

General Pathology



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CELL INJURY (1)

OBJECTIVES

1. DEFINITION OF PATHOGENESIS, ETIOLOGY, ADAPTATION
2. TYPES OF ADAPTATION
3. CELL INJURY TYPES (REVERSIBLE AND IRREVERSIBLE)
4. MORPHOLOGICAL CHANGES IN CELL INJURY
5. CELL DEATH, APOPTOSIS, NECROSIS
6. TYPES OF NECROSIS
7. CAUSES OF CELL INJURY
8. SUBCELLULAR AND BIOCHEMICAL CHANGES IN CELL INJURY
9. INTRACELLULAR ACCUMULATION
10. AGING

Lecture 1

General pathology,

Pathology: is the study “logo i.e. language” of suffering “patho” which will be studied in this course.

It focuses on the fundamental cellular & tissue responses to pathologic stimuli, and it includes the following topics:

1. Cell injury, Adaptation & Cell Death - Chapter 1
2. Inflammation & Repair - Chapters 2 & 3
3. Hemodynamic disorders - Chapter 4
4. Neoplasia - Chapter 6



References :

- ROBBINS BASIC PATHOLOGY
by Kumar et al. 10th Edition (last edition)
- Color Atlas of Histopathology
R.C. CURRAN Oxford University Press.
- Color Atlas of gross pathology.
Oxford University Press.



The study of pathology in general includes:

1. Etiology:

- It is the study of the underlying causes & modifying factors of diseases. To understand the **genetic and the various environmental factors** that are considered to be the causes of diseases, i.e **it describes why the disease occurs.**



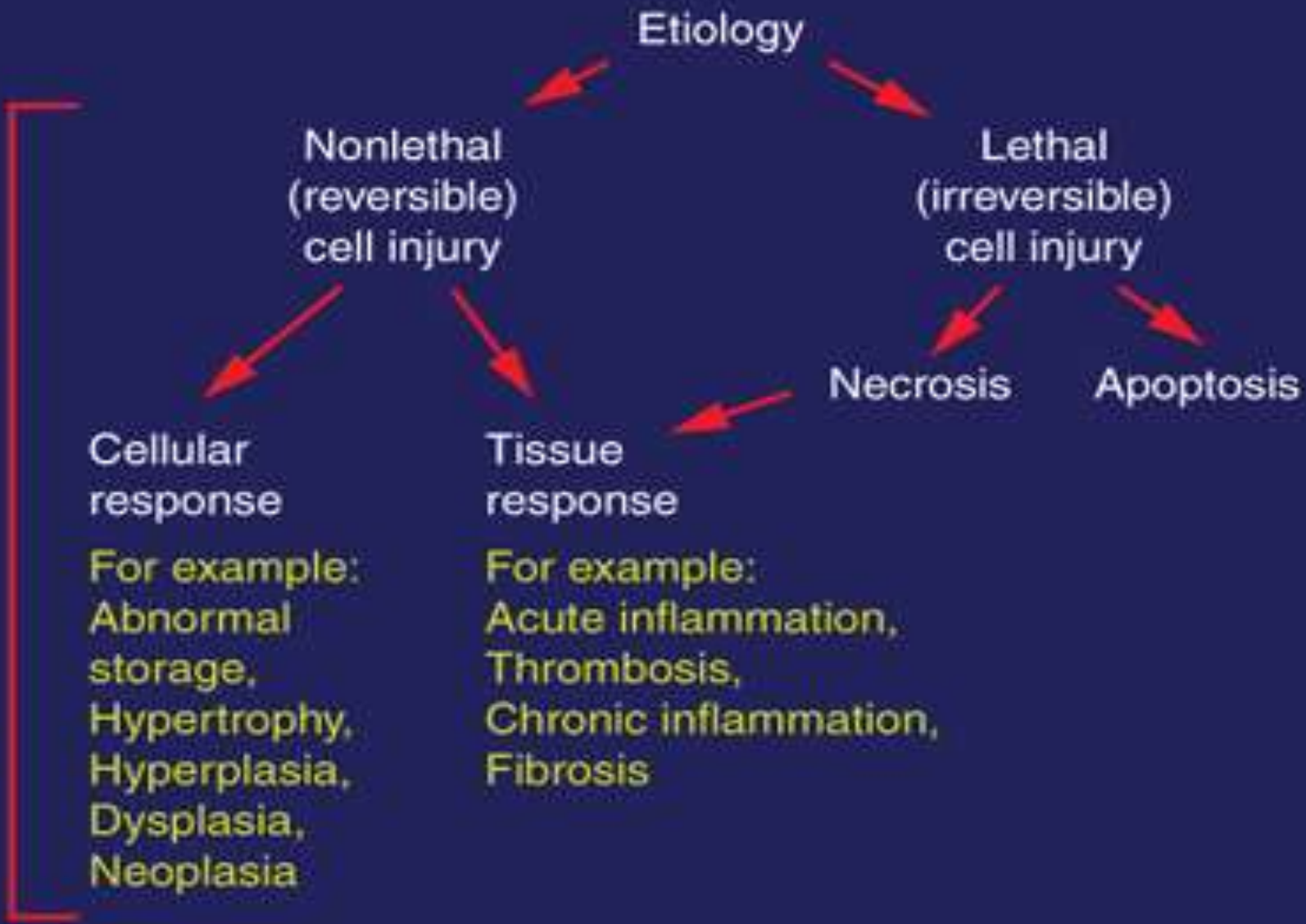
Pathogenesis

2. Pathogenesis :Refers to the steps of development of disease. It describes **how the etiological factors trigger cellular and molecular changes that give rise to the specific functional and structural abnormalities** that characterize the disease i.e it describes how the disease arises.

5- Pathological anatomy and pathology

- ❑ **Pathologists** identify changes in organs and tissues in various diseases, both grossly by naked eye (macroscopically) or microscopically or even at the level of ultrastructural changes using electron microscopy. Both on surgical specimens during life, or Autopsy specimens after death
- ❑ **Pathologists** tend to use a variety of morphological, molecular, microbiological and immunological techniques to reach the correct diagnosis.

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- As cells encounter physiologic stresses or pathologic stimuli, it will start achieving a new steady state and try to preserve viability and function.
- They can undergo **Adaptations** which are **reversible** including changes in the **number, size, phenotype, metabolic activity, or functions of cells** in response to changes in their environment.

Adaptation Can be :

- Physiologic adaptations usually represent responses of cells to normal stimulation by hormones or endogenous chemical mediators (e.g., the enlargement of the breast during puberty & induction of lactation in pregnancy).
- Pathologic adaptations often occurring due to pathological lesions, share the same underlying mechanisms like the physiological adaptations, & allow the cells to modulate their environment & ideally escape injury.

- **I- Hypertrophy:** Increase in size of cells and organ.
- **II- Hyperplasia:** Increase in number of cells in an organ.
- **III – Atrophy:** Decrease in size of cells & organ.
- **IV – Metaplasia:** Change in type of cells into another adult type of cells .

Hypertrophy:

- ❑ Is an increase in the size of an organ due to increase in cell size caused by stress such as increased demand.
- ❑ Increased in functional capacity
- ❑ Hypertrophy is achieved via:
 1. Gene activation
 2. Increased protein synthesis
 3. Increased organelles production within the cells.
 4. Hormonal stimulation
 5. Growth factor stimulation
 6. Increased functional demand

❑ Hypertrophy usually occurs in permanent tissues which cannot undergo cell division .

As the skeletal muscle , cardiac muscle (myocardium) or neurons.

It can be **Physiological** as seen in skeletal muscle during exercise , or in heavy weight lifters ..

❑ Or due to Hormonal stimulation : as in gravid uterus during pregnancy , usually combined with hyperplasia which is increase in cell number .

or **Pathological** due to

a- **increased demand** myocardial cells due to hypertension , or aortic valve disease leading to enlargement of the heart size induced by increase in the size of myocardial cells .

b- Compensatory enlargement of residual viable cardiac myocytes after myocardial infarction (MI) in which the hypertrophy compensate for the death of neighboring ischemic cells .

c- Hormonal stimulation : as seen due to the increase production of growth hormone by pituitary gland leading to gigantism or acromegally.

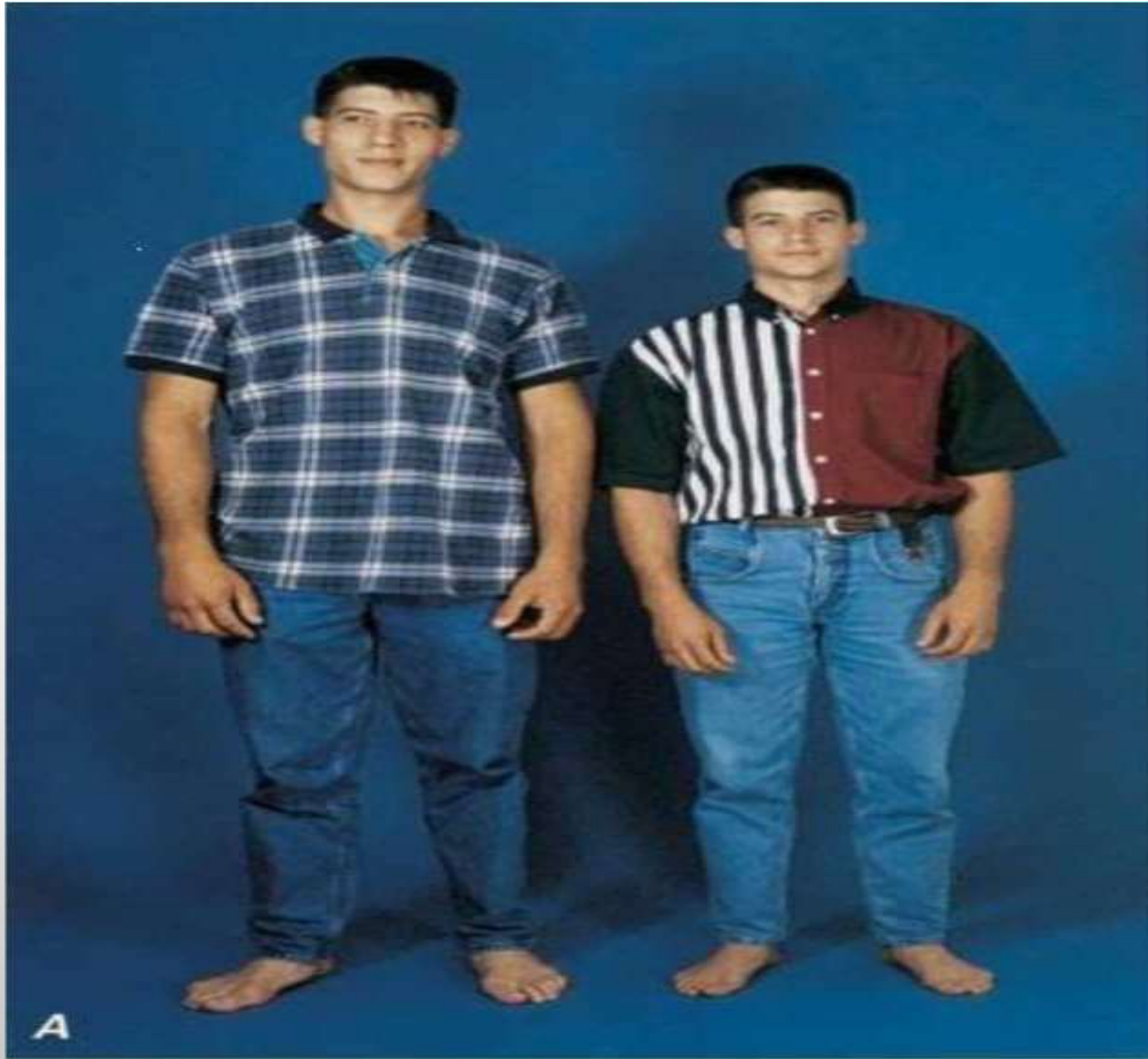


Figure : Gigantism seen in young man (left) compared to normal (age-matched) young man (right) .Due to hypertrophy of body organs & tissues including bones caused by increased growth hormone by pituitary gland before puberty .



