IRREVERSIBLE CELL INJURY AND CELL DEATH

When the damaging effect on the cell severe or persists for longer time, the injury becomes irreversible, and ultimately leads to cell death "Point of no return". The *four* principal intracellular systems targeted during cell injury are the **cell membranes**, **mitochondria (ATP)**, **protein synthesis machinery**, and **DNA** (integrity of genetic apparatus).

Three distinctive molecular changes manifest in irreversible cell death:

1. *Loss of structure and functions of the plasma membrane and intracellular membranes*; The cytoplasm may contain so-called "myelin figures" which are collections of phospholipids resembling myelin sheaths that are derived from damaged cellular membranes.
2. *Mitochondrial changes* such as swelling and the inability to restore mitochondrial function (oxidative phosphorylation and ATP generation) even after the end of the original injury.
3. *Loss of DNA and chromatin* structural integrity.

Features of irreversible cell injury:

- Inability to reverse mitochondrial dysfunction (loss of oxidative phosphorylation and ATP production
- Significant damage to membrane function

CELL DEATH

Cell death will start once the injurious agent is severe and/or it affects the cell for prolonged period of time.

Pathways of cell death

Necrosis

Necrosis is a pathologic uncontrolled but passive type of cell death characterized by inflammatory responses in a pathologic condition. It follows when plasma membrane become exposed to extensive damage exposing cellular contents. This leads to morphologic changes and cell death after irreversible injury due to the effects of degradative enzymes or protein denaturation.

Enzymatic digestion of the cell may be caused by its own lysosomal enzymes (***autolysis***) or the cell is digested by proteolytic enzymes secreted from invading inflammatory cells (***heterolysis***). Necrosis may result from ischemia, toxins, infections, or chemical injury, etc.

Apoptosis

Apoptosis is programmed cell death, that may either be physiologic or pathologic. Apoptosis happens when the damage affects the cells growth factors, DNA or proteins (plasma membrane integrity remains intact) that is considered beyond repair, and the cell kills itself.

Apoptosis is an *active*, energy-dependent, tightly regulated cell death that occur in specific situations. It is most commonly physiological; i.e. programmed, which eliminates unwanted or aging cells. Sometimes apoptosis happens in cells with damaged DNA.

There are 6 major differences in Necrosis and Apoptosis.

Cause of death:	Necrosis is always pathological (infections, heat, or lack of blood, etc).
Cell Size	Necrotic cell size increases while apoptotic cell shrinks.
Nucleus	Necrotic cell nucleus undergo pyknosis, fragmentation and karyolysis. Apoptotic nucleus breaks into nucleosome size fragments.
Plasma membrane	Loss of plasma membrane integrity in necrosis, Intact plasma membrane in apoptosis.
Cellular contents	In necrosis there is leakage of cellular contents
Inflammation	Inflammatory response in necrosis due to rupture of plasma membrane and leakage of contents Apoptotic bodies are phagocytosed so there is no cellular material to induce inflammation.

