

# Examples of cell injury

## II. Ischemia –reperfusion injury

- If cells are reversibly injured, the restoration of blood flow can result in cell recovery.
- However, under certain circumstances, *the restoration of blood flow to ischemic but viable tissues results, paradoxically, in the death of cells that are not otherwise irreversibly injured.*
- This so-called ischemia-reperfusion injury is a clinically important process that may contribute significantly to tissue damage in myocardial and cerebral ischemia.

- Several mechanisms may account for the exacerbation of cell injury resulting from reperfusion into ischemic tissues:
  - a. New damage may be initiated during reoxygenation by increased generation of *ROS* from parenchymal and endothelial cells and from infiltrating leukocytes
  - b. When the supply of oxygen is increased, there may be a corresponding increase in the production of *ROS*, especially because mitochondrial damage leads to incomplete reduction of oxygen, and because of the action of oxidases in leukocytes, endothelial cells, or parenchymal cells

- c. Cellular antioxidant defense mechanisms may also be compromised by ischemia, favoring the accumulation of free radicals.
- d. The *inflammation* that is induced by ischemic injury may increase with reperfusion because of increased influx of leukocytes and plasma proteins.
  - The products of activated leukocytes may cause additional tissue injury
- e. Activation of the *complement system* may also contribute to ischemia-reperfusion injury.
  - Complement proteins may bind in the injured tissues, or to antibodies that are deposited in the tissues, and subsequent complement activation generates by-products that exacerbate the cell injury and inflammation.

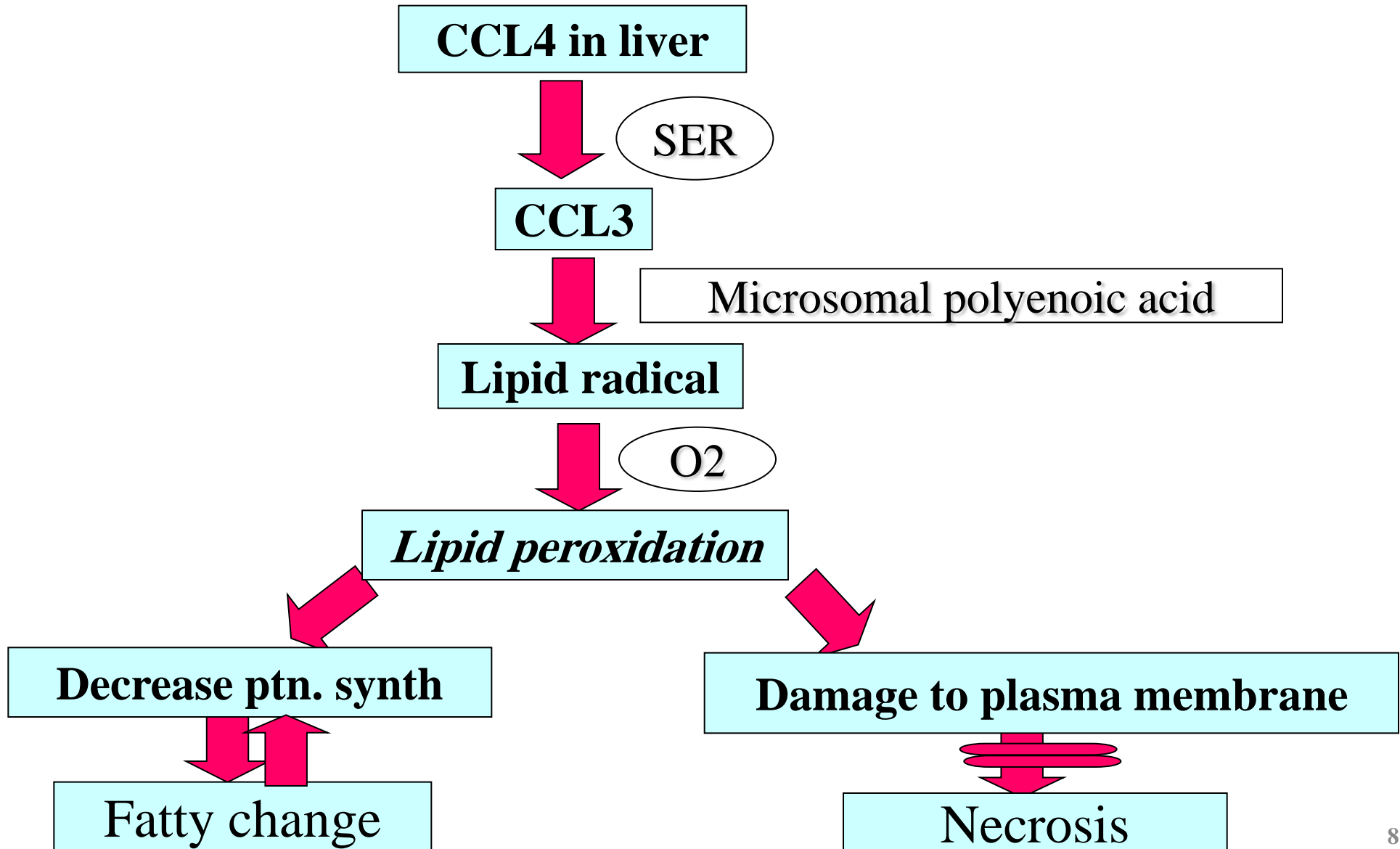
- induce cell injury by one of two general mechanisms:
  - a. *Some chemicals act directly by combining with a critical molecular component or cellular organelle.*
- For example, in mercuric chloride poisoning from ingestion of contaminated seafood , mercury binds to the sulfhydryl groups of various cell membrane proteins, causing inhibition of ATP-dependent transport and increased membrane permeability.
  - b. *Many other chemicals are not intrinsically biologically active but must be first converted to reactive toxic metabolites by the cytochrome P-450 in the smooth ER of the liver and other organs, which then act on target cells.*

