (in stomach)

iii) Intestinal strictures iv) Muscular arteries in hypertension. 3. Hypertrophy of skeletal muscle e.g. hypertrophied muscles in athletes and manual labourers. 4. Compensatory hypertrophy may occur in an organ when the contralateral organ is removed e.g. 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.

MORPHOLOGIC FEATURES. 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 (Fig. 3.41). 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. All body cells do not possess hyperplastic growth potential (Chapter 6). 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 is present. CAUSES. As with other non-neoplastic adaptive disorders of growth, hyperplasia has also been divided into physiologic and pathologic. 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 menstrual 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 nephrectomy 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. v) Intraductal epithelial hyperplasia in the breast in fibrocystic breast disease.

PATHOLOGIC FEATURES. There is enlargement of the affected organ or tissue and increase in the number of cells (Fig. 3.42). This is due to increased rate of DNA synthesis and hence increased mitoses of the cells.

METAPLASIA Metaplasia 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 (Fig. 3.43). Metaplasia is broadly divided into 2 types: epithelial and mesenchymal. A. EPITHELIAL METAPLASIA. This is the more common type. The metaplastic change may be patchy or diffuse and usually results in replacement by stronger but less wellspecialised 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. Depending upon the type epithelium transformed, two types of epithelial metaplasia are seen squamous and columnar: 1. Squamous metaplasia. This is more common. Various types of specialised 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 (Fig. 3.44).

iii) In gallbladder (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) Columnar metaplasia in Barrett's oesophagus, in which there is change of normal squamous epithelium to columnar epithelium (Fig. 3.45).

iii) Conversion of pseudostratified ciliated columnar epithelium in chronic bronchitis and bronchiectasis to columnar type. iv) 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 (Mönckeberg'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 (Fig. 3.46). 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 characterised by cellular proliferation and cytologic changes. These changes include: 1. Increased number of layers of epithelial cells 2. Disorderly arrangement of cells from basal layer to the surface layer

3. Loss of basal polarity i.e. nuclei lying away from basement membrane 4. Cellular and nuclear pleomorphism 5. Increased nucleocytoplasmic ratio

6. Nuclear hyperchromatism 7. Increased mitotic activity. The two most common examples of dysplastic changes are the uterine cervix (Fig. 3.47) 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. This concept is further discussed again in details in Chapters 8, 17, and 24. The differences between dysplasia and metaplasia are contrasted in Table 3.7.

AdaptationsCELLULAR AGING Old age is a concept of longevity in human beings. The consequences of aging appear after reproductive age. However, aging is distinct from mortality and disease although aged individuals are more vulnerable to disease. The average age of death of primitive man was barely 20-25 years compared to life-expectancy now which is approaching 80 years, survival being longer in women than men (3:2). About a century ago, the main causes of death were accidents and infections. But now with greater safety and sanitation, the mortality in the middle years has sufficiently declined. However, the maximum human lifespan has remained stable at about 110 years. Higher life expectancy in women is not due to difference in the response of somatic cells of the two sexes but higher mortality rate in men is attributed to violent causes and greater susceptibility to cardiovascular disease, cancer, cirrhosis and respiratory diseases, for which cigarette smoking and alcohol consumption are two most important contributory factors. In general, the life expectancy of an individual depends upon the following factors: 1. Intrinsic genetic process i.e. the genes controlling response to endogenous and exogenous factors initiating apoptosis in senility. 2. Environmental factors e.g. consumption and inhalation of harmful substances, diet, role of

antioxidants etc. 3. Lifestyle of the individual such as diseases due to alcoholism (e.g. cirrhosis, hepatocellular carcinoma), smoking (e.g. bronchogenic carcinoma and other respiratory diseases), drug addiction. 4. Age-related diseases e.g. atherosclerosis and ischaemic heart disease, diabetes mellitus, hypertension, osteoporosis, Alzheimer's disease, Parkinson's disease etc.

CELLULAR BASIS With age, structural and functional changes occur in different organs and systems of the human body. Although no definitive biologic basis of aging is established, most acceptable theory is the functional decline of non-dividing

cells such as neurons and myocytes. The following hypotheses based on investigations explain the cellular basis of aging: 1. Experimental cellular senescence. By in vitro studies of tissue culture, it has been observed that cultured human fibroblasts replicate for up to 50 population doublings and then the culture dies out. It means that in vitro there is reduced functional capacity to proliferate with age. Studies have shown that there is either loss of chromosome 1 or deletion of its long arm (1q). Alternatively it has been observed that with every cell division there is progressive shortening of telomere present at the tips of chromosomes, which in normal cell is repaired by the presence of RNA enzyme, telomerase. However, due to aging, because of inadequate presence of telomerase enzyme, lost telomere is not repaired resulting in

: Differences between Metaplasia and Dysplasia. Feature Metaplasia Dysplasia

i) Definition Change of one type of epithelial or mesenchymal Disordered cellular development, may be cell to another type of adult epithelial or mesen- accompanied with hyperplasia or metaplasia chymal cell

ii) Types Epithelial (squamous, columnar) and Epithelial only mesenchymal (osseous, cartilaginous)

iii) Tissues affected Most commonly affects bronchial mucosa, uterine Uterine cervix, bronchial mucosa endocervix; others mesenchymal tissues (cartilage, arteries)

iv) Cellular changes Mature cellular development Disordered cellular development (pleomorphism, nuclear hyperchromasia, mitosis, loss of polarity)

v) Natural history Reversible on withdrawal of stimulus May regress on removal of inciting stimulus, or may progress to higher grades of dysplasia or carcinoma in situ

2. Geneticcontrol in invertebrates. Clock (clk) genes responsible for controlling the rate and time of aging have been identified in lower invertebrates e.g. clk-1 gene mutation in the metazoa, Caenorhabditis elegans, results in prolonging the lifespan of the worm and slowing of some metabolic functions. 3. Diseasesof accelerated aging. Aging under genetic control in human beings is supported by the observation of high concordance in lifespan of identical twins. A heritable condition associated with signs of accelerated aging process is termed progeria and is characterised by baldness, cataracts, and coronary artery disease. Another example is Werner's syndrome, a rare autosomal recessive disease, characterised by similar features of premature aging, atherosclerosis and risk for development of various cancers. 4. Oxidative stress hypothesis (free radical-mediated injury).Currently, it is believed that aging is partly caused by progressive and reversible molecular oxidative damage due to persistent oxidative stress on the human cells. In normal cells, very small amount (3%) of total oxygen consumption by the cell is converted into reactive oxygen species. The rate of generation of reactive oxygen species is directly correlated with metabolic rate of the organisms. With aging, there is low metabolic rate with generation of toxic oxygen radicals, which fail to get eliminated causing their accumulation and hence cell damage.