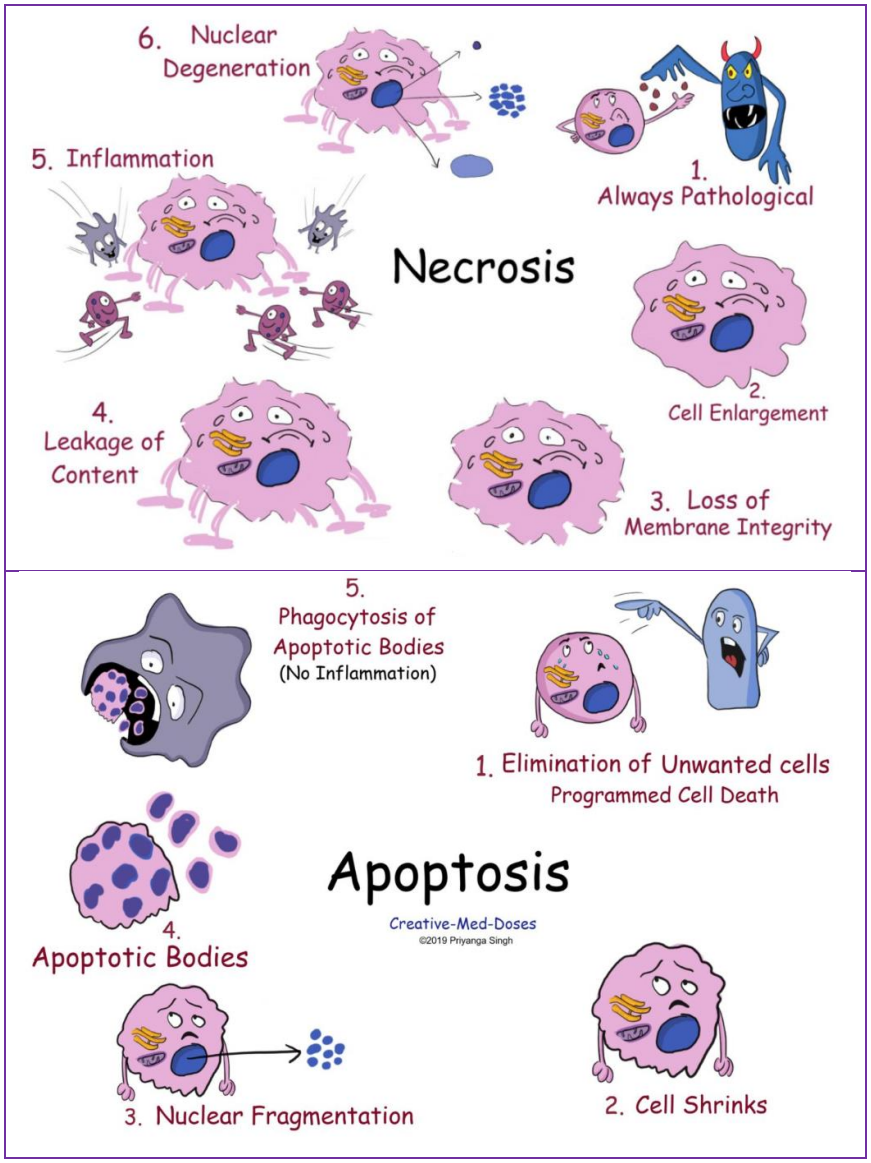


Table 1. Physiological events during apoptosis and necrosis.

		Apoptosis	Necrosis
Morpho-logical changes	Cell	Shrinkage and loss of cell-cell contacts; apoptotic cells phagocytose neighboring cells	Swelling; cell lysis
	Plasma membrane	Blebbing with intact cell integrity, formation of apoptotic bodies at late stages	Loss of integrity; increased permeability
	Organelles	No visible changes	Cell fragmentation
	Nucleus	Chromatin condensation, fragmentation	Condensation of chromatin and disintegration of the nucleus
	Mitochondrion	Decrease in membrane potential, swelling	Non-functional, swelling and fragmentation
Biochemical changes	DNA	Endonuclease-induced cleavage to fragments of specific lengths (DNA laddering)	Random degradation of genomic DNA
	Proteins	Kinases activation; phosphatases, caspases, and nucleases	Unspecific degradation
	Anti-apoptotic proteins	Bcl-2 family proteins, Inhibitor of Apoptosis Proteins (IAPs), caspase inhibitors	Bcl-2 expression in some cases
	Energy	ATP-dependent	ATP-independent
Tissue response		Restricted to individual cells Induced by physiological changes (e.g., Ca++, free radicals, hormones, toxins, depletion of growth factors) Usually no inflammatory response	Necrosis often affects groups of cells rather than a single cell Induced by external and internal pathological conditions Enhancement of inflammatory response
Post-death clearance		Phagocytosis	Cell lysis

Morphology (microscopic feature) of irreversible cell injury

CELL DEATH BY NECROSIS

Necrosis is a series of changes that accompany cell death, resulting from the digestive action of enzymes.

It may show the following morphological features:

A. Cytoplasm changes in include

- Increased eosinphilia: due to increase binding of eosin (pink) to denatured cytoplasmic protein
- Reduced basophilia (that is normally imparted by the ribonucleic acid (RNA) in the cytoplasm) of ribosome & Glycogen depletion.

B. Nuclear change (pathgnomonic)

- Pyknosis (nuclear shrinkage with increase basophilia of the nucleus, the DNA condenses into a solid shrunken mass).
- Karyorrhexis (nuclear dust fragmentation, then the nucleus completely disappears).
- Karyolysis (nuclear loss with fading of chromatin basophilia, secondary to deoxyribonuclease (DNase) activity).

C. Calcification may occur. Dead cells may be replaced by large, phospholipid masses which then either phagocytized by other cells or degraded into fatty acids which will be calcified resulting in the appearance of calcified dead cells.

D. The cell may have a glassy and more homogeneous appearance than viable cells, due to the loss of glycogen particles.

E. The cytoplasm may become vacuolated and appears "motheaten", due to digestion of cytoplasmic organelles by digestive enzymes.

