LETHAL CELL INJURY - ONCOTIC NECROSIS AND APOPTOSIS

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- Some injuries to cells are too severe to permit their survival and repair.
- Irreversible cell injury -> **cell death**

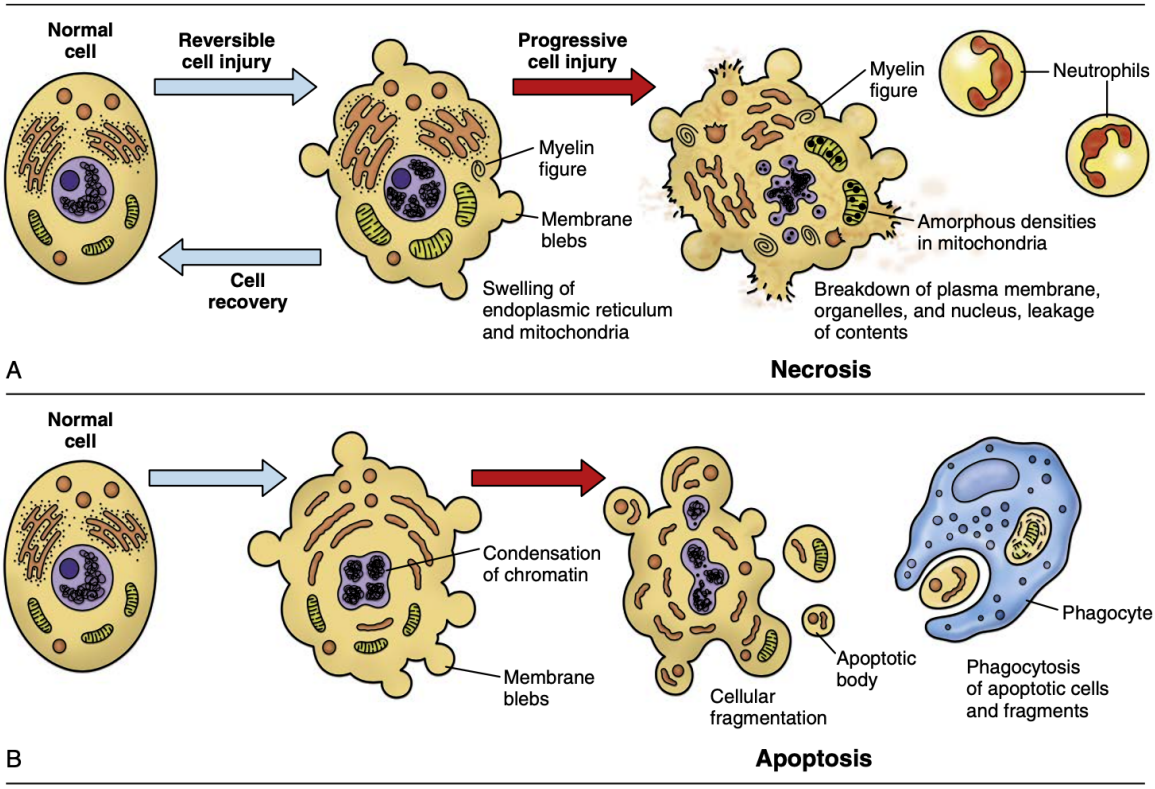
Necrosis = the term used traditionally to describe the **death of cells in a living organism** (i.e. Ante mortem cell death) *and* the gross and microscopic morphological changes that are indicative of this event

TYPES OF NECROSIS

- Two major forms of cell death in living organisms are recognised (**Figure 1**):

Oncosis (or **oncotic necrosis**) **= ante mortem cell death via swelling** (*onco*= swelling)
Apoptosis (or **apoptotic necrosis**) **= ante mortem cell death via shrinkage** (*apo*= off, *ptosis* = falling or dropping)

Figure 1 – Oncotic Necrosis versus Apoptosis



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- **Oncotic necrosis is more common than apoptosis.**
- Some causes of cell injury can induce cell death via **both oncosis and apoptosis.**
- Moreover, it is **often impossible to distinguish oncosis from apoptosis at the light microscopic level.**

- Accurate identification of apoptosis requires electron microscopy or agar gel electrophoresis of cell extracts (neither of which is done routinely)

NOTE – Many scientists use the term **necrosis** in its broadest traditional sense (i.e., death of cells in a living organism), irrespective of whether the process involved is oncosis or apoptosis.

