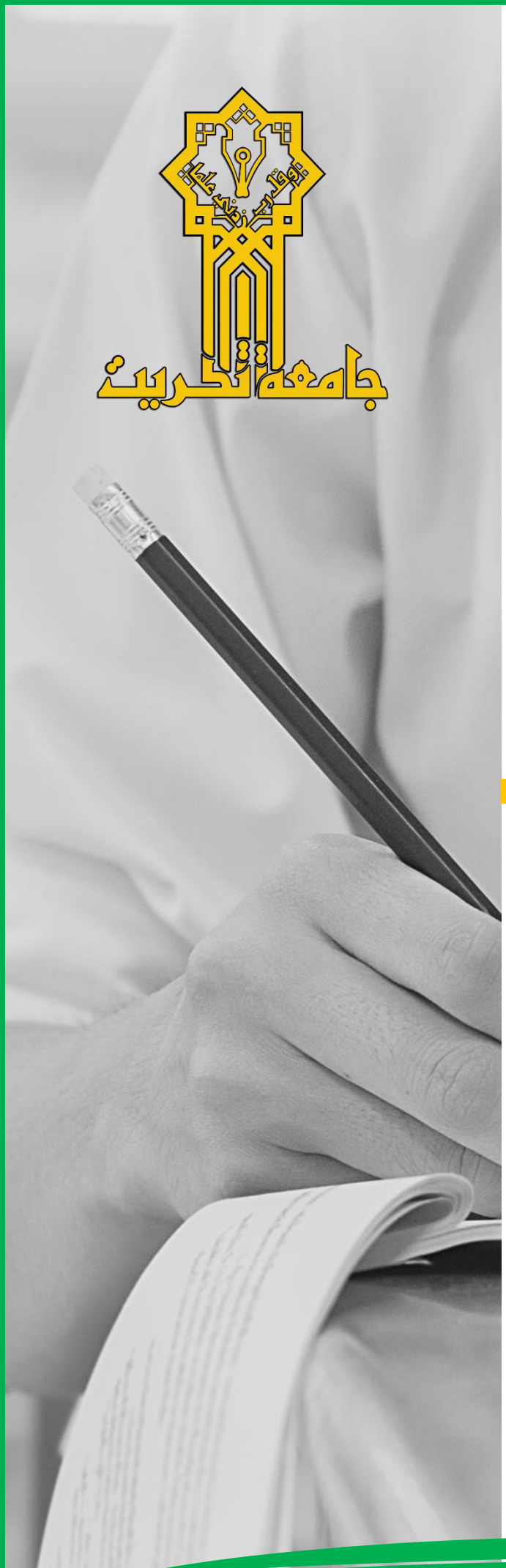

Tikrit University
College of Veterinary Medicine

NECROSIS &APOPTOSIS

Subject name: Pathology

Subject year: Third

Lecturer name: Assist. Prof. Hassan Hadi Khorsheed

AcademicEmail:dr_patho80@tu.edu.iq

SCAN ME
Lecturers link

Irreversible cell injury

There are two morphological types of cell death

1. Apoptosis

2. Necrosis

Apoptosis is an active, energy-dependent, regulated type of cell death**.** It serves many normal functions and is not necessarily pathological is the mode of cell death when

a. The cell is deprived of its growth factors or

b. The cell's DNA or proteins are damaged beyond repair

Necrosis is a reflection of the morphological changes that accompany cell death due to the digestion of cellular contents by the liberated degradative lysosomal enzymes. It is associated with an inflammatory response (unlike apoptosis), due to leakage of the cellular contents through the damaged plasma membrane. The lysosomes of the inflammatory cells also contribute to the digestion of the dying cells. Necrosis is the major pathway of cell death in many commonly encountered injuries, such as those resulting from ischemia, exposure to toxins, & various infections.