Figure 4 : Acromegaly , photographic view . Showing enlargement of jaw bones and soft tissues , due to excessive production of growth hormone by pituitary gland after puberty & closure of epiphyseal plates of bones.

- ❑ Here the mechanism of hypertrophy occurs due to **mechanical trigger** as **stretch in skeletal muscle** during exercise , or in myocardial cells of the heart in **hypertension** . Or induced by a **trophic trigger** which is due to release of soluble mediators that stimulate cell growth **as growth factors and adrenergic hormones** .
- ❑ These stimuli turn on signal transduction pathways that lead to induction of a number of genes , which in turn stimulate synthesis of many cellular proteins , including growth factors and structural proteins .
- ❑ The increased proteins & myofilaments per cell, will increase the force generated with each contraction , enabling the cells to meet the increased work demands .

□ Whatever the exact mechanism or mechanisms of hypertrophy, **a limit is reached beyond which the enlargement of muscle mass can no longer compensate for the increased burden & in the heart, cardiac failure follows** due to number of “degenerative changes” occur in the myocardial fibers, like fragmentation & loss of the contractile elements .

These degenerative changes occur as a result of:

- (1) **Limited blood supply** that is required to supply the enlarged fibers.
- (2) **Inadequate** mitochondrial supply of **ATP**.
- (3) **Failure** of the biosynthetic machinery to provide the contractile **proteins** & cytoskeletal elements.



Figure Skeletal muscle hypertrophy in heavy weight -lifter (physiological hypertrophy).

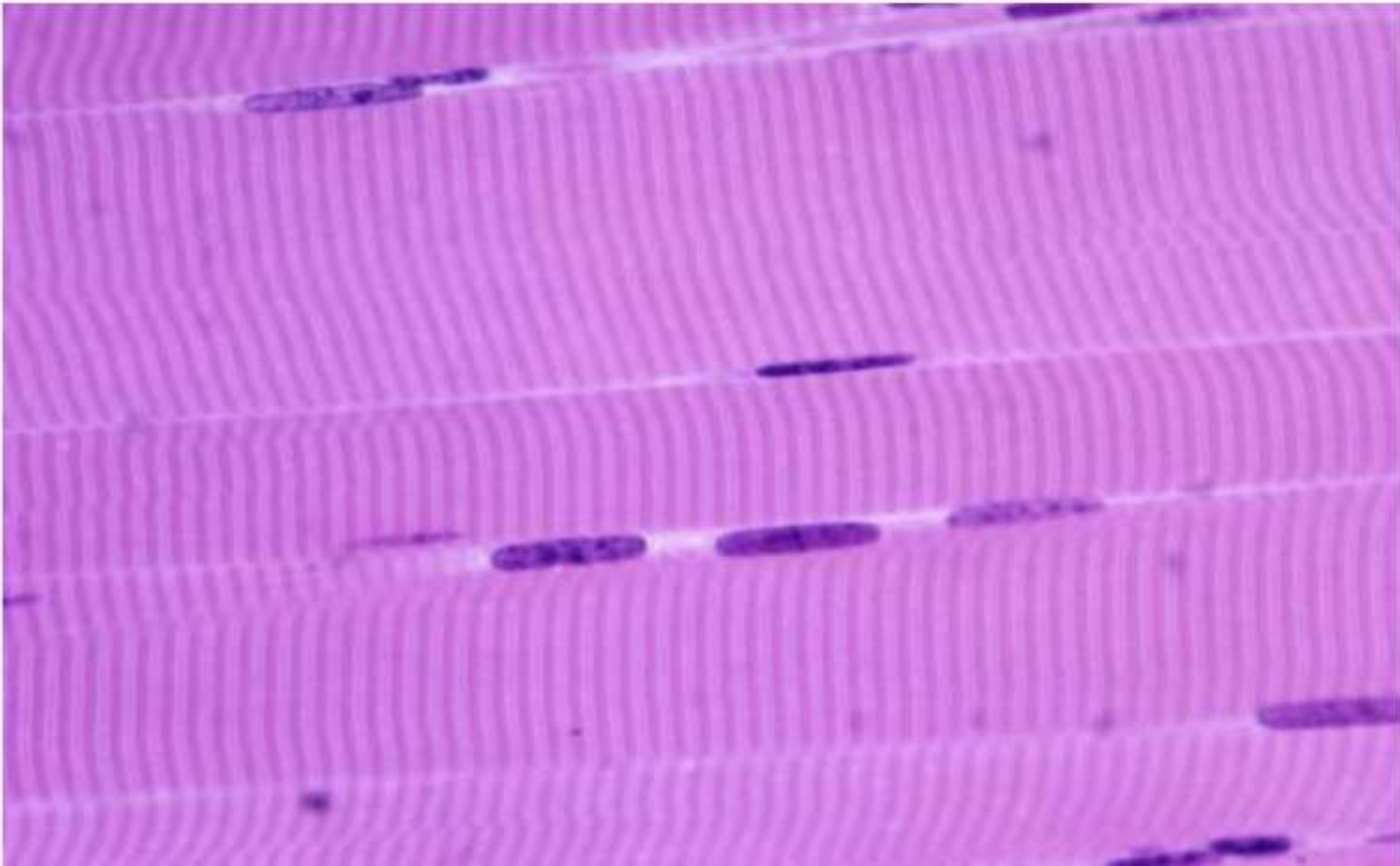


Figure 6 - Microscopic view of normal skeletal muscle (Longitudinal view) .

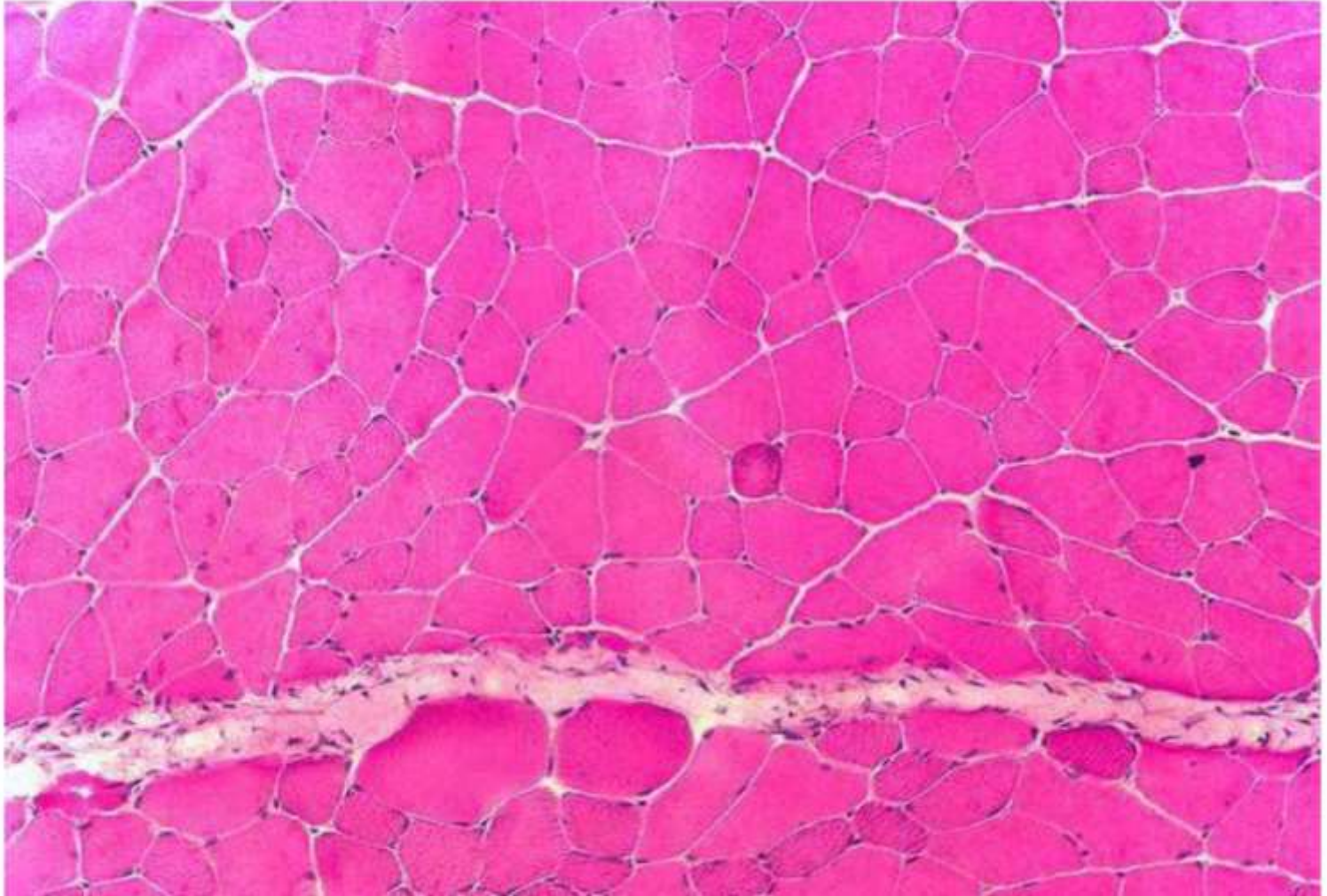


Figure - Normal skeletal muscle (Cross section) H&E stain , microscopic view .

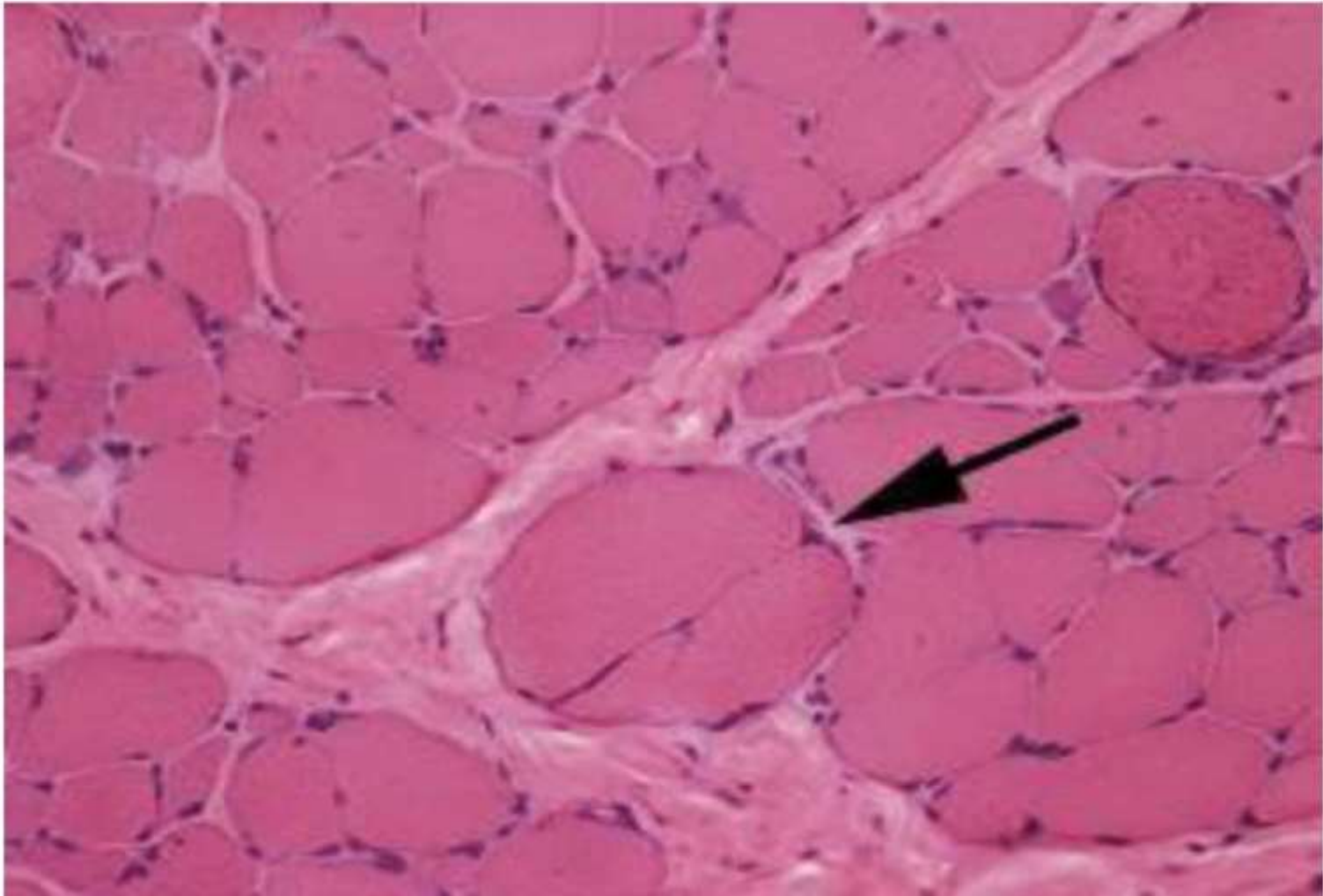


Figure - Hypertrophied skeletal muscle fibers



Figure 9 - Hypertrophy of skeletal muscle.