ONCOSIS (ONCOTIC NECROSIS)

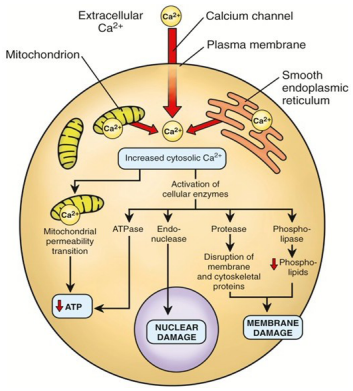
Oncotic necrosis = cell death by swelling in a living organism

- Caused by **severe cell membrane injury** or **sustained cell hypoxia/anoxia.**
- Cell death is **preceded by acute cell swelling** (Figure 1A)
- Release of products of cell membrane phospholipid degradation (e.g. Arachidonic acid) -> chemoattraction of leukocytes -> **inflammatory response** from adjacent viable tissue (c.f. Apoptosis - no inflammatory response)

Pathogenesis of Oncotic Necrosis

- The point at which a cell can no longer recover from injury is difficult to define precisely.
- However, irreversible injured cells often show **severe cell membrane damage** and/or **Severe mitochondrial damage**
- Experimental models of reperfusion injury following restoration of blood supply to ischaemic myocardium have clearly demonstrated that **ionised calcium (Ca++) plays a pivotal role in the final demise of many lethally injured cells.**
- Direct injury to cell membranes or failure of membrane ion pumps -> influx of Ca++ into cells
- Damaged mitochondria and endoplasmic reticulum may also release sequestered Ca++ into the cell cytoplasm.
- Free cytosolic Ca++ acts as an intracellular messenger and enzyme activator (**Figure 2**)

Figure 2 – Consequences of High Intracellular Calcium Concentrations



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- **Free cytosolic Ca^{++} can cause activation of**:
  - **Membrane-bound phospholipases** -> enzymatic destruction of membrane phospholipids of mitochondria and other organelles
  - **ATPases** -> accelerated depletion of remaining cell ATP stores
  - **Proteases** -> enzymatic destruction of membranes and cytoskeletal proteins

- **Endonucleases** -> degradation of nuclear chromatin

- If lysosomal membranes become leaky, **lysosomal enzymes** may contribute to the final moments of the cell (but they are more important in the subsequent degradation of the dead cell)

- After cell death by oncotic necrosis, the cells are **degraded by hydrolytic lysosomal enzymes**, with denaturation of cell proteins and lysis of cell components.
- The lysosomal enzymes involved are derived from:
  - The **dead cells** themselves (= **autolysis** = self-digestion), **AND**
  - **Leukocytes** recruited into sites of oncotic necrosis (= **heterolysis** = digestion by others)
- Most of the morphological patterns that are recognisable grossly or microscopically as indicative of oncotic necrosis are due to the effects of these autolytic and heterolytic enzymes and to the presence of infiltrating leukocytes which phagocytose the cell debris.
- These secondary events, together with the fact that many thousands of cells may die by oncotic necrosis, mean **oncotic necrosis may be grossly obvious** (c.f. Apoptosis)

NOTE - Most pathologists, medical doctors and veterinarians use the term **autolysis** synonymously with the postmortem decomposition changes that occur after death of the whole organism (**somatic death**). The latter is **postmortem autolysis**. Oncotic necrosis involves **antemortem autolysis**.

Gross Features of Oncotic Necrosis

- Depending on the tissue involved, it may take **12 to 24 hours after onset for gross lesions of oncotic necrosis to become visible.**
  - I.e. **Lesions of peracute to early acute oncotic necrosis may be invisible during a post-mortem examination.**
- In general, foci of oncotic necrosis display gross:
  - Pallor (unless blood oozes into the affected zone)
  - Softness
  - Friability
  - Sharp demarcation from adjacent viable tissue, especially as the host inflammatory response develops +/- dystrophic mineralisation (see below)
- Foci of oncotic necrosis become **more sharply defined over time** as the host inflammatory reaction develops in adjacent viable tissue.
- A red border develops due to arteriolar vasodilation and increased perfusion of viable capillary beds (= **tissue hyperaemia**)
- Internal to the red band is a white band of massed leukocytes emerging from the dilated capillary beds and entering the necrotic zone.
- Incoming leukocytes may release lysosomal enzymes to liquefy the necrotic tissue and/or they may phagocytose and digest it.