- The main mechanism of cell injury involves the formation of free radicals and examples include *Carbon tetrachloride* ( $\text{CCl}_4$ )-once widely used in the dry cleaning industry but now banned-and the analgesic *acetaminophen*
- $\text{CCl}_4$  is converted to the toxic free radical principally in the liver, and this free radical is the cause of cell injury, mainly by membrane phospholipid peroxidation
- In less than 30 minutes after exposure to  $\text{CCl}_4$ , there is breakdown of ER membranes with a decline in hepatic protein synthesis of enzymes and plasma proteins;
- Within 2 hours, swelling of the smooth ER and dissociation

of ribosomes from the smooth ER have occurred.

- There is reduced lipid export from the hepatocytes, as a result of their inability to synthesize apoprotein to form complexes with triglycerides and thereby facilitate lipoprotein secretion; the result is the fatty liver .
- Mitochondrial injury follows, with diminished ATP stores result in defective ion transport and progressive cell swelling;
- The plasma membranes are further damaged by fatty aldehydes produced by lipid peroxidation in the ER.
- The end result is calcium influx and eventually cell death.

# Carbontetrachloride(CCL4) poisoning





# Apoptosis

- Is a pathway of cell death in which cells activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins
- Fragments of the apoptotic cells then break off, giving the appearance that is responsible for the name (*apoptosis*, "falling off").

## Notes

1. The plasma membrane of the apoptotic cell remains intact, but is altered in such a way that the cell and its fragments become avid targets for phagocytes.

2. - The dead cell and its fragments are rapidly cleared before cellular contents have leaked out, so apoptotic cell death does not elicit an inflammatory reaction in the host
- Apoptosis and necrosis sometimes coexist, and apoptosis induced by some pathologic stimuli may progress to necrosis.

### **Causes of Apoptosis** :.

**I. Apoptosis in Physiologic Situations** :-Is a normal phenomenon that serves to eliminate cells that are no longer needed to maintain a constant number of cells in tissues and is important in the following situations

- a. The programmed destruction of cells during embryogenesis: Normal development is associated with the death of some cells and the appearance of new cells
- b. Involution of hormone-dependent tissues upon hormone deprivation:
1. Endometrial cell breakdown during the menstrual cycle
  2. Regression of the lactating breast after weaning
- c. Cell loss in proliferating cell populations: Such as intestinal crypt epithelia, in order to maintain a constant number
- d. Elimination of cells that have served their useful purpose

1. *Such* as neutrophils at the end of an acute inflammation
  2. Lymphocytes at the end of an immune response .
- e. Elimination of potentially harmful self-reactive lymphocytes*, in order to prevent reactions against the body's own tissues

## *II. Apoptosis in Pathologic Conditions*

- a. DNA damage* by radiation, cytotoxic anticancer drugs, can damage DNA, either directly or through production of free radicals and if repair mechanisms cannot cope with the injury, the cell triggers apoptosis

- In these situations, elimination of the cell may be a better alternative than risking mutations in the damaged DNA, which may progress to malignant transformation
- These injurious stimuli cause apoptosis if the insult is mild, but larger doses of the same stimuli result in necrosis
- Inducing apoptosis of cancer cells is a desired effect of chemotherapeutic agents, many of which work by damaging DNA.

*b. Accumulation of misfolded proteins.*

- Improperly folded proteins may arise because of mutations in the genes encoding these proteins or

because of free radicals damage

- Excessive accumulation of these proteins in the ER leads to *ER stress*, which results in apoptotic death of cells

*c. Cell injury in certain infections* : Such as viral infections, in which loss of infected cells is mainly due to apoptotic death that may be induced by the virus (as in adenovirus and human immunodeficiency virus infections) or by the host immune response (as in viral hepatitis).

***Mechanisms of Apoptosis*** :- Apoptosis results from the activation of enzymes called caspases ( cysteine proteases that cleave proteins after *aspartic* residues).