Morphologic features of necrosis: these consists of cytoplasmic & nuclear changes

Cytoplasmic changes: the necrotic cells show increased cytoplasmic eosinophilia i.e. it appears deep pink in color than normal cells. This is attributable in part to increased binding of eosin to denatured cytoplasmic proteins and in part to loss of the basophilia that is normally imparted by the RNA in the cytoplasm (basophilia is the blue staining from the hematoxylin dye). The cell may have a more homogeneous appearance than viable cells, mostly because of the loss of glycogen particles. The latter is responsible in normal cells for the granularity of the cytoplasm.

Nuclear changes: these assume one of three patterns, all due to breakdown of DNA and chromatin

a. Karyolysis i.e. the basophilia of the chromatin become pale secondary to deoxyribonuclease (DNase) activity

b. Pyknosis characterized by nuclear shrinkage and increased basophilia; the DNA condenses into a solid shrunken mass.

c. Karyorrhexis**,** the pyknotic nucleus undergoes fragmentation.

Patterns of Tissue Necrosis

There are several morphologically patterns of tissue necrosis, which may provide clues about the underlying cause.

1. Coagulative necrosis is characterized grossly by firmness of the affected tissue due to denaturation of structural proteins and microscopically by loss of the cellular fine structural details but preservation of the basic tissue architecture.

The necrotic cells show homogeneously eosinophilic cytoplasm and are devoid of nuclei. Ultimately the crotic cells are removed through the degradtative enzymes released from both the dead cells themselves as well as from the already present inflammatory cells. The latter also contribute by phagocytosing the cellular debris. Coagulative necrosis is characteristic of ischemic damage in all solid organs except the brain.

2. Liquefactive necrosis is characterized by complete digestion of the dead cells, resulting in transformation of the affected tissue into thick liquid mass (hence the name liquefactive). Eventually, the liquefied necrotic tissue is enclosed within a cystic cavity. This type of necrosis is seenin two situations

a. Focal pyogenic bacterial infections. These bacteria stimulate the the accumulation of inflammatory cells & the enzymes of leukocytes digest ("liquefy") the tissue. This process is associated acute suppurative inflammation (abscess); the liquefied material is frequently creamy yellow and is called pus.

b. Ischemic destruction of the brain tissue: for unclear reasons, hypoxic death of cells within the central nervous system often induces liquefactive necrosis.

3. Gangrenous necrosis (**gangrene)** is not a distinctive pattern of cell death; however, the term is still commonly used in clinical practice. It is usually applied to a limb, usually a leg that has lost its blood supply and has undergone coagulative necrosis involving multiple tissue layers (dry gangrene). When bacterial infection is superimposed, coagulative necrosis is modified by the liquefactive action of the bacteria and the attracted leukocytes (wet gangrene). Intestinal gangrene (the consequences of mesenteric vascular occlusion) and gangrenous appendix are also commonly used terms; they signify ischemic necrosis of these structures with superimposed bacterial infection.

4. Caseous necrosis, unlike coagulative necrosis, the tissue architecture is completely lost and cellular outlines cannot be detected. It is encountered most often in foci of tuberculous infection. The term "caseous" (cheese-like) is derived from the friable yellow-white appearance of the area of necrosis .

Microscopically, the necrotic focus appears as pinkish, and granular in appearance. Caseous necrosis is often bordered by a granulomatous inflammation.

5. Fat necrosis is typically seen in acute pancreatitis and results from release of activated pancreatic lipases into the pancreas and the peritoneal cavity. Pancreatic enzymes that have leaked out of acinar cells liquefy the membranes of fat cells in and outside the pancrease. Lipases split the liberated triglycerides into fatty acids that combine with calcium to produce grossly visible chalky white areas . **Microscopically**,

the foci of necrosis contain vague outlines of necrotic fat cells with bluish calcium deposits. The necrotic foci are surrounded by an inflammatory reaction. Another

example of fat necrosis is seen in female breasts; at least some of these cases are preceded by a history of trauma (traumatic fat necrosis).