Light Microscopic Features of Oncotic Necrosis

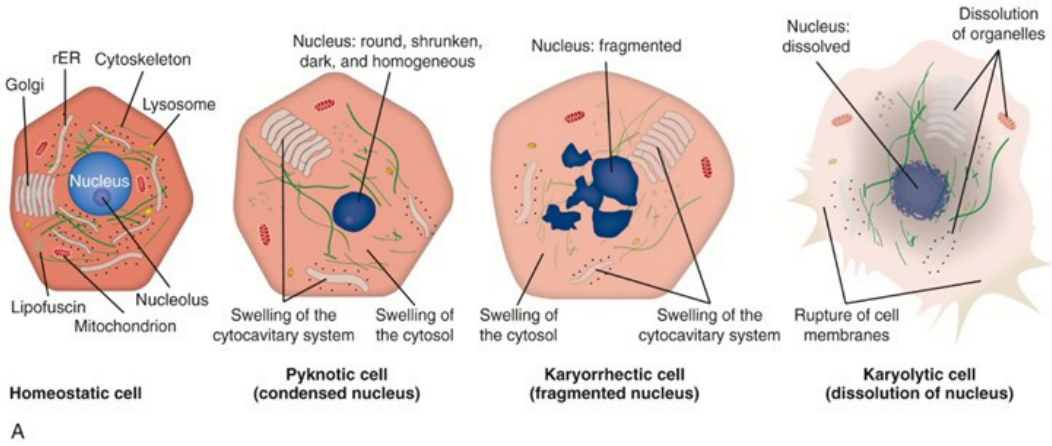
- Depending on the tissue involved, it may take **4-12 hours after onset before light microscopic evidence of oncotic necrosis appears.**
- I.e. **Lesions of peracute oncotic necrosis may be invisible by routine light microscopic examination of affected tissues.**

- **Cytoplasmic changes** include:
  - Progressively severe cell swelling
  - Initially, **increased cytoplasmic eosinophilia** (due to loss of basophilic ribosomal RNA, denaturation of cytoplasmic proteins +/- consolidation of collapsing cytoplasmic components)
  - Then, **cytoplasmic pallor** with a moth-eaten vacuolated appearance
  - Finally, **detachment** from basement membranes and surrounding cells (-> sloughing) or simply **rupture** -> ghost outlines of cell debris -> **disappearance.**

- **The appearance of the nucleus is the more important feature used to determine whether a cell was viable or not by light microscopy.**
- In oncotic necrosis, **cell nuclei may undergo one of the following three morphological changes:**

  - **Pyknosis** = a shrunken, darkly staining (basophilic, hyperchromatic) nucleus (Figure 3) (also seen in apoptosis)
  - **Karyorrhexis** = rupture of the nuclear envelope with extrusion of dark nuclear fragments (Figure 4) (also seen in apoptosis)
  - **Karyolysis** = fading of the nucleus (due to the actions of activated RNAases and DNAases) -> eventual **disappearance**

Figure 3 – Nuclear Pyknosis and Karyorrhexis



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Sequelae (Consequences) of Oncotic Necrosis

- In **small areas of oncotic necrosis** involving tissues composed of **stable or labile cells**, healing eventually involves regenerative cell hyperplasia (via mitotic division) with a variable degree of **fibrosis** (scar tissue formation)
- In **large areas of oncotic necrosis in any tissue**, regeneration and repair are much less effective.
- Significant scar tissue formation is typical.
- Sometimes large foci of oncotic necrosis may be walled off (**sequestration**) from viable tissue by a capsule of scar tissue and then persist (e.g. Bone sequestrum, lung sequestrum)

- In **small areas of oncotic necrosis** involving tissues composed of **permanent cells** (e.g. Neurons or myocardial fibres), tissue regeneration is impossible, and repair involves fibrosis (especially in the myocardium) or attempted filling of the defect by astrocyte processes (CNS)

SPECIFIC TYPES OF ONCOTIC NECROSIS

- Several specific types of oncotic necrosis are recognisable grossly and/or microscopically.
- These types are influenced by the **cause of the necrosis** (and therefore their recognition provides important diagnostic clues to the cause) and also by the local conditions within the tissue in which oncotic necrosis develops.