- Two distinct pathways converge on caspase activation:
  - a. Mitochondrial pathway
  - b. *Death receptor pathway*
- A. The Mitochondrial (Intrinsic) Pathway of Apoptosis:**
  - The mitochondria contain several proteins that are capable of inducing apoptosis which include
    1. Cytochrome c
    2. Proteins that neutralize endogenous inhibitors of apoptosis.
  - The choice between cell survival and death is determined by the permeability of mitochondria, which is controlled by a family of many proteins, the prototype of which is Bcl-2



## Triggering stimuli for mitochondrial pathway

- a. Deprivation of growth factors and other survival signals,
- b. Or exposure to agents that damage DNA,
- c. Accumulation of unacceptable amounts of misfolded proteins

## Mechanism : The following occur:

1. A number of sensors are activated: These sensors are members of the Bcl-2 family called "BH3 proteins").
2. Then sensors activate two pro-apoptotic members of the Bcl2 family called Bax and Bak, which dimerize

3. Then insert into the mitochondrial membrane, and form channels through which cytochrome c escape into the cytosol

NOTE:- These sensors also inhibit the anti-apoptotic molecules Bcl-2 and Bcl-x<sub>L</sub> enhancing the leakage of mitochondrial proteins

4. Cytochrome c, activates caspase-9.
5. Other proteins that leak out of mitochondria block the activities of caspase antagonists that function as physiologic inhibitors of apoptosis.
6. The net result is the activation of the caspase cascade, ultimately leading to nuclear fragmentation

## Notes:

- Conversely, if cells are exposed to growth factors and other survival signals, they synthesize anti-apoptotic members of Bcl-2 family, the two main ones of which are Bcl-2 itself and Bcl-x<sub>L</sub>
- These proteins antagonize Bax and Bak, and thus limit the escape of the mitochondrial pro-apoptotic proteins
- Cells deprived of growth factors not only activate the pro-apoptotic Bax and Bak but also show reduced levels of Bcl-2 and Bcl-x<sub>L</sub>, thus tilting the balance toward death
- The mitochondrial pathway seems to be the pathway that

is responsible for apoptosis in most situations

## B. The Death Receptor (Extrinsic) Pathway of Apoptosis :

- Many cells express surface molecules, called death receptors, that trigger apoptosis and most of are members of the tumor necrosis factor (TNF) receptor family
- The death receptors contain in their cytoplasmic regions a conserved domain called "death domain,"
- The prototypic death receptors are the type I TNF receptor and Fas (CD95).
- Fas ligand (FasL) is a membrane protein expressed

mainly on activated T lymphocytes.

- The mechanism of death

1. When these T cells recognize Fas-expressing targets,
2. Fas molecules are cross-linked by FasL and bind adaptor proteins via the death domain.
3. Adaptor proteins in turn recruit and activate caspase-8.

Note; In many cell types caspase-8 may cleave and activate a pro-apoptotic member of the Bcl-2 family called Bid, thus feeding into the mitochondrial pathway

- A caspase antagonist called FLIP, block activation of

- . caspases downstream of death receptors and some viruses produce FLIP homologues it is suggested that this is a mechanism that viruses use to keep infected cells alive.
- The death receptor pathway is involved in elimination of self-reactive lymphocytes and in killing of target cells by some cytotoxic T lymphocytes.

## **Activation and Function of Caspases**

1. The mitochondrial and death receptor pathways lead to the activation of the *initiator caspases*, caspase-9 and -8, respectively.

2. So active forms of these enzymes are produced
3. The active forms cleave and thereby activate another series of caspases that are called the *executioner caspases and the active forms* cleave numerous targets, culminating in activation of nucleases that degrade DNA and nucleoproteins.
  - Caspases also degrade components of the nuclear matrix and cytoskeleton, leading to fragmentation of cells.

### Clearance of Apoptotic Cells:

- In normal cells phosphatidylserine is present on the inner leaflet of the plasma membrane, But in apoptotic cells

this phospholipid "flips" to the outer leaflet, where it is recognized by tissue macrophages and leads to phagocytosis of the apoptotic cells

- Cells that are dying by apoptosis also secrete soluble factors that recruit phagocytes facilitating clearance of the dead cells before they undergo secondary membrane damage and release their cellular contents (which can induce inflammation)
- Some apoptotic bodies express adhesive glycoproteins that are recognized by phagocytes, and macrophages may produce proteins that bind to apoptotic cells (but not to live cells) and target the dead cells for engulfment.



- Numerous macrophage receptors have been shown to be involved in the binding and engulfment of apoptotic cells.
- This process of phagocytosis of apoptotic cells is so efficient that dead cells disappear without leaving a trace, and inflammation is virtually absent.
- Although, necrosis and apoptosis are distinct from each other, they may coexist , For instance, DNA damage (seen in apoptosis) activates an enzyme called poly-ADP(ribose ) polymerase, which depletes cellular supplies of nicotinamide adenine dinucleotide, leading to a fall in ATP levels and ultimately necrosis

- **Apoptosis**

- Active process
- Physiological & pathological
- Occur in single cells
- The cell size is small
- The nucleus is fragmented into small fragments
- The plasma membrane is intact but with altered structure
- The cellular contents are intact but may be released in apoptotic bodies
- No inflammatory reaction

- **Necrosis**

- Passive process
- Always pathological
- Affects mass of cells
- The cell is enlarged (swelling)
- The nucleus show pyknosis→karyorrhexis→karyolysis
- The plasma membrane is disrupted
- The cellular contents show enzymatic digestion and may leak out of the cell
- stimulates Inflammation

# INTRACELLULAR ACCUMULATIONS

**1. Fatty Change (Steatosis)** : Refers to any abnormal accumulation of triglycerides within parenchymal cells.

- It is most often seen in the liver, since this is the major organ involved in fat metabolism, but it may also occur in heart, skeletal muscle, kidney, and other organs.