Figure 10 : Normal heart (left) .Hypertrophied heart . (right) .

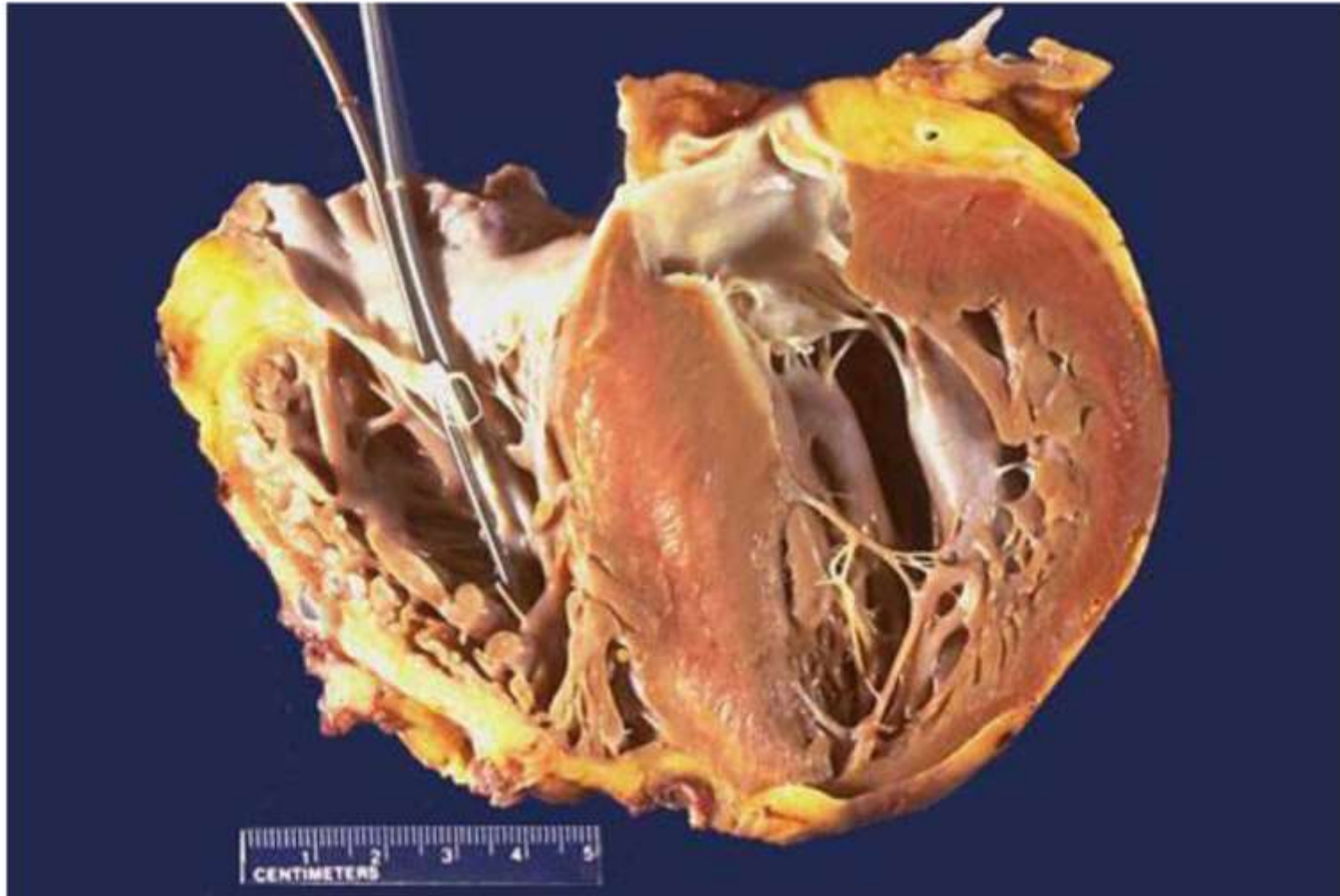


Figure 11 : Gross view of the heart showing left ventricular hypertrophy seen in hypertension ,(pathological) .

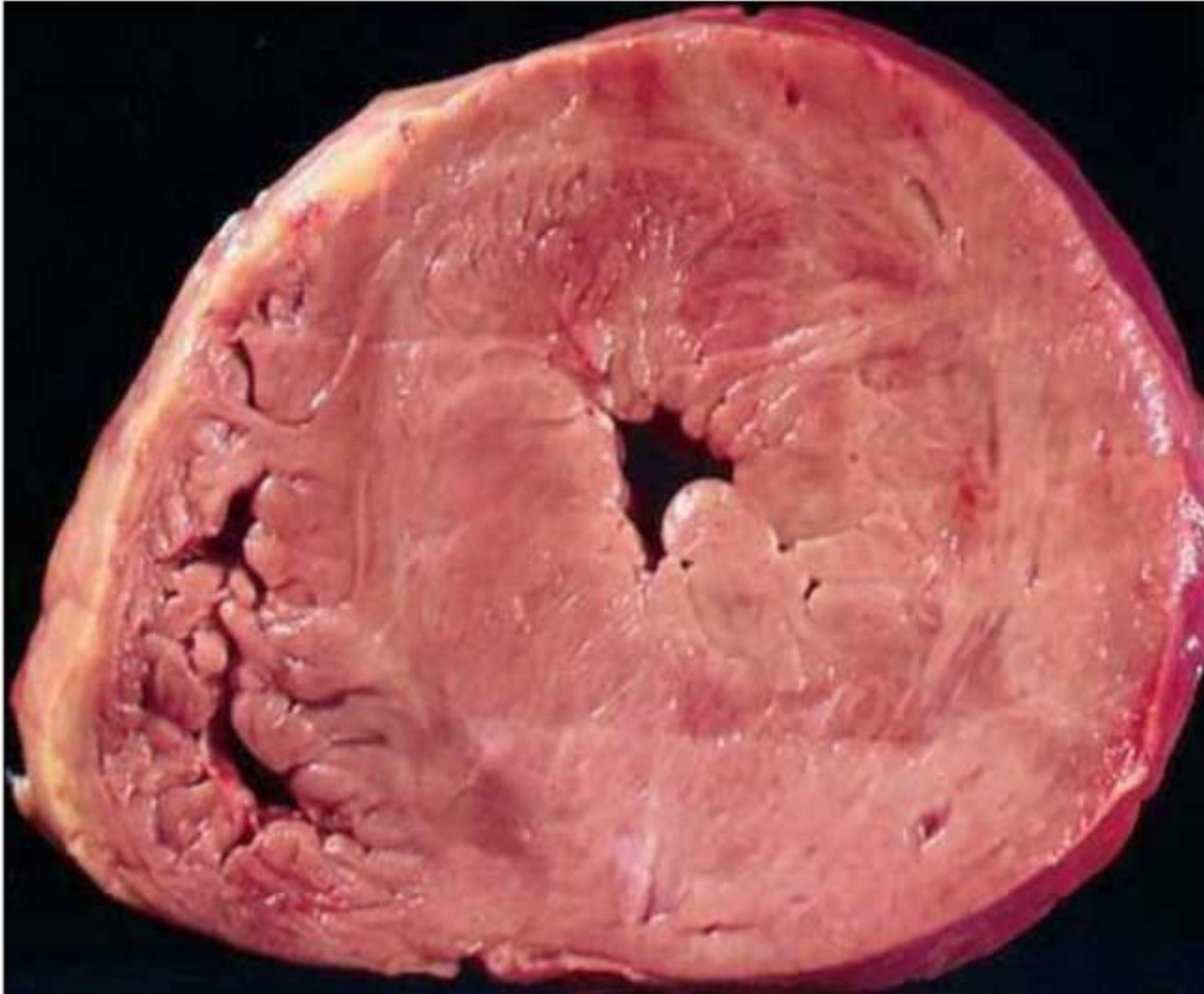


Figure 12 - Hypertrophy of left ventricle in hypertension.

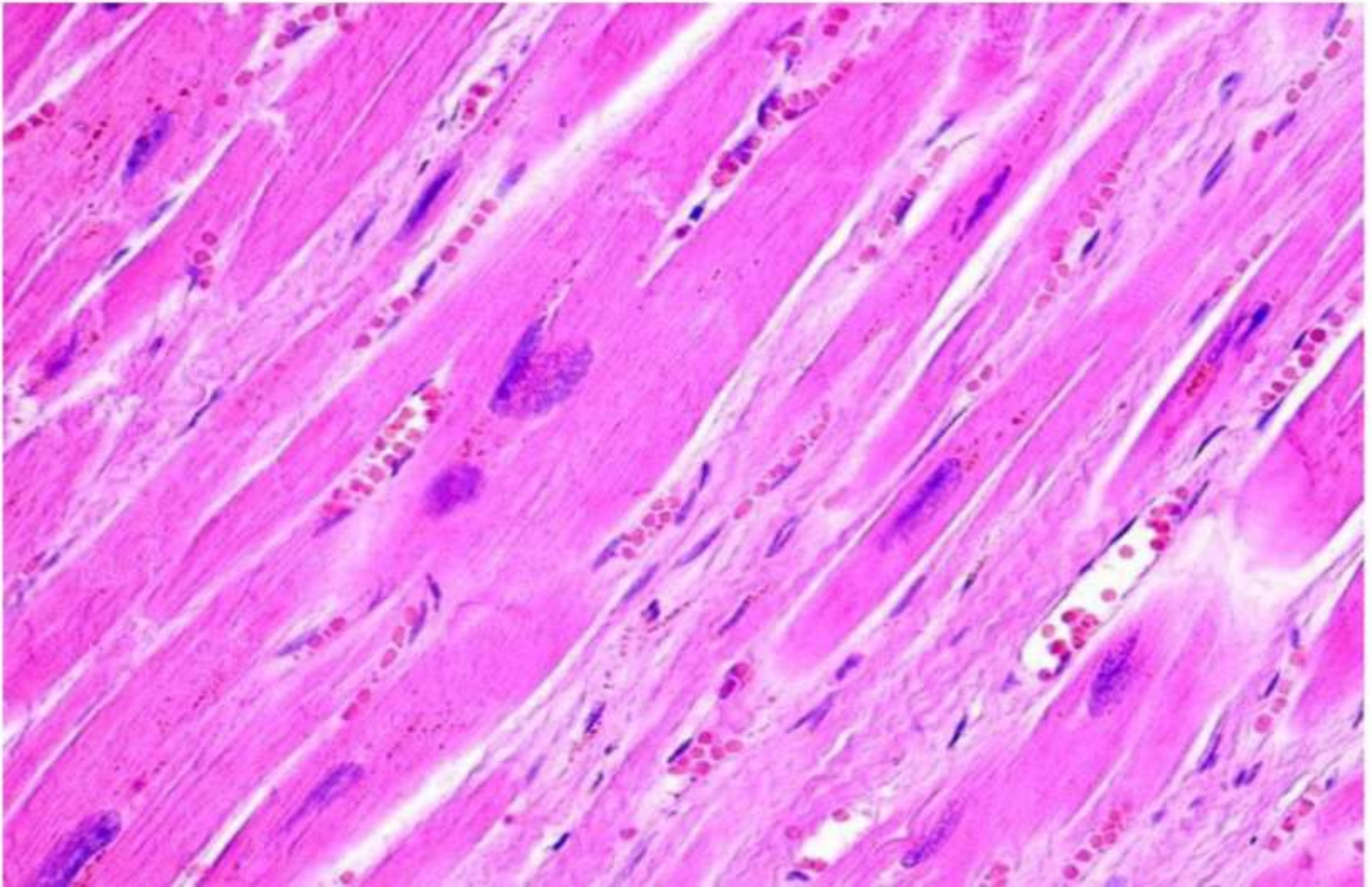


Figure 14 - Hypertrophy of cardiac muscle cells

Hyperplasia :

□ Is an increase in the number of cells leading to increase in size of the organ .