Coagulative (Coagulation) Necrosis

- Coagulative necrosis is typical of **lethal hypoxic injury** in **all body tissues except the central nervous system** (CNS - brain and spinal cord) (although it can affect the cell bodies of brain/spinal neurons)
- Hypoxic/anoxic insult or subsequent intracellular acidosis -> **denaturation** of both structural and enzymatic proteins -> proteolysis of the dead cells is prevented.
- Affected tissues may appear pale (with a parboiled or cooked appearance) (e.g. Arterial infarcts) or haemorrhagic (e.g. Venous infarcts) (**infarct** = a localised zone of ischaemic necrosis)
- The necrotic cells appear shrunken and hypereosinophilic, with the nuclei pyknotic, karyolytic or karyorrhectic.
- The basic outline of the dead cells persists for at least several days.
- Affected cells ultimately lyse (fragment)
- Debris is removed by phagocytosis by macrophages (that also release their proteolytic enzymes into the necrotic zone to liquefy the mummified cells)
- Coagulative necrosis can also be induced by certain **exotoxins of anaerobic bacteria(e.g. *Clostridium* species, *Fusobacterium necrophorum*)**

Gangrenous Necrosis (Gangrene)

- **Gangrene** is an ancient term that is still used to describe tissues that have undergone. **Coagulative necrosis**
- There are two main forms of gangrene:

  Dry gangrene

  = coagulative necrosis induced by ischaemia (i.e. Infarction)

  - The affected tissue eventually mummifies due to dehydration -> shrivelled, dry and brown to black -> eventual sloughing.
  - E.g. Frostbite
  - E.g. Too tight a tourniquet
  - E.g. Ergot poisoning (a mycotoxicosis in which a fungal toxin induces sustained vasoconstriction)

  Wet gangrene or **gas gangrene**

  = necrosis of tissue (usually of coagulative type) that is then colonised by bacteria > liquefaction and putrefaction

- The bacteria are saprophytes derived from the soil, air, skin or gastrointestinal tract)
- Affected tissue becomes moist, soft, red brown to black and is malodorous due to gas production by the bacteria.
- E.g. Gangrenous pneumonia following inhalation of rumen contents into the lungs.
- E.g. Gangrenous mastitis (inflammation of the udder)
- E.g. Bacterial invasion of intestinal infarcts
- E.g. Deep clostridial wound infections.

Liquefactive Necrosis

- In liquefactive necrosis, there is **rapid enzymatic degradation of the dead cells**(involving both autolysis and heterolysis) -> obliteration of the original tissue architecture and formation of **liquid.**
- Microscopically, see amorphous eosinophilic debris containing pyknotic or karyorrhectic nuclear remnants.
- Typical of **abscesses** caused by **pyogenic (pus-forming) bacteria** (e.g. *Staphylococci*, *streptococci*, *Arcanobacterium* species) -> rapid accumulation of neutrophils and release of their proteolytic enzymes into the infected area -> pocket of **pus** (= dead and dying neutrophils and tissue debris)
- Liquefactive necrosis is also the typical form of oncotic necrosis seen in the **CNS** (-> **malacia** = **softening** of affected parts of the brain or spinal cord) -> formation of a cavity filled with fluid and lipid debris
- Probably favoured by the large volume of cell membranes in the CNS
- Liquefactive necrosis is also the typical form of oncotic necrosis in the **intestines** and **Pancreas** due to the local abundance of proteolytic enzymes

Caseous (Caseation) Necrosis

- In caseous necrosis, the dead tissue is converted into a **grossly dry, granular, cream-white to yellow, friable coagulum** of the consistency of mature cheese (*caseous =cheesy*)
- Histologically, see obliteration of tissue architecture and fragmentation of the dead cells to form a mass of amorphous to granular nuclear and cytoplasmic debris, surrounded by arim of active inflammation (often granulomatous – i.e. Macrophage-rich) and a fibrous tissue capsule.
- Foci of caseous necrosis often undergo **dystrophic mineralisation** (see below), with the mineralised debris persisting indefinitely.
- Alternatively, the foci may eventually be lysed or phagocytosed by macrophages.
- Caseous necrosis is often seen in foci of **infection by bacteria with complex cells walls and poorly degradable lipid components** (e.g. Tuberculosis caused by *Mycobacterium tuberculosis* or *M. Bovis*; e.g. Caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis*)
- Also seen in some **fungal infections** and within **rapidly growing tumours**
- Also seen in **chronic abscesses** caused by pyogenic bacteria, after gradual reabsorption of the water component of the pus

Fat Necrosis

Fat necrosis = necrosis of fat

(**NOTE** - This form of necrosis cannot affect tissues other than adipose tissue!!!)