6. Fibrinoid necrosis is typically seen in immune reactions involving blood vessels. Deposits of immune complexes, together with fibrin that has leaked out of vessels result in a homogeneous bright pink appearance. This type is exemplified by the necrosis seen in polyarteritis nodosa . The leakage of intracellular proteins through the damaged cell membrane and ultimately into the blood provides means of detecting necrosis of specific tissues using blood or serum samples. The measurement of
the levels of these specific enzymes in the serum is used clinically to assess damage to these tissues. Cardiac muscles contain a unique enzyme creatine kinase (CK) and the contractile protein troponin. The serum levels of both are elevated after acute myocardial infarction. Hepatocytes contain transaminases & these are elevated in the serum following an episode of hepatitis (viral or otherwise).

SUBCELLULAR RESPONSES TO INJURY

Some of these alterations occur in acute lethal injury, others in chronic cell injury, and still others are adaptive responses.

Autophagy refers to lysosomal digestion of the cell's own components. It is a survival mechanism whenever there is nutrient deprivation; the starved cell lives by eating its own contents. In this process, organelles are sequestered from the cytoplasm in an autophagic vacuole. The vacuole fuses with lysosomes to form phagolysosome, & the cellular components are digested by lysosomal enzymes. Lysosomes with undigested debris may persist within cells. Lipofuscin pigment is indigestible material; it is seen as brownish-yellow granules within parenchymal cells e.g. of the liver & heart in old age & in atrophy of these organs. Carbon particles inhaled from the atmosphere and inoculated pigment in tattoos can persist in phagolysosomes of
macrophages for decades. In hereditary lysosomal storage diseases there are deficiencies of enzymes that degrade certain macromolecules; the result is an abnormal collection of these in the lysosomes
of cells all over the body.

Induction (hypertrophy) of smooth endoplasmic reticulum (SER): the SER is involved in the metabolism of various chemicals including some drugs, and cells exposed to these chemicals show hypertrophy of the SER as an adaptive response that may have important functional consequences for e.g. induction of hepatic drug-metabolizing activity.

Mitochondrial Alterations: mitochondria may show

1. An increase in their number in cellular hypertrophy.
2. A decrease in number during cellular atrophy (probably via autophagy).
3. Extremely large and abnormal shapes (megamitochondria), e.g. in hepatocytes in association with nutritional deficiencies and alcoholic liver disease.

Cytoskeletal Abnormalities:

Cytoskeletone is the cellular scaffold, which is represented by myosin**,** intermediate filaments and microtubules Examples of cytoskeletal abnormalities are seen in

1. Alcoholic liver disease: Mallory bodies are eosinophilic, collections of intermediate filamentsd that
accumulate within the cytoplasm of hepatocytes.
2. Kartagener (immotile cilia) syndrome: due to disorganization of microtubules, which is associated with sterility due to inhibition of sperm motility? There are also chronic infections of the lung due to defective motility of cilia of the respiratory epithelium, and thus accumulation of the secreted mucus & impaired clearance of inhaled bacteria.

MECHANISMS OF CELL INJURY

The outcomes of the interaction between injurious agents & cells depend on

1. The injurious agent: its type, severity, and the duration of its application to the cells.
2. The cells exposed to the injury: its type, adaptability, and their genetic makeup.

The above are exemplified by the following facts Low doses of toxins or a brief duration of ischemia may lead to reversible cell injury, whereas larger toxin
doses or longer ischemic intervals may result in irreversible injury and cell death. The same injury has different outcomes depending on the cell type; thus, striated skeletal muscles in the leg resist complete ischemia for 2 to 3 hours without irreversible injury (as in applying a tornique to stop severe uncontrable bleeding from a limb trauma), whereas cardiac muscles die after only 20 to 30 minutes of severe acute ischemia. Individuals who inherit variants of the same gene that encod an enzyme that degrades a particular toxin show differences in the speed (rate) of toxins degredation. This explains the different outcomes that may occur when different indivuals are exposed to the same dose of a given toxin.

The most important targets of injurious agnets are

1. Mitochondria (the sites of ATP generation)
2. Cell membranes, which influence the ionic and osmotic homeostasis of the cell
3. Protein synthesis (ribosomes)
4. The cytoskeleton (microtubules, and various filaments)
5. The genetic apparatus of the cell (nuclear DNA)

ATP Depletion

ATP, the energy fuel of cells, is produced mainly by oxidative phosphorylation of ADP within the mitochondria. In addition, the glycolytic pathway can generate ATP in the absence of oxygen using glucose derived either from the circulation or from the hydrolysis of intracellular glycogen (anaerobic glycolysis).