- Steatosis may be caused by :

- a. Toxins,
- b. Protein malnutrition
- c. Diabetes mellitus
- d. Obesity
- e. Anoxia.

- *Alcohol abuse and diabetes associated with obesity are the most common causes of fatty change in the liver (fatty liver) in industrialized nations.*

- **Morphology of fatty liver**

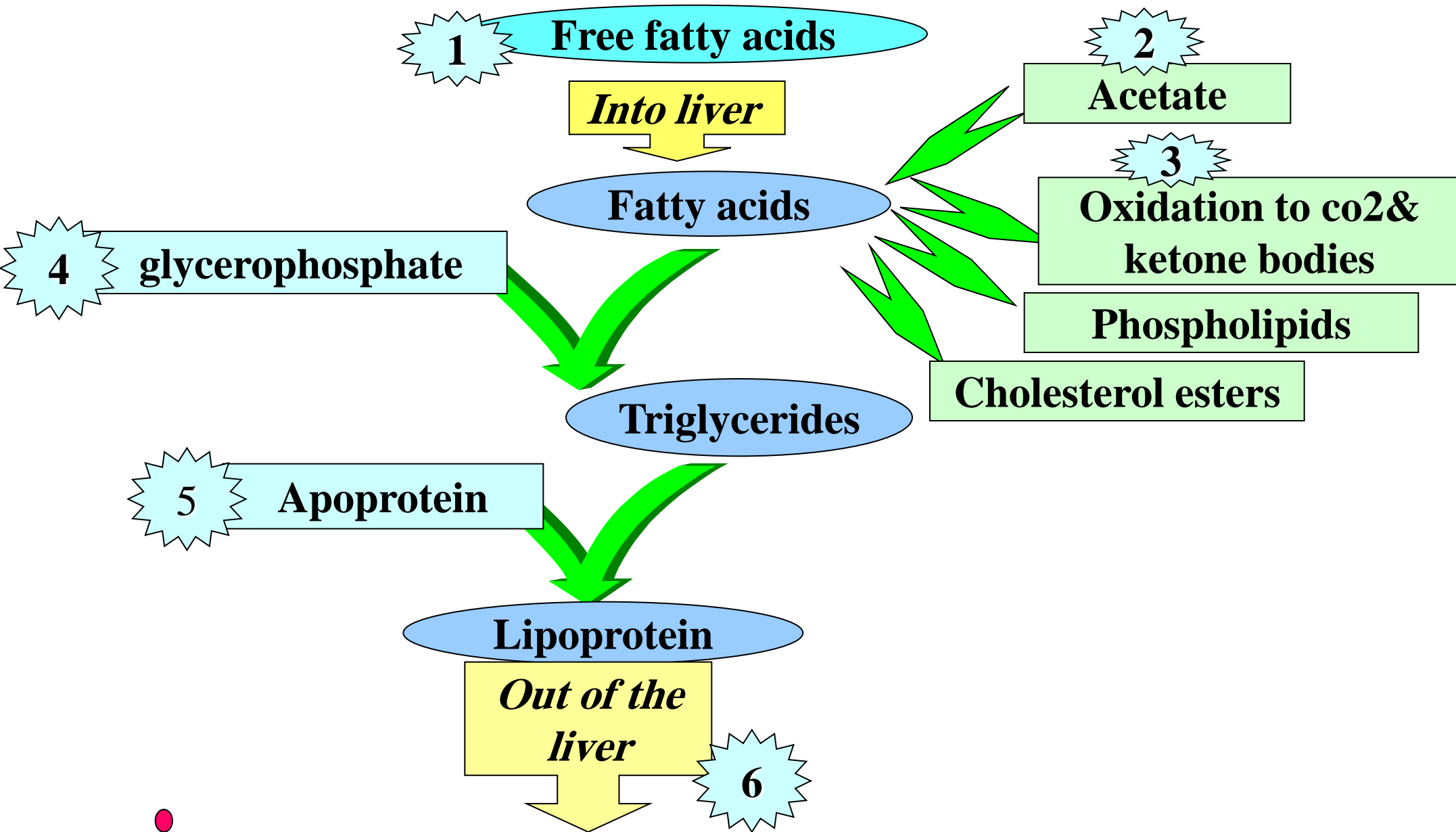
**Gross appearance depends on severity**