Usually achieved by production of new cells by the of stem cells .

Can be :

I- **Physiological:** Can be induced by :

a- **Hormonal stimulation** : as in female uterus during pregnancy hyperplasia is accompanied by hypertrophy or in the female breast during puberty or lactation .

b- **Compensatory physiological hyperplasia** :

that is, hyperplasia which occurs when a portion of the tissue is removed or diseased.

Examples :

In Surgical resection :

- ❑ For example ; when the liver is partially resected , mitotic activity in the remaining cells begins as early as 12 hours later, eventually restoring the liver to its normal weight, within three months .**

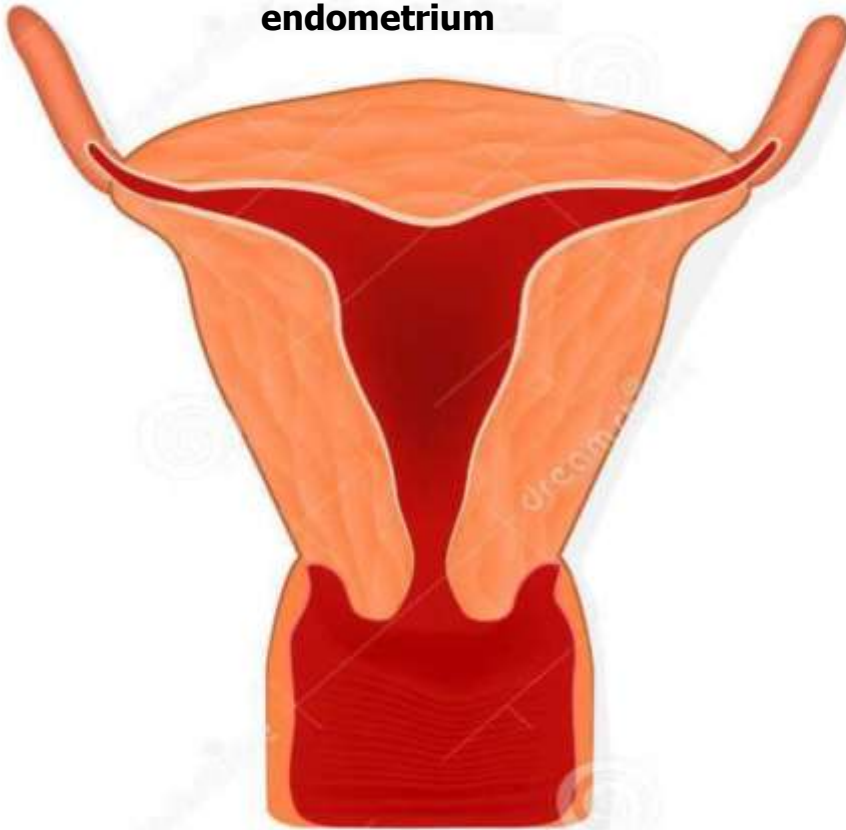
- ❑ The hyperplasia in this setting is due to stimulation by polypeptide growth factors (GF) produced by remnant hepatocytes as well as non-parenchymal cells found in the liver.**

II- **Pathological** : due to hormonal stimulation as in endometrial hyperplasia due to estrogen hormone stimulation, leading to thickening of uterine endometrium ,clinically presents as a dysfunctional uterine bleeding called menorrhagia .

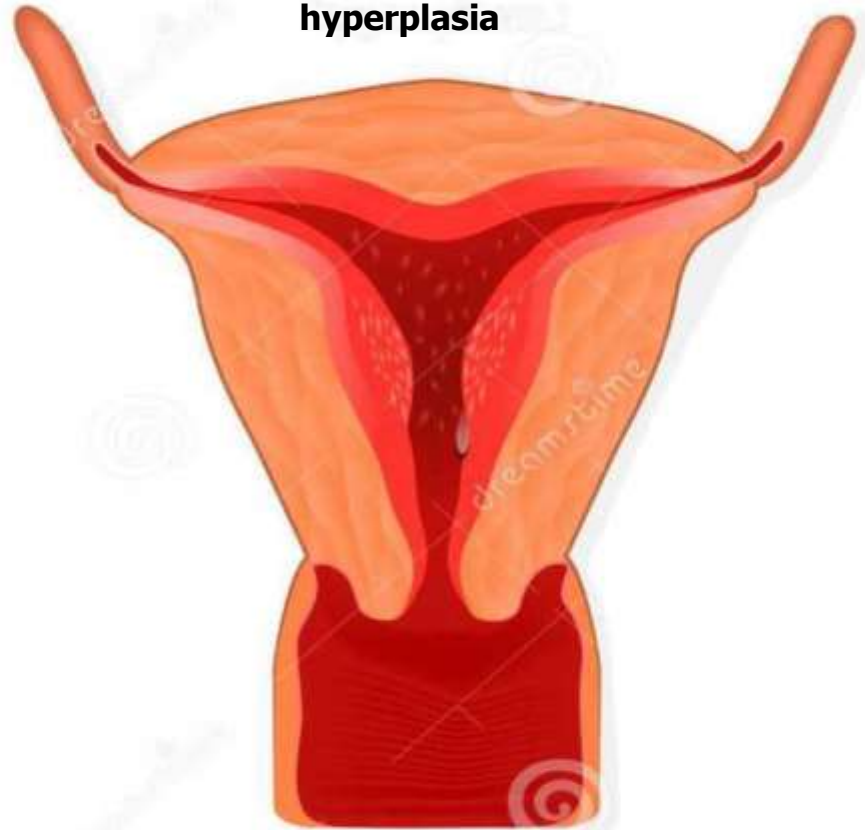
❑ Or in benign prostatic hyperplasia in men causing urinary tract obstruction due to enlargement of the prostate .

❑ **In wound healing : the GF-stimulated fibroblasts & blood vessels (BV) endothelial cells (EC) hyperplasia to facilitate repair . In this case growth factors are produced by white blood cells (WBCs) that accumulate during tissue injury.**

**Normal
endometrium**



**Endometrial
hyperplasia**



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Figure 16 : Diagrammatic view of normal uterus (left) showing normal endometrium & endometrial hyperplasia (right)

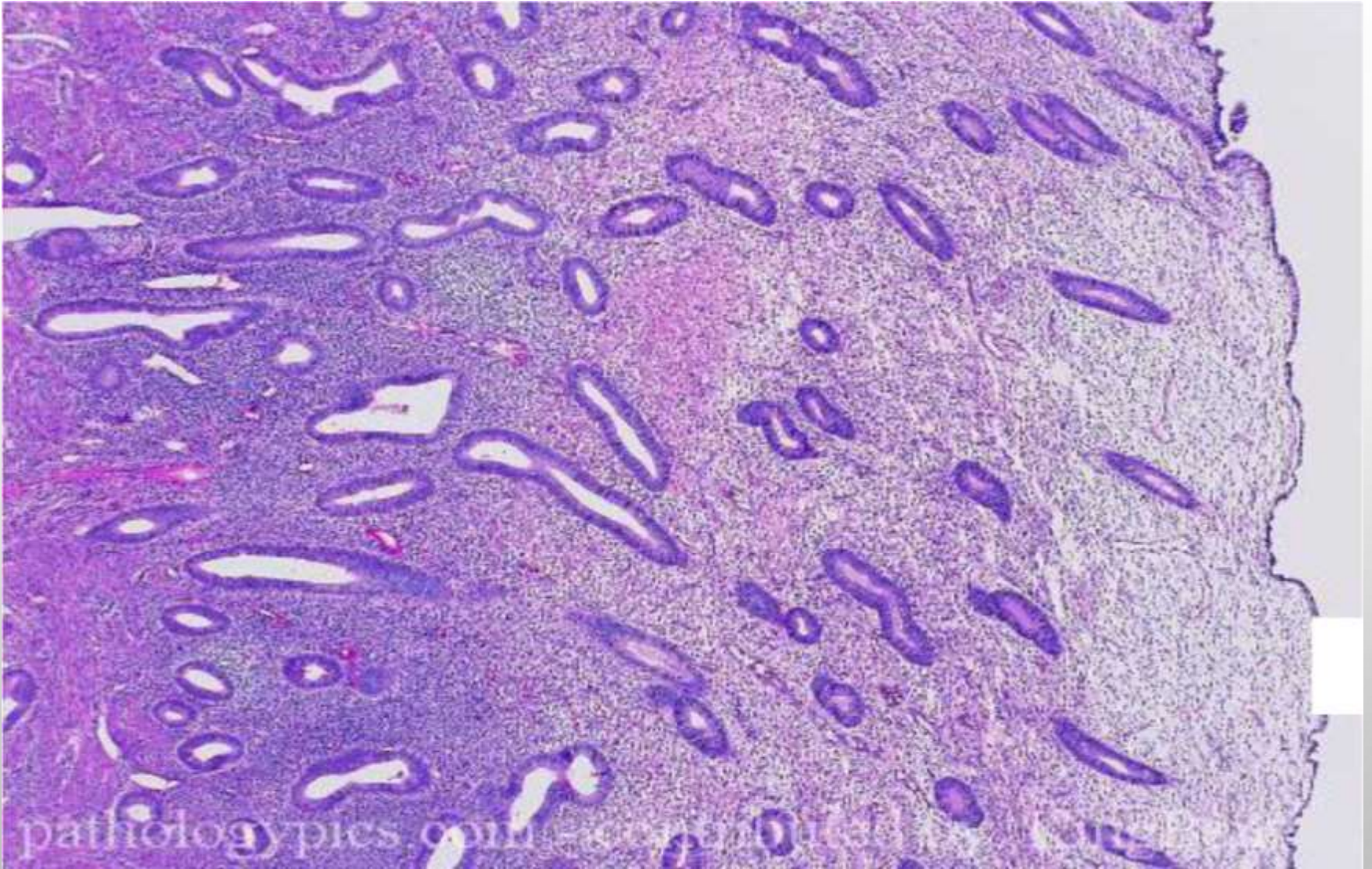


Figure 17 : Microscopic view of normal endometrium.