The major causes of ATP depletion are

1. Reduced supply of oxygen and nutrients
2. Mitochondrial damage

3. The actions of some toxins (e.g., cyanide)

High-energy phosphate in the form of ATP is required for virtually all synthetic and degradative processes within the cell, including membrane transport, protein synthesis, phospholipid turnover, etc. Depletion of ATP to less than 5% to 10% of normal levels has widespread effects on many critical cellular systems. a. The activity of the plasma membrane energy-dependent sodium pump is reduced, resulting in intracellular accumulation of sodium and efflux of pot The net gain of solute is accompanied by iso-osmotic gain of water, causing cell swelling.

b. There is a compensatory increase in anaerobic glycolysis in an attempt to maintain the cell's energy sources. As a consequence, intracellular glycogen stores are rapidly depleted, and lactic acid accumulates, leading to decreased intracellular pH and decreased activity of many cellular enzymes.

c. Failure of the Ca2+ pump leads to influx of Ca2+, with damaging effects on numerous cellular components (described below).

d. Structural disruption of the protein synthetic apparatus manifested as detachment of ribosomes from the rough endoplastic reticulum (RER), with a consequent reduction in protein synthesis.

e. Ultimately, there is irreversible damage to mitochondrial and lysosomal membranes, and the cell undergoes necrosis.

Mitochondrial Damage

Mitochondria can be damaged by increases of cytosolic Ca2+, reactive oxygen species, and oxygen deprivation, and so they are sensitive to virtually all types of injurious stimuli, including hypoxia and toxins. There are two major consequences of mitochondrial damage: Failure of oxidative phosphorylation with progressive depletion of ATP, culminating in necrosis of the cell. Leakage of cytochrome c that is capable of activating apoptotic pathways.

Influx of Calcium

Cytoplasmic free calcium is normally maintained by ATP-dependent calcium pump (transporter) at concentrations that are10, 000 times lower than the concentration of extra-cellular calcium or intracellular mitochondrial and ER calcium. Ischemia and certain toxins cause an increase in cytoplasmic calcium concentration, initially because of release of Ca2+ from the intracellular stores, and later resulting from increased influx across the plasma membrane.

Increased cytosolic Ca2+ leads to

1. Activates a number of enzymes including phospholipases (which cause membrane damage), proteases (which break down both membrane and cytoskeletal proteins), endonucleases (which are responsible for DNA and chromatin fragmentation), and ATPases (worsen ATP depletion).
2. Induction of apoptosis, by direct activation of certain enzymes called caspases.

Accumulation of Oxygen-Derived Free Radicals (Oxidative Stress)

These are designated as **reactive oxygen species (ROS)** & are units with a single unpaired electron in their outer orbit. When generated in cells they

enthusiastically attack nucleic acids, cellular proteins and lipids. ROS are produced normally in cells during mitochondrial respiration and energy generation, but they are degraded and removed by cellular defense systems. When their production increases or the defense systems are ineffective, the result is an excess of these free radicals, leading to a condition called oxidative stress. Cell injury in many circumstances involves damage by free radicals; these include
Reperfusion injury
Chemical and radiation injury
Toxicity from oxygen and other gases
Cellular aging
Inflammatory cells mediated tissue injury

DEFECTS IN MEMBRANE PERMEABILITY

Early loss of selective membrane permeability leading ultimately to overt membrane damage is a consistent
feature of most forms of cell injury (except apoptosis). The plasma membrane can be damaged by ischemia,
microbial toxins, complement components-mediated lysis, etc.

Biochemical mechanisms contribute to membrane damage include: 1. Decreased phospholipid synthesis due to a fall in ATP levels. This affects all cellular membranes including mitochondrial, which worsen the loss of ATP.
2. Degradation of membrane phospholipids due to activation of intracellular phospholipases through increased levels of intracellular Ca2+.
3. Injury to cell membranes by Oxygen free radicals **(**ROS)
4. Damage to the cytoskeleton through activation of proteases by increased cytoplasmic Ca2+
5. They detergent effect of free fatty acids on membranes**.** These products result from phospholipid degradation. The most important sites of membrane damage during cell injury are the mitochondrial membrane, the plasma membrane membranes of lysosomes.