- Normal to large size, looks yellow and greasy when severe

**Histology**

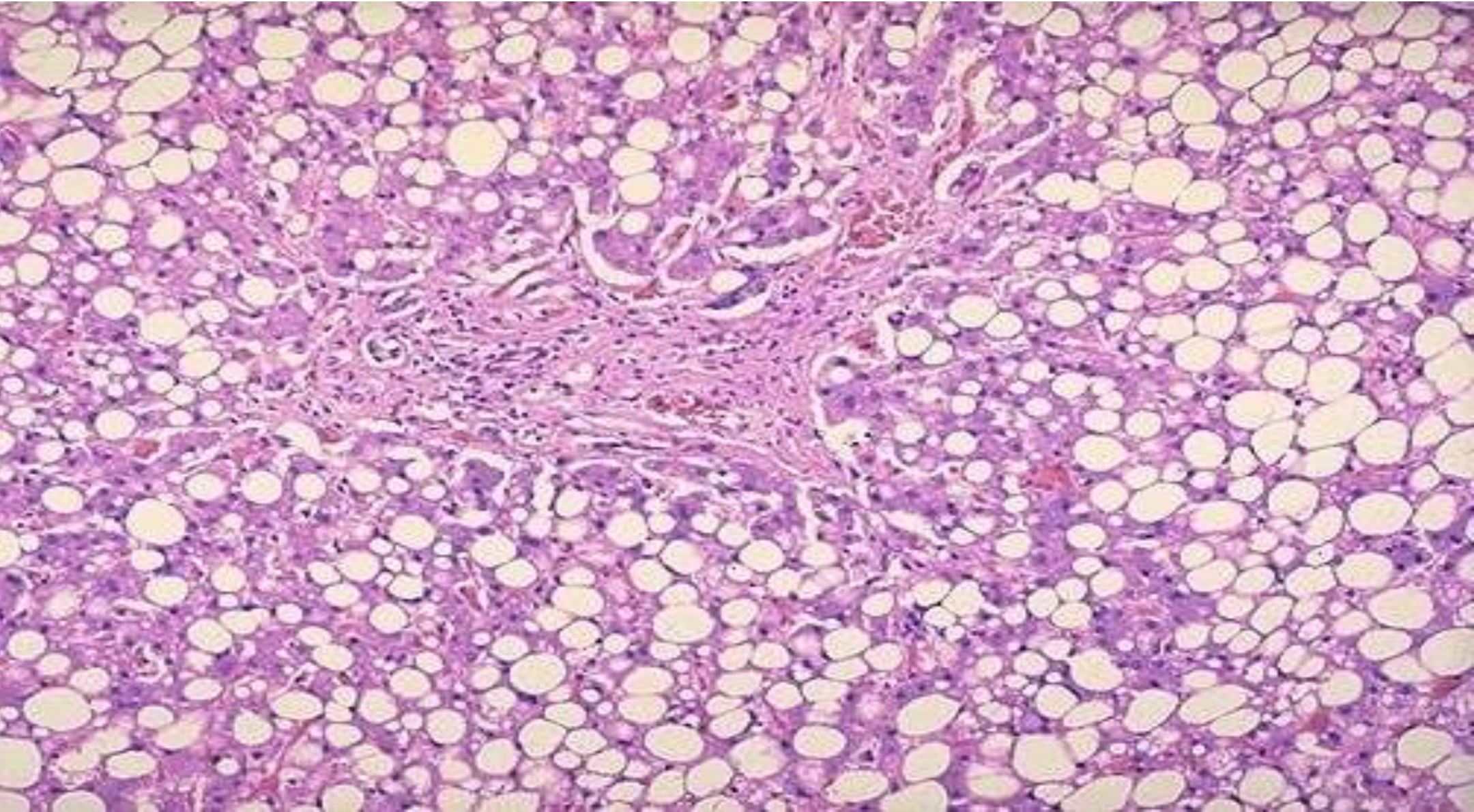
- Fat accumulates in hepatocytes as small vacuoles in cytoplasm with nucleus in the center  
**((Microvesicular fatty change )**

# Mechanism of fatty liver



- The whole cytoplasm is replaced by fat and nucleus is pushed to one cell side ( Macrovesicular fatty change)
- Special stains are needed to distinguish fatty change from intracellular water
- To identify fat microscopically, tissue must be processed for sectioning without the organic solvents used routinely, so portions of the tissue are therefore frozen then the tissue is stained with sudan black or oil red O (stain fat orange red)

# Fatty liver





## **2. Cholesterol and Cholesteryl Esters :**

- Cellular cholesterol metabolism is tightly regulated to ensure normal cell membrane synthesis without significant intracellular accumulation.
- However, phagocytic cells may become overloaded with lipid (triglycerides, cholesterol, and cholesteryl esters) in several different pathologic processes. Of these, atherosclerosis is the most important