PATTERNS OF NECROSIS

1. COAGULATIVE NECROSIS

The commonest type of necrosis. Occurs in almost all solid organs EXCEPT the CNS, usually results from hypoxia of severe ischemia (infarct). Protein denaturation alters its molecular structure, thereby converting albumin into a gelatinous, transparent to opaque, firm coagulated structure.

Grossly

The tissue appears whitish-gray or red-hemorrhagic firm wedge shape area of infarction.

Histology

Preservation of the tissue architecture & cellular outline. Loss of internal details including nuclei.

Infarction

It's an ischemic necrosis caused by occlusion of either the arterial supply or the venous drainage. Coagulative necrosis is characteristic of infarcts (areas of ischemic necrosis).

2. LIQUEFACTIVE NECROSIS:

Commonly seen in ischemic necrosis (infarction) of *CNS*, in focal bacterial, sometimes fungal infections or abscess formation in pyogenic infections in *all tissues*.

Enzyme digestion & autolysis leads to protein denaturation.

Grossly

Softening & liquefaction of the necrotic tissue.

Microscopically

Complete loss of tissue architecture (no original tissue), contains necrotic debris & macrophage**.**

3. CASEOUS NECROSIS:

It is a special form of necrosis usually seen in ***tuberculosis***. But it may be seen in other lesions; therefore, it's not pathognomonic of tuberculosis.

Grossly

It is soft & yellow-white, appears as *cheese-like*.

Microscopically:

Tissue architecture is completely loss. The necrotic area appears as amorphous structure less granular eosinophilic material enclosed within an inflammatory border.

4. FAT NECROSIS

Fat necrosis has two types:

1- **Enzymatic fat necrosis**: usually follow acute pancreatitis due to release of pancreatic enzyme (lipases) causing necrosis of pancreatic tissue & releasing free fatty acid, which combine with calcium to produce grossly visible ***chalky-white areas*** **(fat saponification)**.

2- **Traumatic fat necrosis**: occur in the *breast* after trauma that caused the release of fatty acid from cells. This stimulate macrophage infiltration, which engulf fat leading to inflammation and massive fibrosis, so the lesion grossly become hard and mimic carcinoma.

5. FIBRINOID NECROSIS

It is characterized by deposition of fibrin like material in the tissue e.g. Fibrinoid necrosis of blood vessels in malignant hypertension & vasculitis**.**

Microscopically

Bright eosinophilic material seen in the wall of blood vessel or in the luminal surface of peptic ulcer. Fibrinoid necrosis (is caused by immune-mediated vascular damage). Its marked by deposition of fibrin-like proteinaceous material in arterial walls, which appears eosinophilic under light microscopy.

6. GANGRENOUS NECROSIS (Gangrene)

Gangrenous necrosis is not a specific pattern of cell death, but the term is commonly used in clinical practice. It is usually affects *a limb*, the lower leg, that has *lost its blood supply* and has undergone necrosis (typically *coagulative necrosis*) involving multiple tissue planes.

Gangrene may be classified as ***dry*** or ***wet***.

Dry gangrene

In dry gangrene, the part becomes dry and shrinks with dark brown or black.

It spreads slowly and remain localized. The line of inflammatory reaction between the dead gangrenous and healthy tissues (called line of demarcation). Dry gangrene usually results from interference with arterial blood supply to a part without interference with venous return and *is a form of coagulation necrosis*.

Wet gangrene

In wet gangrene, the area is cold, swollen, and pulseless. The skin is moist, black. Blebs form on the surface, liquefaction occurs, and a foul odor is caused by bacterial action.

There is *no line of demarcation* between the normal and diseased tissues, and the spread of tissue damage is *rapid* and the lesion may extend *proximally*.

Wet gangrene primarily results from *interference with venous return* from the part. *Bacterial invasion* plays an important role in the development of wet gangrene.

Dry gangrene is confined almost exclusively to the extremities, but wet gangrene may affect the internal organs or the extremities.

Fate of necrotic tissue

The body treats necrotic tissue *as a foreign material.*

- It can induce acute inflammatory cell infiltrate in the surrounding tissue (Neutrophils) followed by macrophage infiltration, which try to remove the dead tissue then healing can occur which is either:
  - by the same type of cell (regeneration) … or
  - by fibrosis (organization).
- Calcification: deposition of calcium seen in necrotic tissue.
- Gangrene

CELL DEATH BY APOPTOSIS

Its programmed suicide cell death, when activated enzymes degrade DNA and cytoplasmic proteins in cells that are destined to die. The plasma membrane remains intact but the cell is fragmented into apoptotic bodies to be highly "edible" by phagocytes. The apoptotic cell death *does not* elicit an inflammatory reaction.