Damage to DNA & proteins

Cells have mechanisms that repair damage to DNA, but if this damage is too severe to be corrected (e.g., after radiation injury or oxidative stress), the cell initiates its suicide program and dies by apoptosis. A similar reaction is triggered by improperly folded (configured) proteins (see unfolded protein response), which may be the result of inherited mutations or through free radicals. These mechanisms of cell injury typically cause apoptosis.

EXAMPLES OF CELL INJURY AND NECROSIS

Ischemic and Hypoxic Injury

Ischemia is the most common cause of cell injury in clinical medicine.
Unlike hypoxia, in which energy generation by anaerobic glycolysis can continue, in ischemia the delivery of the substrates for glycolysis is also interfered with. Consequently, anaerobic energy generation ceases in ischemic tissues.

Therefore, ischemia injures tissues faster than does hypoxia. The fundamental biochemical abnormality in hypoxic cells that leads to cell injury is reduced intracellular
generation of ATP, as a consequence of reduced supply of oxygen.

Loss of ATP leads to the failure of many energy-dependent systems of the affected cell; these include

1. Paralysis of ion pumps (leading to cell swelling, and influx of Ca2+)
2. Depletion of glycogen stores with accumulation of lactic acid (anerobic glycolysis)
3. Reduction in protein synthesis

The functional consequences may be severe. For instance, heart muscle ceases to contract within 60 seconds of coronary occlusion. However, loss of contractility does not mean cell death. If hypoxia continues, worsening ATP depletion causes further deterioration, for e.g., in renal tubular epithelium, there is loss of
microvilli and the formation of "blebs". At this time, the entire cell and its organelles (mitochondria, ER) are markedly swollen, with increased concentrations of water, sodium, and chloride and a decreased concentration of potassium. If oxygen is restored, all of these disturbances are reversible. If ischemia persists, irreversible injury and necrosis ensue. Irreversible injury is associated with severe swelling of mitochondria, extensive damage to plasma membranes, and swelling of lysosomes. Massive influx of calcium into the cell may occur. The cell's components are progressively degraded, and there is widespread leakage of cellular enzymes into the extracellular space.

APOPTOSIS

This form of cell death is a regulated suicide program in which the relevant cells activate enzymes capable of degrading the cells' own nuclear DNA and other nuclear and cytoplasmic proteins.

The plasma membrane of the apoptotic cell remains intact, but is altered in such a way that the cell becomes avid targets for phagocytes. The dead cell is rapidly cleared before its contents have leaked out, and therefore cell death by this pathway does not elicit an inflammatory reaction in the host. Thus, apoptosis differs from necrosis; the latter is characterized by loss of membrane integrity, leakage of cellular contents, and frequently a host reaction.

Causes of Apoptosis

Apoptosis occurs in physiologic situations; it serves to eliminate potentially harmful cells and cells that are no longer useful to the wellbeing of the body.

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

Apoptosis in Physiologic Situations

Death by apoptosis is a normal phenomenon that serves to eliminate cells that are no longer needed. It is important in the following physiologic situations:

1. During embryogenesis (organogenesis and involution).
2. Involution of hormone-dependent tissues (hormone deprivation, as endometrial cell breakdown during the menstrual cycle, and regression of the lactating breast after weaning)
3. In proliferating cells, such as intestinal crypt epithelia (to maintain a constant number).
4. In cells that have served their useful purpose (as neutrophils in an acute inflammation).

Apoptosis in Pathologic Conditions

Apoptosis eliminates cells that are genetically altered or injured beyond repair without eliciting a host reaction, thus keeping the damage as restricted as possible.