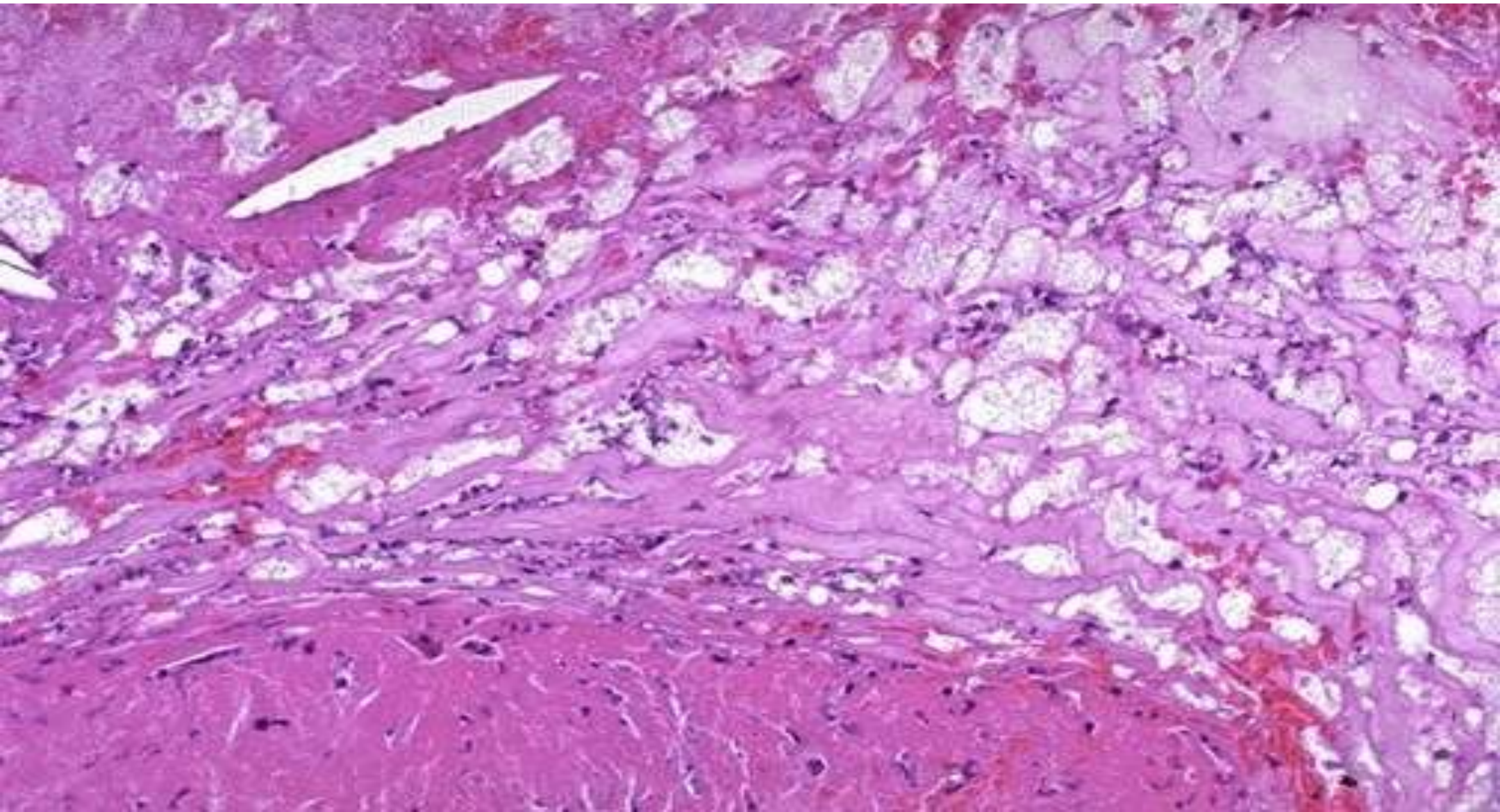
**3. Proteins:** Are much less common than lipid accumulations;:

A. Nephrotic syndrome: A disorder characterized by heavy protein leakage across the glomerular filter there is a

much larger reabsorption of the protein, and vesicles containing this protein accumulate, giving the histologic appearance of pink, hyaline cytoplasmic droplets.

- The process is reversible: If the proteinuria abates, the protein droplets are metabolized and disappear
- B. Marked accumulation of newly synthesized immunoglobulins that may occur in the RER of some plasma cells, forming rounded, eosinophilic called *Russell bodies*
- C. Alcoholic hyaline bodies in the liver;
- D. Neurofibrillary tangles in neurons in Alzheimer disease