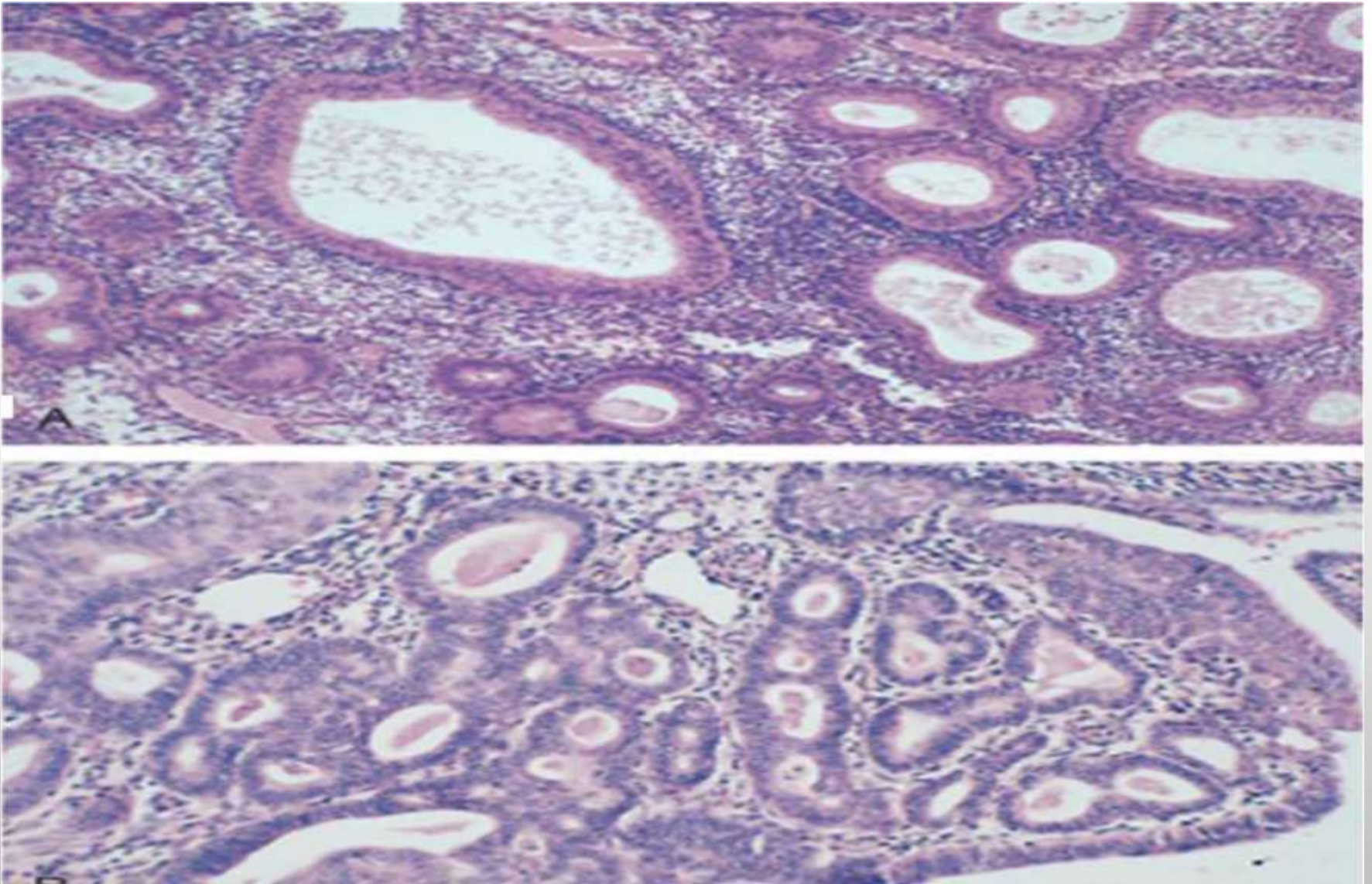
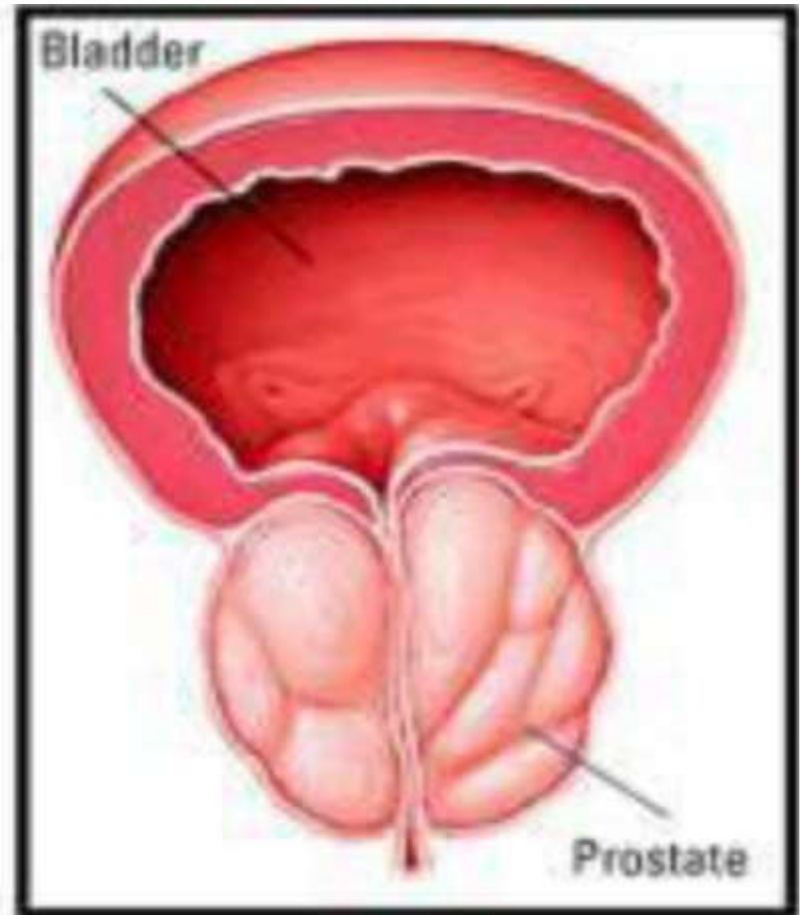
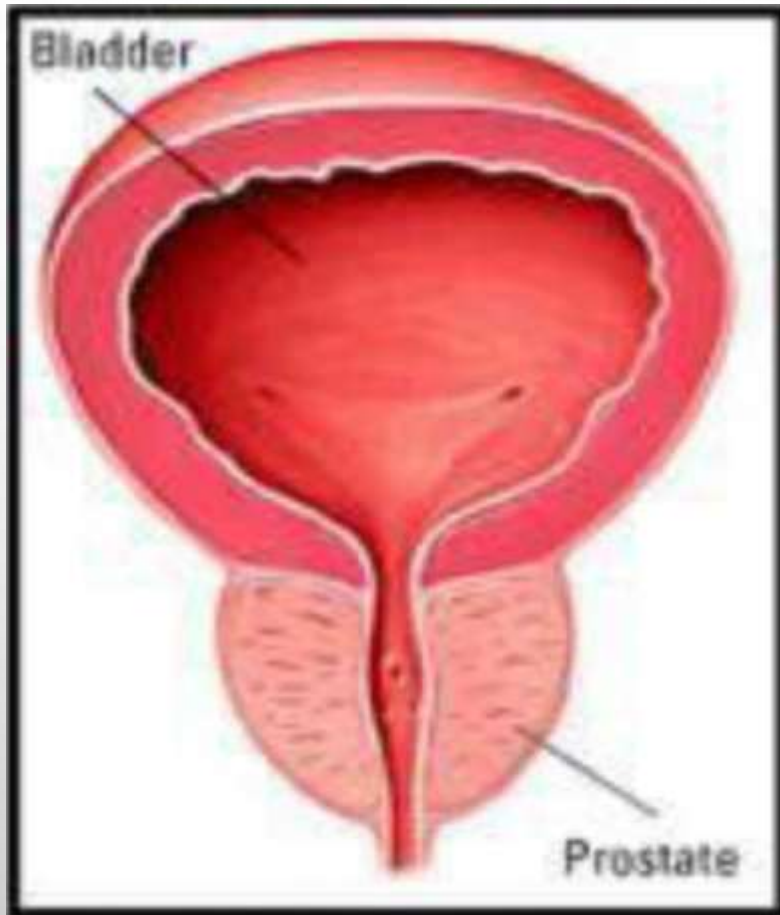


Figure 18 : Upper view : Microscopic view of endometrial hyperplasia , showing cystic glands . Lower view : Endometrial hyperplasia showing numerous glands .



Normal Prostate Enlarged Prostate (BPH)

Figure 20 : Diagrammatic view of normal prostate (left) and benign prostatic hyperplasia (right) .

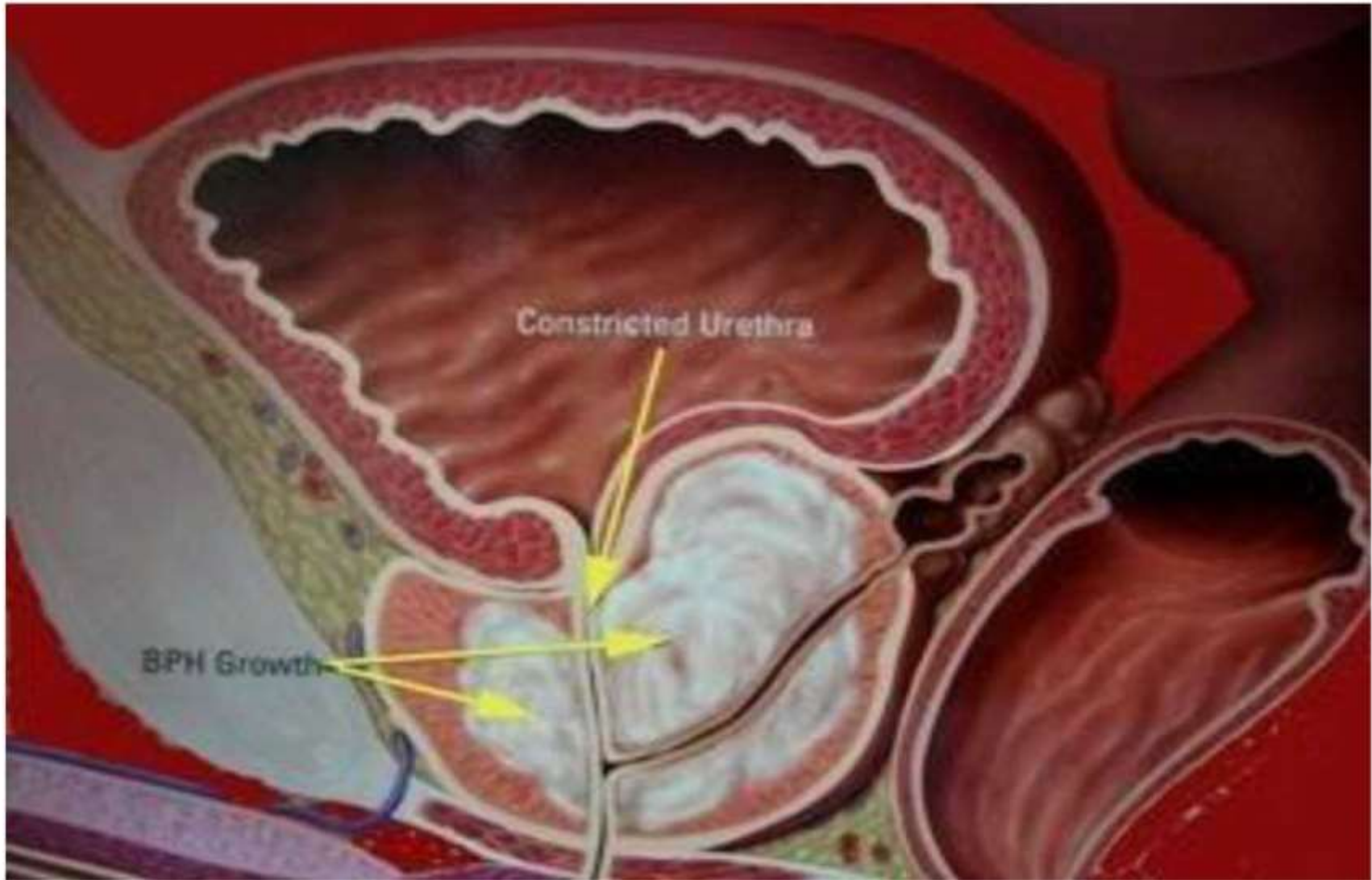


Fig. 21 - Benign Prostatic Hyperplasia (BPH)