Microscopic features Apoptotic cells may appear as round or oval masses with intensely eosinophilic cytoplasm. Nuclei show chromatin condensation and, ultimately fragmentation (karyorrhexis). The cells rapidly shrink, and fragment into apoptotic bodies that are composed of membrane-bound vesicles of cytoplasm and organelles. These fragments are quickly extruded and phagocytosed without eliciting an inflammatory response. The fundamental event in apoptosis is the activation of enzymes caspases that culminate in activation of nucleases with DNA degredation.

Examples of Apoptosis

1. Growth factor deprivation: hormone-sensitive cells deprived of the relevant hormone, lymphocytes that are not stimulated by antigens and cytokines, and neurons deprived of nerve growth factor die by apoptosis. These are attributable to activation of pro-apoptotic members of the Bcl-2 family.
2. DNA Damage**:** exposure of cells to radiation or cytotoxic anticancer chemotherapeutic agents & extremes of temperature induces DNA damage, and if this is too severe to be repaired it triggers apoptotic death. When DNA is damaged, the p53 protein accumulates in cells. It first arrests the cell cycle (at the G1 phase) to allow time for repair. However, if the damage is too great to be repaired successfully, p53 triggers apoptosis by stimulating synthesis of pro-apoptotic members of the Bcl-2 family. When p53 is mutated or absent (as it is in certain cancers), it is incapable of inducing apoptosis, so that cells with damaged DNA are allowed to survive. This enhances the possibility of mutations or translocations that lead to neoplastic transformation and subsequently providing the tumor cells with a growth advantage.
3. Accumulation of Misfolded Proteins

During normal protein synthesis, chaperones (escorters) in the ER control the proper folding of newly synthesized proteins, and misfolded polypeptides are targeted for proteolysis. If, however, unfolded or misfolded proteins accumulate in the ER because of inherited mutations or stresses, they induce "**ER stress**" that triggers a number of cellular responses, collectively called the **unfolded protein response**. This response activates signaling pathways that increase the

production of chaperones and retard protein translation, thus reducing the levels of misfolded proteins in the cell. However, if this response is unable to cope with the accumulation of misfolded proteins, the result is the activation of caspases that lead to apoptosis. Intracellular accumulation of abnormally folded proteins is now recognized as a feature of a number of neurodegenerative diseases, including Alzheimer, Huntington, and Parkinson diseases, and possibly type II diabetes.

4. Apoptosis of Self-Reactive Lymphocytes: lymphocytes capable of recognizing self antigens are normally produced in all individuals. If these lymphocytes encounter self antigens, the cells die by apoptosis. Failure of apoptosis of self-reactive lymphocytes is one of the causes of autoimmune diseases.

5. Cytotoxic T Lymphocyte-Mediated Apoptosis**:** cytotoxic T lymphocytes (CTLs) recognize foreign antigens presented on the surface of infected host cells and tumor cells. Upon activation, CTL granule proteases called granzymes enter the target cells. Granzymes are able to activate cellular caspases. In this way, the CTL kills target cells by directly inducing the effector phase of apoptosis.

6. Cell injury in certain infections, particularly viral infections, in which loss of infected cells is largely due to apoptotic death that may be induced by the virus (as in HIV infection) or by the host immune response (as in viral hepatitis)

7. Pathologic atrophy in parenchymal organs after duct obstruction as occurs in the pancreas, parotid gland,
and kidney.

INTRACELLULAR ACCUMULATIONS

Cells may accumulate abnormal amounts of various substances; these may be harmless or associated with
injury. The locations of these substances are either cytoplasmic within organelles (typically lysosomes), or in the nucleus. There are three main pathways of abnormal intracellular accumulations :

1. In adequate removal of a substance i.e. the metabolic rate of its removal is inadequate. An example of this type of process is fatty change in the liver.

2. Defective transport of a substance: endogenous substance accumulates because of genetic or acquired defects in its folding, packaging, transport, or secretion. Mutations may lead to accumulation of proteins
(e.g., α1-antitrypsin deficiency).

3. Failure to degrade a metabolite either because of

a. an inherited defect in an enzyme (as in **storage diseases)** or

b. the cell has neither the enzymatic machinery to degrade an abnormal exogenous substance nor the ability to transport it to other sites. Accumulations of carbon or silica particles **.**