# Atherosclerosis



#### **4. Glycogen:**

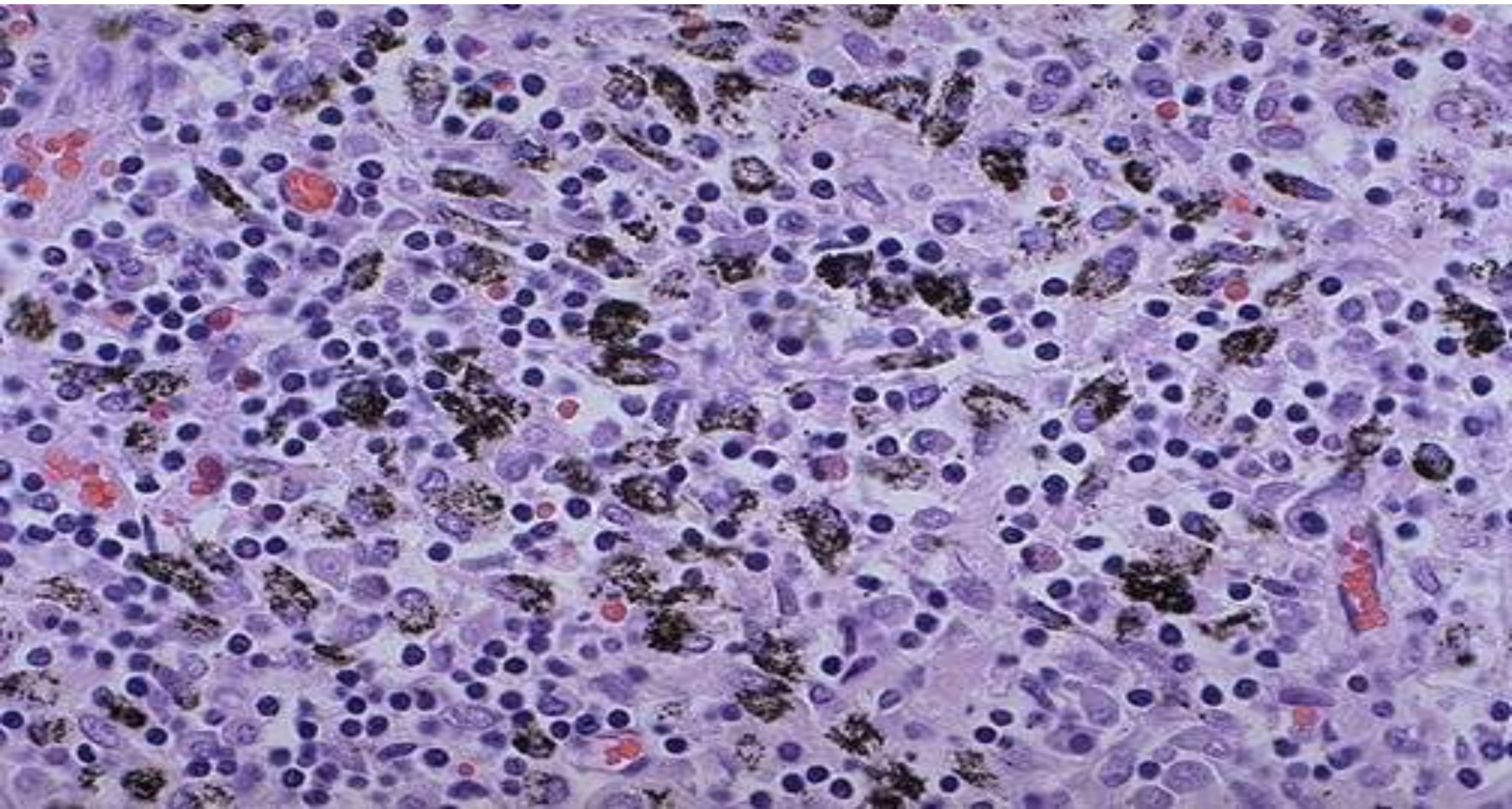
- a. In poorly controlled diabetes mellitus, glycogen deposits in renal tubular epithelium and cardiac myocytes
- b. Glycogen storage diseases called glycogenosis

#### **5. Pigments:**Are colored substances that are either:

**A. Exogenous,** coming from outside the body,

**Carbon,** is the most common exogenous pigment (coal dust), and when inhaled, it is phagocytosed by alveolar macrophages and transported through lymphatic channels to the regional tracheobronchial lymph nodes

# Anthracosis



leaving a trace, and inflammation is virtually absent.

- Although, necrosis and apoptosis are distinct from each other, they may coexist , For instance, DNA damage (seen in apoptosis) activates an enzyme called poly-ADP(ribose) polymerase, which depletes cellular supplies of nicotinamide adenine dinucleotide, leading to a fall in ATP levels and ultimately necrosis.
- In fact, even in common situations such as ischemia, it has been suggested that early cell death can be partly attributed to apoptosis, with necrosis supervening later as ischemia worsens.

- Aggregates of the pigment blacken the draining lymph nodes and pulmonary parenchyma (*anthracosis* )

B. Or endogenous: Synthesized within the body itself,

**1. *Lipofuscin***, or "wear-and-tear pigment," :

- Is an insoluble brownish-yellow granular intracellular material that accumulates in tissues (particularly the heart, liver, and brain) as a function of age or atrophy.
- Lipofuscin represents complexes of lipid and protein that derive from the free radical-catalyzed peroxidation of polyunsaturated lipids of subcellular membranes
- It is not injurious to the cell

- but is a marker of past free radical injury.
- The brown pigment, when present in large amounts, imparts an appearance to the tissue that is called *brown atrophy*.
- **2. Melanin** : Is an endogenous, brown-black pigment that is synthesized by melanocytes located in the epidermis and acts as a screen against harmful ultraviolet radiation
- Although melanocytes are the only source of melanin adjacent basal keratinocytes in the skin can accumulate the pigment (e.g., in freckles)