Features

Reduced cell size, eosinophilic cytoplasm
Chromatin condensation (chromatin aggregates peripherally)
Cytoplasmic blebs and apoptotic bodies
Phagocytosis of apoptotic cells by macrophages

Apoptosis in different conditions

Physiologic conditions

- Fetal development (embryogenesis)
  - Cells die off after their purpose has been fulfilled.
  - Removal of supernumerary cells during development
- Involution of tissues with hormone withdrawal:
  - Endometrial shedding in menstrual cycle
  - Lactating breast regression (weaning)
- Removal of self-reactive lymphocytes (may cause autoimmune disease)
- Removal of neutrophils in an inflammatory response
- Control of cell proliferation, maintaining a constant number of cell populations (as in immature lymphocytes in bone marrow)

Pathologic conditions

- DNA damage: prevents survival of cells with DNA mutations (protective effect).
- Removal of improperly folded proteins
- Ductal obstruction (e.g., kidney, parotid gland): Atrophy occurs by apoptosis.
- Infections (esp. viral illness): Cytotoxic T lymphocytes eliminate infected cells.
- Mechanisms of Apoptosis

Phases of apoptosis:

Initiation: activation of caspases → cascade of other caspases
  Intrinsic pathway
  Extrinsic pathway
Execution: terminal caspases → cellular fragmentation

Mechanisms of Apoptosis

Apoptosis is regulated by death- and survival-inducing signals that activate groups of enzymes called ***caspases***.

Caspases:

- Cystein aspartic acid proteases
- Exist in inactive form, requiring enzymatic cleavage to be activated
- Active caspases: a marker for cells undergoing apoptosis

Phases of apoptosis

Initiation: activation of caspases → cascade of other caspases
- Intrinsic pathway
- Extrinsic pathway

Execution: terminal caspases → cellular fragmentation

Two distinct pathways converge on caspase activation:

A. The mitochondrial (intrinsic) pathway

This pathway is functioning in most physiologic and pathologic situations.

Events:

- Several Mitochondria proteins are able to induce apoptosis. Increased permeability of the mitochondrial outer membrane → release of **cytochrome c** into the cytoplasm
- Cytochrome c initiates apoptosis
- In the cytoplasm, cytochrome c binds with apoptosis-activating factor-1 (**APAF-1**), forming a structure, **apoptosome**.
- Apoptosome leads to self-cleavage and activation of caspase-9, the initiator caspase.
- **Activated caspase-9** → cascade of executioner caspases

B. The death receptor (extrinsic) pathway

The extrinsic pathway is responsible for removing self-reactive lymphocytes and damage by CTLs. Plasma membrane death receptors initiate this pathway. A surface molecules called death receptors trigger apoptosis by tumor necrosis factor (TNF) receptor and Fas (CD95) that bind with intracellular death domain and activate caspase-8.

Events:

- FasL (Fas ligand on T cellsand cytotoxic T lymphocytes) binds to Fas → a signal for apoptosis is given to the cell.
- 3 or more Fas molecules combine to form the protein, Fas-associated death domain (**FADD**).
- FADD binds pro-caspase-8.
- **Caspase-8 (or caspase-10)** is activated → stimulates executioner caspases

Clearance of apoptotic cells

Apoptotic fragments producing a number of "eat-me" signals and secrete soluble factors to attract phagocytes. A phospholipid in normal cells, phosphatidylserine is normally found on the inner leaflet of the plasma membrane. In apoptosis, this phospholipid "flips" to the outer leaflet, thereby identified by macrophages, leading to phagocytosis and apoptosis.

Necroptosis

A new term that indicate newly observed type of necrosis that differs from classical necrosis by being ***regulated*** cell death with necrotic phenotype. Necroptosis is highly immunogenic in nature and is often utilized by host cells as a defense mechanism against pathogens.

Pyroptosis

In this form of death, certain danger-sensing protein complex in the cytosol, known as the inflammasome, become activated. This inflammasome, in turn will activate caspases thereby initiate inflammation through cytokines production. The process is applicable in some infectious diseases, where it leads to cell explosion through formation of pores in the plasma membrane.

Autophagy (self-eating)

When the cells lysosomal digest the cells own components to help the cells to survive in time when nutrients are not available and cellular activities require energy. Therefore, the cells initiate a recycling process of some components to provide the needed energy. Autophagy may be needed in atrophy adaptation process to avoid certain challenges. Once the cells become severely starved and unable to maintain energy and normal function, this may end in apoptosis.