- Common in the subcutaneous fat and in omental and mesenteric fat of the peritoneal cavity
- Affected fat stores are **firm** (due to reactive inflammation, fibrosis +/- mineralisation)and **Chalky-white** (or occasionally yellow due to ceroid pigment)
- **Active lesions** of fat necrosis are surrounded by a **red hyperaemic border** indicative of inflammation.
- Microscopically, necrotic adipocytes appear eosinophilic with wispy or bubbly cytoplasm and pyknotic, karyorrhectic or karyolytic nuclei.
- Free lipid released from dead adipocytes may become complexed with sodium or calcium salts -> basophilic **saponified fat** (the latter may undergo dystrophic mineralisation)
- Free cholesterol may precipitate to form clear, needle-like **cholesterol crystals.**
- Release of free fat is also irritant -> a **chronic "foreign body" inflammatory reaction** characterised by infiltrating macrophages and multinucleate giant cells and by fibrosis (-> permanent scarring)
- Fat necrosis in animals can be induced by:
  - **Lipolytic enzymes**
    - E.g. Local release of activated lipases from the necrotic exocrine pancreas
    - May only involve peripancreatic fat or may be widely scattered throughout the abdominal cavity.
  - **Trauma**
    - E.g. Crushing of fat pads in the pelvic canal of heifers during a difficult calving
    - E.g. Subcutaneous fat in recumbent cattle (downer cows)
  - **Reactive oxygen species (free radicals)**
    - E.g. In deficiency of vitamin E and/or selenium (antioxidants)
    - Ceroid pigment accumulates in macrophages in areas of peroxidation of adipocyte phospholipid membranes -> gross yellow discolouration ("yellow fat disease")
  - **Hypoxia**
    - E.g. Within large fat stores in obese sheep
  - **Unknown causes**
    - E.g. Massive necrosis of peritoneal fat stores in cattle

APOPTOSIS (APOPTOTIC NECROSIS)

- A distinctive form of cell death in a living organism
- Can be a **normal (physiological) process** or an **abnormal (pathological) process.**
- Occurs **rapidly.**
- **Does not induce an inflammatory response.**
- **Involves individual cells or small clusters of cells** that are being selectively eliminated.
- Therefore, **apoptosis is never grossly visible.**

Physiological Apoptosis

- Physiological apoptosis is often referred to as **programmed cell death** and occurs in the following circumstances:

  - **During embryogenesis, foetal development and post-natal growth**
    - Allows scheduled destruction of certain cell populations.

  - **During involution of tissues**

- E.g. Thymus at puberty
- E.g. Mammary parenchyma after cessation of lactation

- **Day-to-day loss of labile cell populations**
  - E.g. Surface epidermal keratinocytes
  - E.g. Superficial small intestinal villous enterocytes

- **Elimination of cells that are superfluous to the body's needs.**
  - E.g. Neutrophils after successful removal of the cause of an acute inflammatory reaction
  - E.g. Excess parenchymal cells after the stimulus for tissue hyperplasia ceases

- **Elimination of cells that are potentially damaging to the body.**
  - E.g. Self-reactive lymphocytes
  - E.g. Virus-infected or neoplastic cells after attack by cytotoxic-lymphocytes