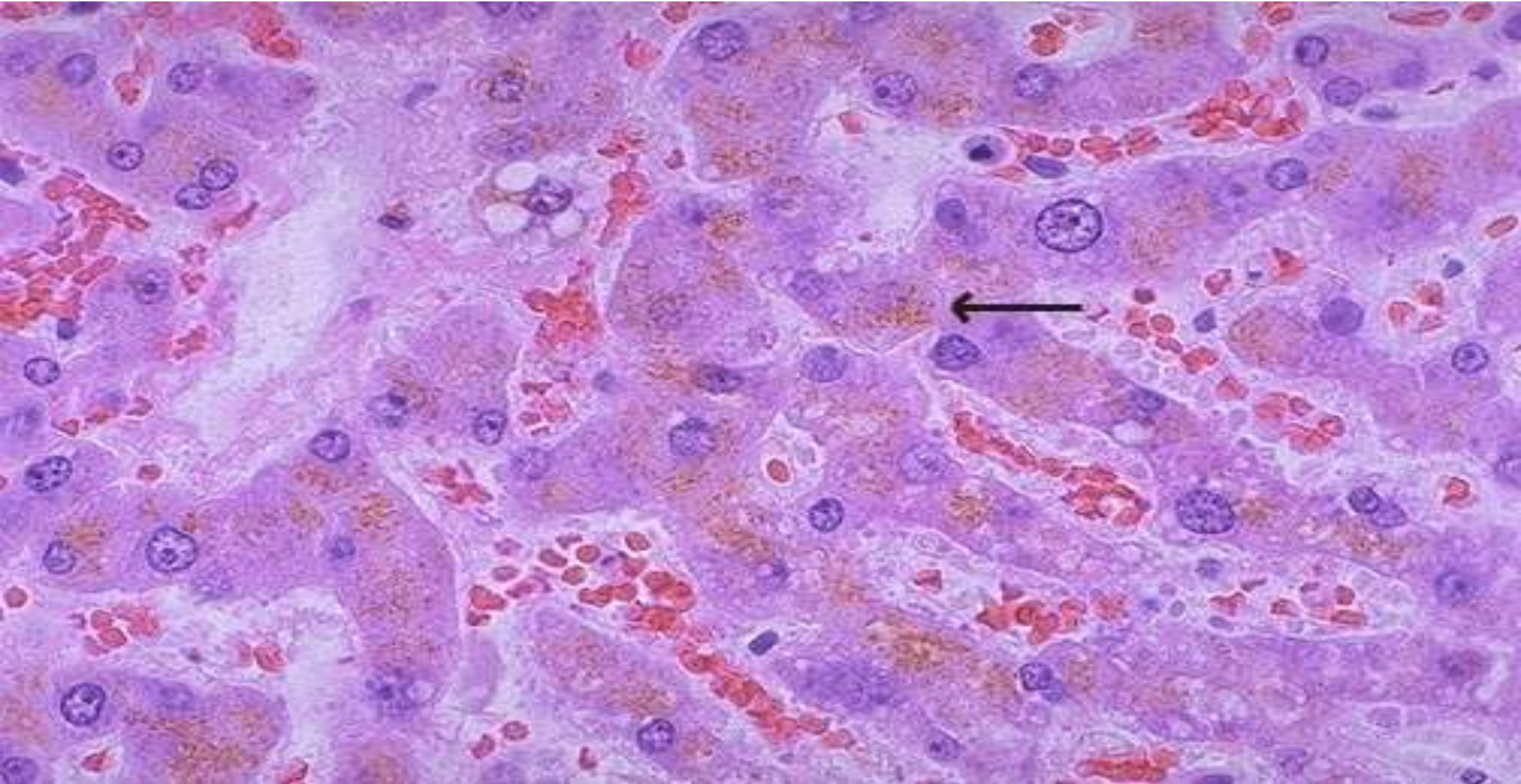


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**3. Hemosiderin:** Is a hemoglobin-derived granular pigment that is golden yellow to brown and accumulates in tissues when there is a local or systemic excess of iron.

- Iron is normally stored within cells in association with the protein *apoferritin*, forming ferritin micelles.
- Excessive deposition of hemosiderin, called *hemosiderosis*, and more extensive accumulations of iron seen in *hereditary hemochromatosis*

# Lipofuscin



# PATHOLOGIC CALCIFICATION

- It implies the abnormal deposition of calcium salts, together with smaller amounts of iron, magnesium, and other minerals.
- There are two types of pathologic calcification:-

## A. dystrophic calcification :

- When the deposition occurs in dead or dying tissues
- The serum levels of calcium are normal.
- Examples
  1. It is seen in the *atheromas* of advanced atherosclerosis,
  2. It can develop in aging or damaged heart valves and is an important cause of aortic stenosis in elderly persons.
  3. Is common in areas of caseous necrosis in tuberculosis

NOTE:- While hypercalcemia is not a prerequisite for dystrophic calcification, it can exacerbate it

B. Metastatic calcification : Occur in normal tissues whenever there is hypercalcemia and causes of hypercalcemia are :

1. Hyperparathyroidism
2. Destruction of bone due to the effects of tumors such as multiple myeloma, leukemia, or metastasis
3. Vitamin D intoxication
4. *Sarcoidosis*
5. *Renal failure,*

. MORPHOLOGY

- Calcium salts are seen on gross examination as fine white

- . granules or clumps, often felt as gritty deposits
- On histologic examination, it appears as intracellular and/or extracellular basophilic deposits and Over time, heterotopic bone may be formed

**NOTE:** Metastatic calcification principally affects the vessels, kidneys, lungs, and gastric mucosa.

- Although they generally do not cause clinical dysfunction, extensive calcifications in the lungs may be evident on radiographs and may produce respiratory deficits, and massive deposits in the kidney (**nephrocalcinosis**) can lead to renal damage.