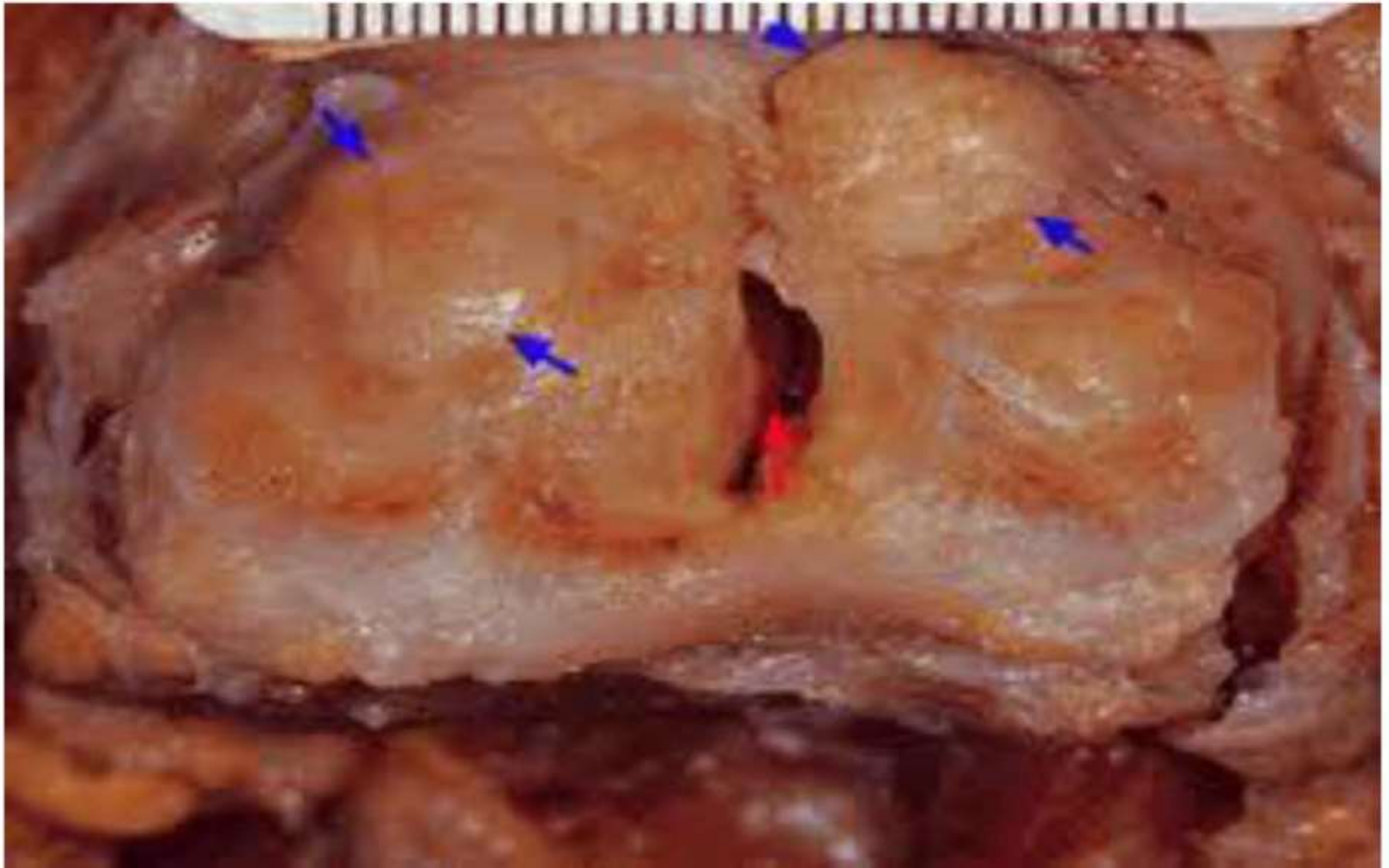
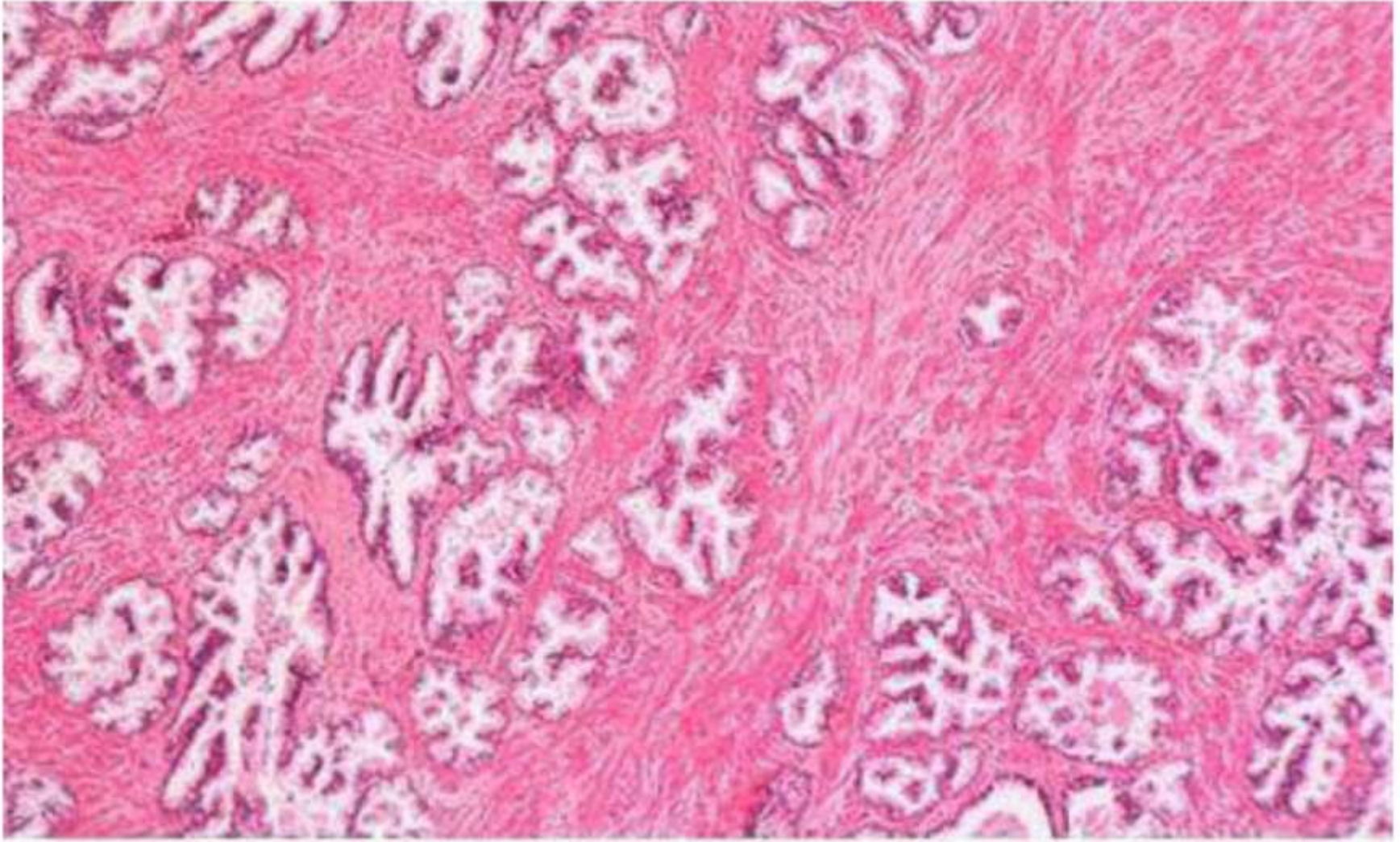


Fig. 22 - Gross appearance of prostate gland showing benign prostatic glandular hyperplasia .



Prostate 40x

Figure 23 : Microscopic view of normal prostate .

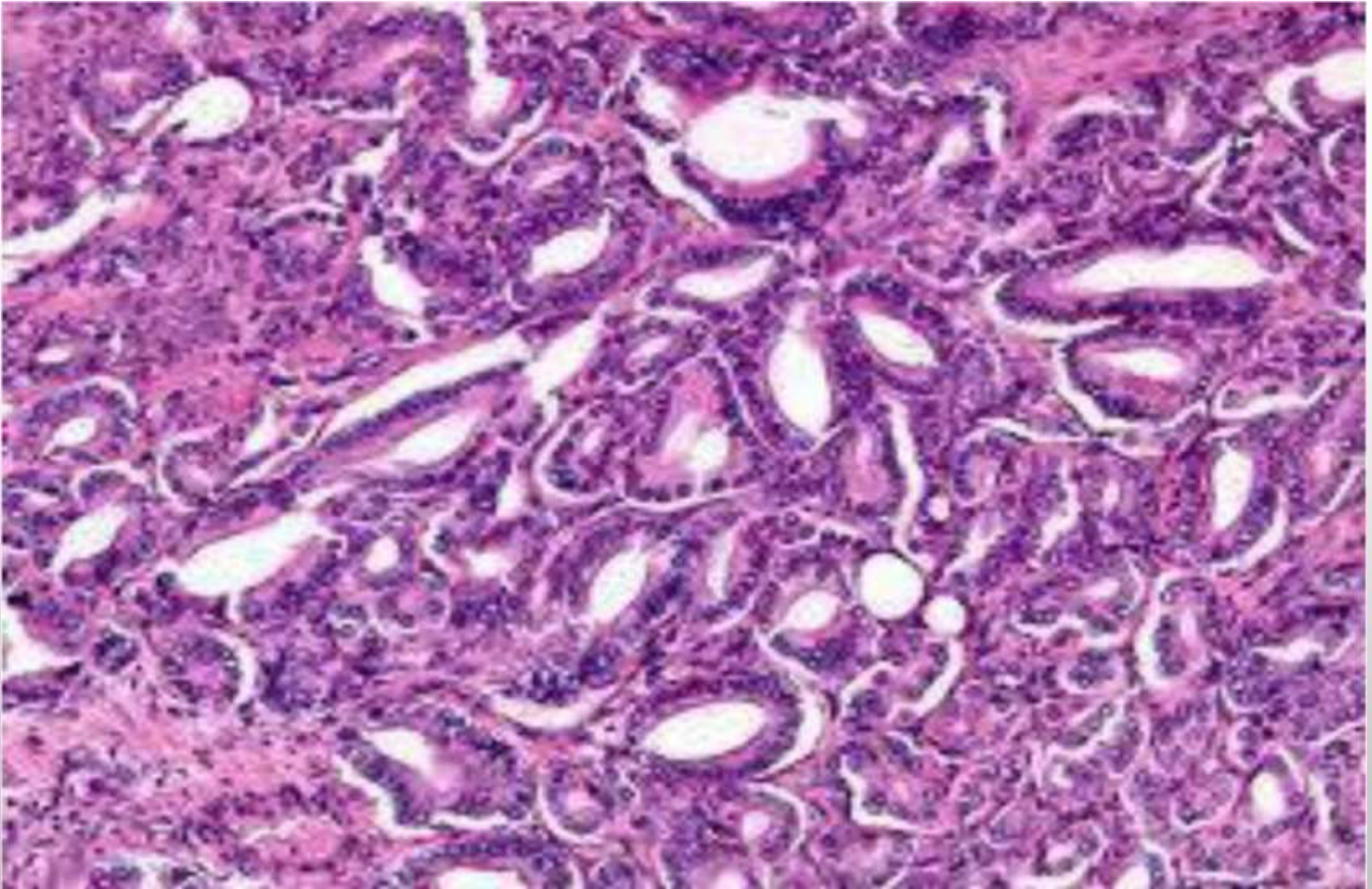
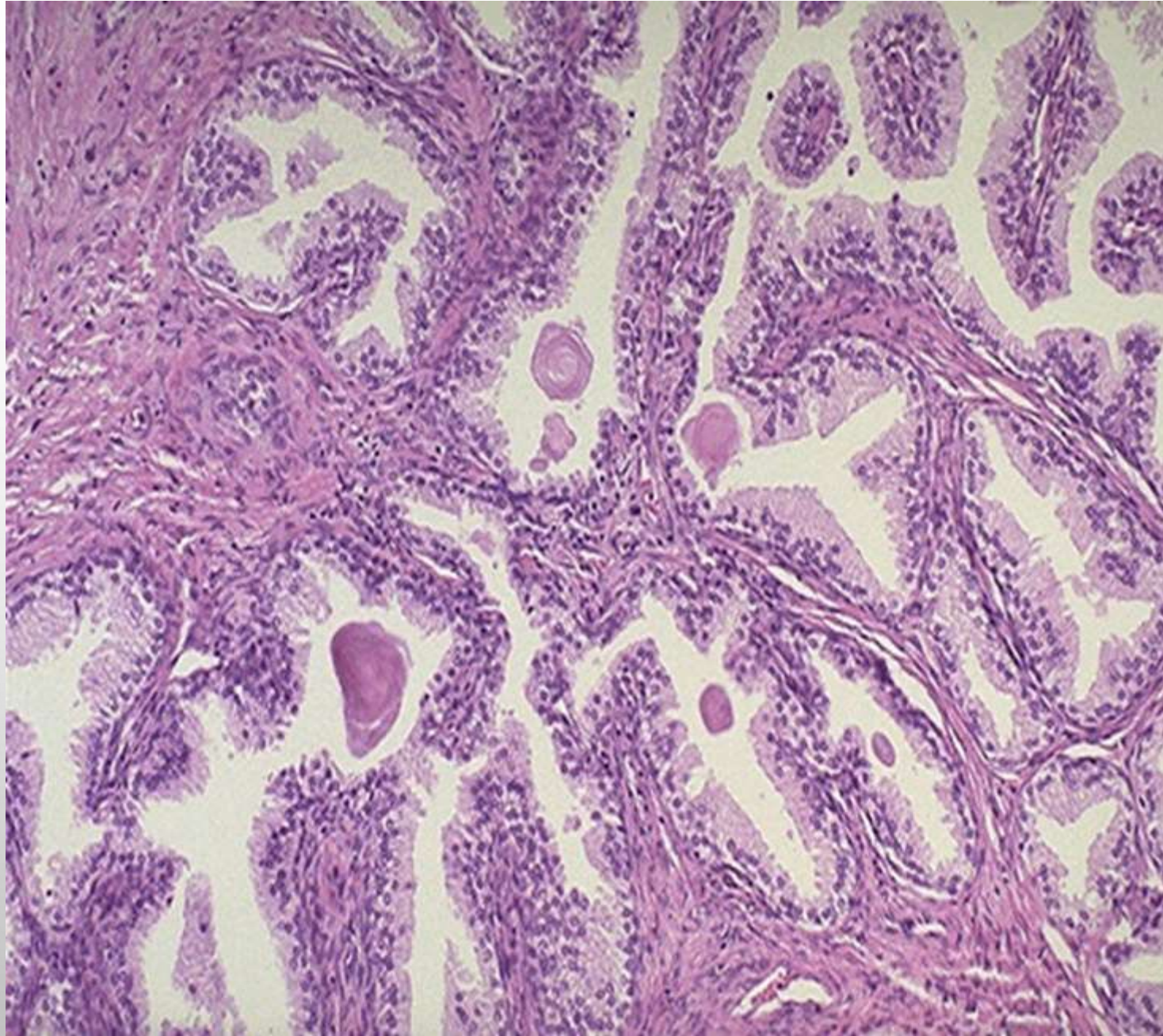