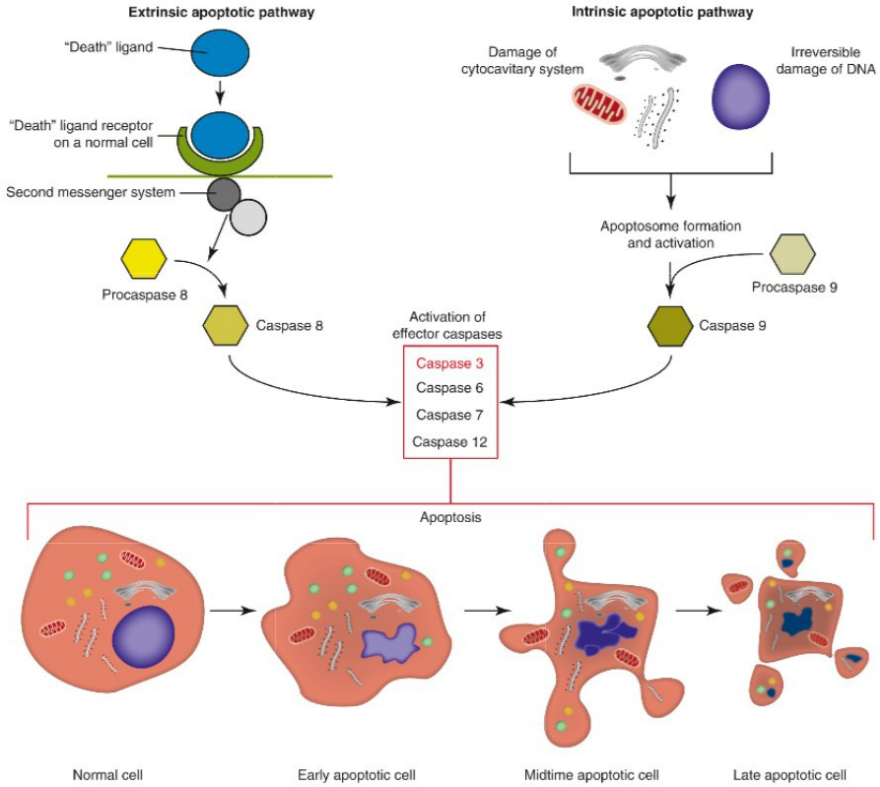
Pathological Apoptosis

- Apoptosis can be a feature of the following disease processes:
  - Radiation injury
  - Cell damage caused by reactive oxygen species.
  - Atrophy of glands following obstruction of secretory/excretory ducts
  - Some viral infections
  - Tissue injury induced by cell-mediated immune responses.
  - Some intoxications
  - Malignant neoplasia
  - Tissue reactions to certain administered drugs - e.g. Corticosteroids, cytotoxic chemotherapeutic agents

Mechanisms of Apoptosis

- Apoptosis is sometimes referred to as **cellular suicide** because the cells involved play a significant molecular part in their own demise (**Figure 4**)
- Cell death via apoptosis involves an **intracellular proteolytic cascade** (sometimes referred to as the **execution phase** of apoptosis) mediated by the cell's own **caspase enzymes.**
- Once activated, **executioner caspases** cleave cytoskeletal proteins and activate endonucleases to cleave nuclear proteins involved in DNA replication, repair and transcription -> cell death
- Fragmentation and budding of apoptotic cells -> flipping of interior phosphatidylserine molecules of phospholipid cell membranes to the exterior of the membrane-bound apoptotic bodies -> prompt recognition by neighbouring cells as being abnormal -> **rapid phagocytosis.**
- Lack of accompanying release of pro-inflammatory cell components (e.g. Arachidonic acid from degradation of cell and organelle membrane phospholipids) -> **no significant inflammatory response to apoptosis**

Figure 4 – Mechanisms of Apoptosis



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DYSTROPHIC MINERALISATION

- Dystrophic mineralisation is the **deposition of calcium salts in tissues that have undergone oncotic necrosis.**
- The process occurs despite **normal serum calcium and phosphate concentrations** and in the absence of derangements in calcium and phosphate metabolism (in contrast to **metastatic mineralisation**)
- A **common phenomenon**
- The mineral deposited is usually calcium phosphate or calcium carbonate.
- Grossly, large mineral deposits may be detectable as **gritty to hard, chalky-white foci.**
- Mineral deposits appear histologically in H&E-stained sections as **dark blue purple (basophilic), granular, amorphous deposits.**
- The deposits may be intracellular or extracellular.
- The pathogenesis of dystrophic mineralisation is not completely understood.
- **Intracellular dystrophic mineralisation** begins with accumulation of calcium within mitochondria of dead and dying cells.
- Initiators of **extracellular dystrophic mineralisation** include acidic phospholipids (especially phosphatidylserine) in fragments derived from disintegrating cells; calcium binds to these phospholipids; membrane phosphatases generate phosphates that bind to the calcium > microcrystal formation > propagation > extracellular deposition of mineral.