

## CHAPTER ONE CELL INJURY

### CELL INJURY AND CELL DEATH

#### Morphologic examples of reversible injury

1. **Cellular swelling (hydropic change or vacuolar degeneration):** this is due to paralysis of energy-dependent ion pumps of the plasma membrane. This leads to influx of sodium (with water) into the cell and departure of potassium out. It is the first manifestation of almost all forms of cell injury. Microscopically, there are clear vacuoles (of water) within the cytoplasm.

#### Hydropic Change due to Ischemia

1. Loss of blood supply leads to decreased oxygen tension inside cell and results in ATP depletion. There is also loss of oxidative phosphorylation causing decreased ATP generation and failure of Na<sup>+</sup>K<sup>+</sup> pump. This leads to increased intracellular sodium and water along with increased extracellular potassium, leading to cellular swelling.
2. Activation of anaerobic pathway causes accumulation of catabolites (phosphates and lactates) causing increased osmotic load and cellular swelling.

#### Hydropic Change due to Chemical Agents

##### a. Directly Acting Chemicals

They cause increased cell membrane injury leading to cellular injury e.g. cyanide poisoning, mercuric chloride, antineoplastic drugs and antibiotics.

##### b. Indirectly Acting Chemicals

They release highly toxic and reactive free radicals causing lipid peroxidation and cell membrane damage with increased influx of sodium and water causing cellular swelling e.g. carbon tetrachloride poisoning.

**Gross Damaged organ** e.g. kidney increases in weight, becomes swollen and pale in colour.

#### Microscopic

1. It mainly involves renal tubules. Glomeruli are usually spared.

2. Tubular cells become swollen and lightly stained.
3. Small, clear vacuoles are seen in the cytoplasm, which may be water vacuoles or represent distended endoplasmic reticulum.
4. There may be granular appearance of cells due to presence of swollen mitochondria.
5. Due to swelling of cells, lumen of renal tubules becomes narrow or completely obliterated. Swollen tubules compress the microvasculature present between them.

### Ultramicroscopic

Electron microscopic changes of reversible cell injury include:

1. Plasma membrane alterations such as blebbing, blunting and loss of microvilli.
2. Mitochondrial changes include swelling and appearance of small, amorphous densities
3. Dilation of endoplasmic reticulum and intra-cytoplasmic myelin figures may be present.
4. Nuclear alteration with dis aggregation of granular and fibrillar elements.

Cells may be separated from basement membrane, nuclei pushed to one side, cells may become anucleated and there is no space between the tubules in very severe forms.

**2. Fatty change Steatosis:** this is manifested by the appearance of lipid vacuoles in the cytoplasm. It is principally encountered in cells participating in fat metabolism such as hepatocytes; as in alcoholic liver disease, malnutrition & total parenteral nutrition.

represents the intracytoplasmic accumulation of triglyceride (neutral fats) of parenchymal organs, such as: liver, myocardium and kidney.

Mechanisms : increase of free fatty acids (starvation, diabetes and chronic ethylism/alcoholism), reduction of free fatty acids oxidation (hypoxia, toxins, chronic ethylism/alcoholism), increase of esterification of free fatty acids into triglycerides (due to increased free fatty acids or reduction of their oxidation, chronic ethylism/alcoholism) and reduced export of tryglicerides due to deficiency of lipid binding apoprotein (starvation/malnutrition, toxins). Initially, fatty change does not impair the cells function, being reversible. At the beginning, the hepatocytes present small fat vacuoles in the

vicinity of the endoplasmic reticulum (liposomes) - microvesicular fatty change (photo). In the late stages, the size of the vacuoles increases pushing the nucleus to the periphery of the cell - macrovesicular fatty change. These vesicles are well delineated and optically "empty" because fat solves during tissue processing (paraffin embedding). Large vacuoles may coalesce, producing fatty cysts - which are irreversible lesions.

### **Irreversible cell injury**

There are two morphological types of cell death

1. Necrosis
2. Apoptosis

#### **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 necrotic cells are removed through the degradative 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 seen in 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 and has undergone to a limb, usually a leg that has lost its blood supply 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 pancreas. 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:**

Cytoskeleton 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 filaments 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.

## 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  $Ca^{2+}$ )
2. Depletion of glycogen stores with accumulation of lactic acid (anaerobic 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.

### **Outcomes of Necrosis**

In cases of necrosis, the damaged tissue can recover from the necrotic process if:

- the cause of the necrosis is neutralized or removed AND
- the animal remains alive during the recovery period AND
- new cells are produced to replace the necrotic cells.

### **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

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 degradation.

### 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. Inadequate 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.,  $\alpha$ 1-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 .

	. Apoptosis	Necrosis
<b>Histology</b>	Single cells	Groups of cells; disruption of tissue structure
<b>Cytology</b>	Shrunken cells Cell fragmentation (apoptotic bodies) Chromatin condensed in the periphery of nuclei Generally morphologically intact mitochondria	Generally swollen, enlarged cells Pyknotic or fragmented nuclei Dilated ER; high amplitude swelling of mitochondria Outline of the cell initially maintained
<b>Effects on Tissue</b>	No inflammation Phagocytosis by adjacent cells	Disrupted membrane permeability; leakage of cellular products into the blood  Acute inflammatory response Possible scar formation