Figure 24 : Microscopic view of benign prostatic hyperplasia , showing increased number of glands.

Here is one of the nodules of **hyperplastic prostate**, with many glands along with some intervening stroma. The cells making up the glands are normal in appearance, but there are just too many of them



- ❑ It is important to note that:
- ❑ In both above examples of pathologic hyperplasia, the hyperplastic process "remains controlled" i.e., if hormonal or GF stimulation ends , the hyperplasia disappears.
- ❑ This differentiates these hyperplastic processes from neoplastic process (neoplasm or tumors) , whether benign or malignant in which cells continue to grow despite the absence of hormonal stimuli.
- ❑ Nevertheless, pathologic hyperplasia constitutes a fertile soil in which cancerous proliferation may eventually arise.

Atrophy

- ❑ Decrease in number of cells ,or shrinkage (decrease) in the size of the cell by the loss of the cell substance.__
- ❑ When a large number of cells are affected, the entire tissue or organ diminishes in size, i.e., becoming atrophic.

Causes of atrophy include:

- (1) Decreased workload (e.g., immobilized muscles in a paralysed limb)
- (2) Decreased or Inadequate nutrition .
- (3) Decreased blood supply as in atherosclerosis .
- (4) Aging .
- (5) Loss of endocrine stimulation .
- (6) Loss of nerve supply (Denervation).

Loss of cells may occur in :

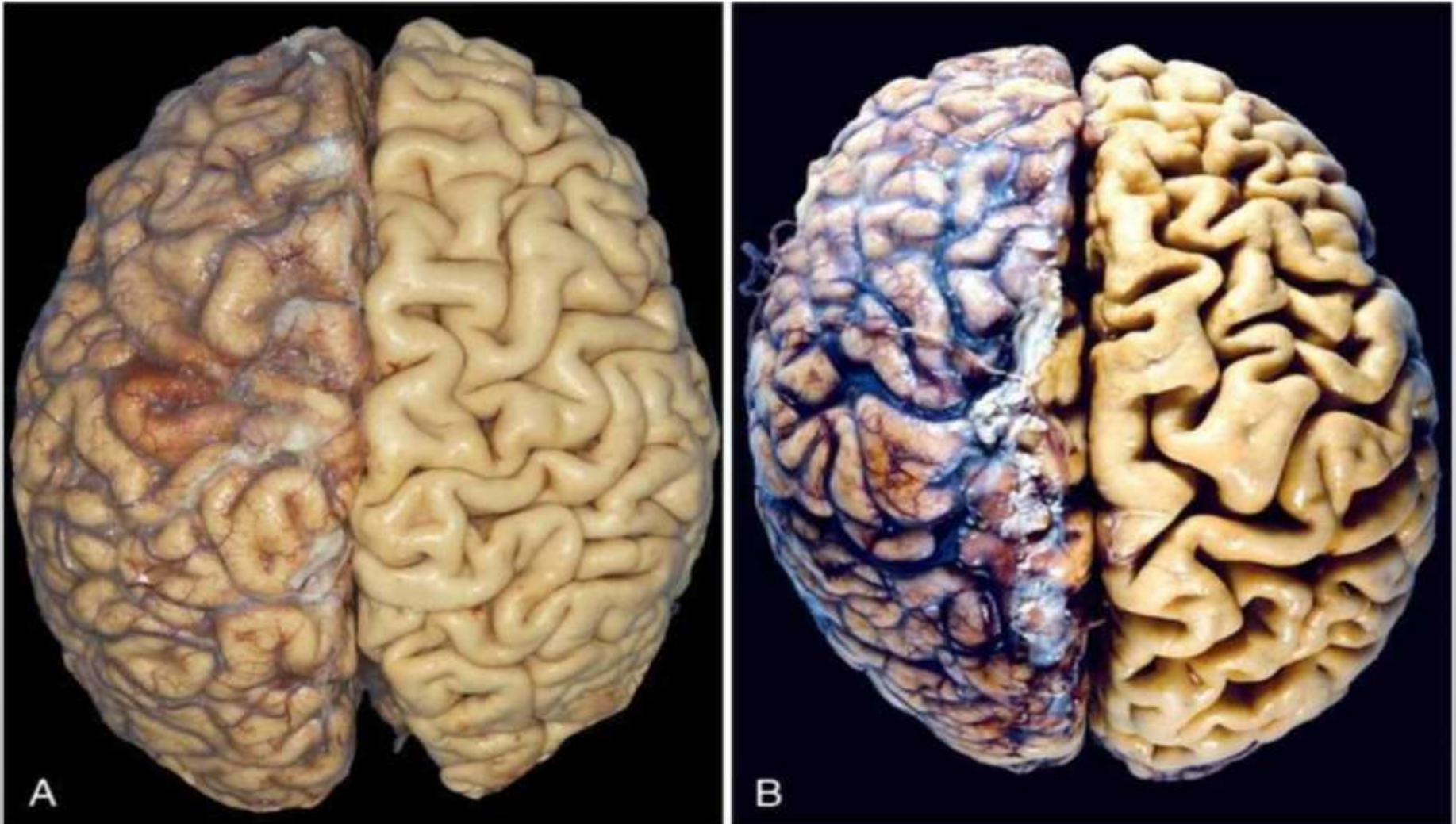
1- **Apoptosis** : a special type of cell death also decrease the number of cells of a tissue or organ .

2- **via Ubiquitin& proteasome pathway** :

☐ Nutrient deficiency and disuse may activate ubiquitin ligases enzyme which enhances attachment of multiple copies of small peptide ubiquitin molecules to cellular proteins as the cellular intermediate filaments of cytoskeleton and target them to degradation by proteasomes .

3- Also the cells may undergo **autophagocytosis** i.e phagocytosis of cellular components which involves generation of autophagic vacuoles that fuse with lysosomes whose hydrolytic enzymes breakdown cellular components .

F 25 : A, left: Normal young adult brain., B, right: Atrophy of the brain in an 82 years-old male with atherosclerotic disease. Note that loss of the brain substance (due to aging & reduced blood supply) narrows the gyri & widens the sulci.



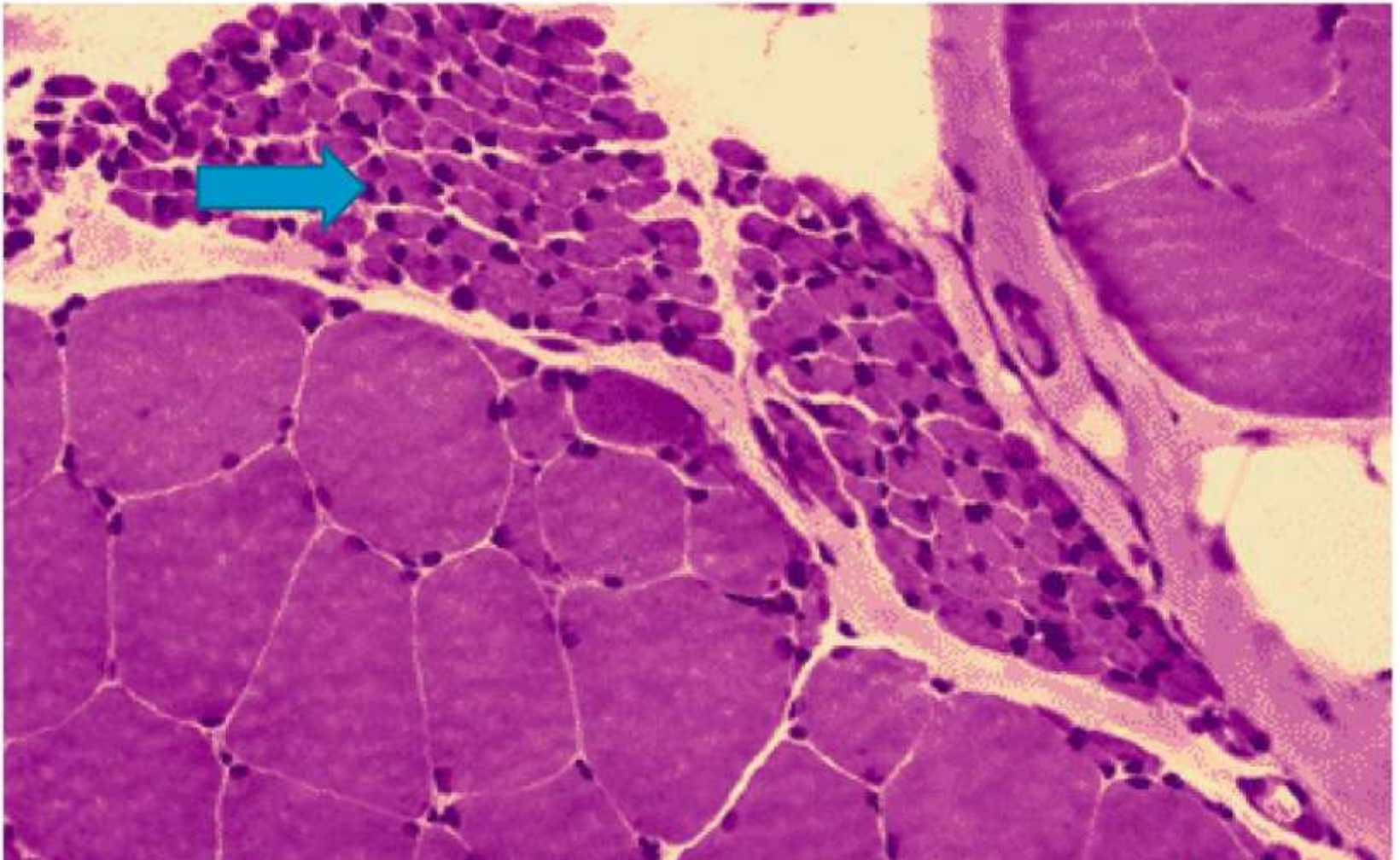


Figure 27 -Atrophy of skeletal muscle fibers due to denervation (center) compared to normal skeletal muscle fibers seen at both sides of section.

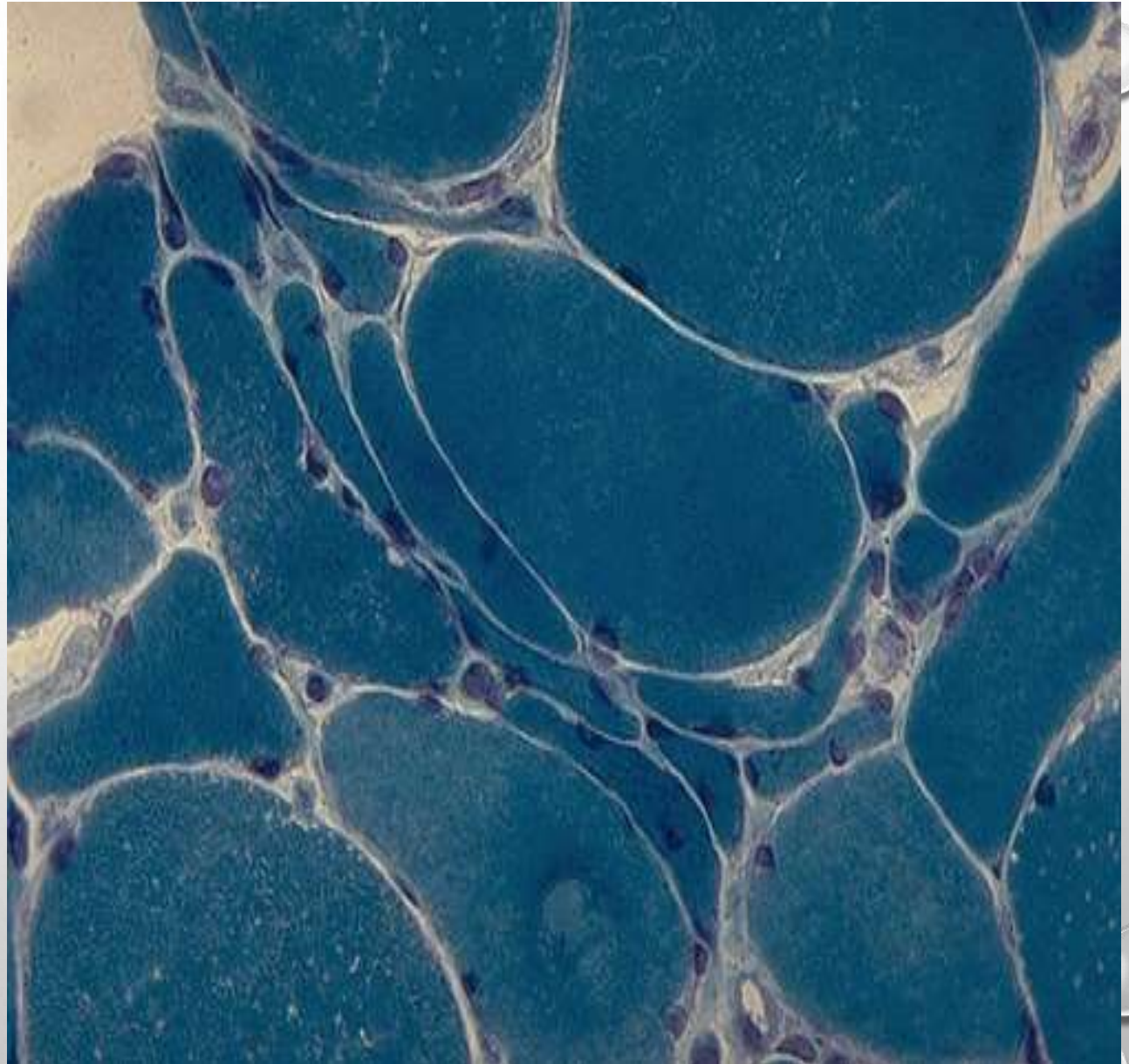
❑ some of these skeletal muscle fibers here show atrophy, compared to normal fibers. The number of cells is the same as before the atrophy occurred, but the size of some fibers is reduced. This is a response to injury by "downsizing" to conserve the cell. In this case, innervation to the small, atrophic fibers was lost.

❑ **(This is a trichrome stain.)**

❑ The most common cause is disuse

❑ In healthy people **lack of exercise**

❑ **In bedridden people they have considerable atrophy**



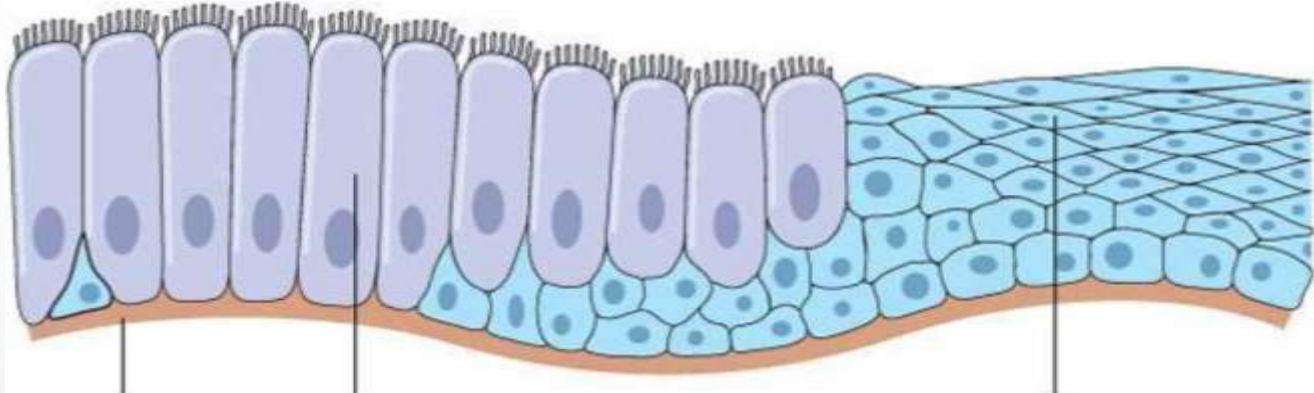
Metaplasia :

- ❑ Is a reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type.
- ❑ Cells sensitive to a particular stress are replaced by other cell types better able to withstand the adverse environment; this arises by the genetic “reprogramming” of the epithelial reserve (Stem) cell .
Examples:

I- Squamous metaplasia that may occurs in the respiratory epithelium in habitual cigarette smokers , in which normal ciliated columnar epithelial cells of the trachea & bronchi are focally or widely replaced by stratified squamous epithelial cells.

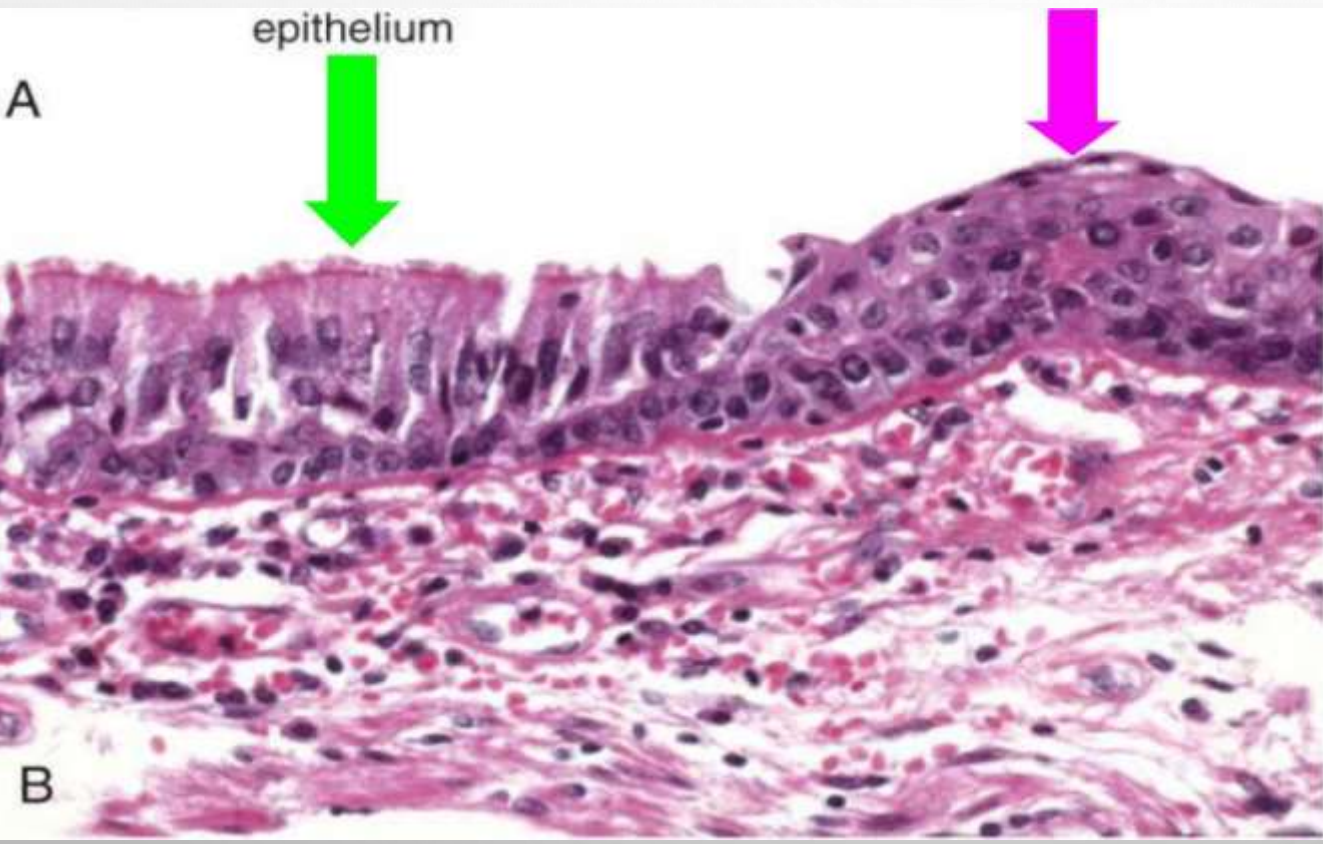
Vitamin A deficiency may also induce identical change .

- ❑ In the respiratory tracts this **metaplastic change seen among smokers is beneficial as presumably**, the more resistant, stratified squamous epithelium is able to survive under such circumstances more than the more fragile specialized ciliated columnar epithelium which would not tolerate such irritation .
- ❑ Although the adaptive metaplastic epithelium probably has survival advantages , while the important protective mechanisms, such as mucus secretion & ciliary clearance of particulate matter , are lost therefore, epithelial metaplasia is considered as a double-edged sword
- ❑ Moreover, the influences that induce metaplastic transformation, if persistent, **may induce** cancer transformation in the metaplastic epithelium.



Basement Normal Squamous
membrane columnar metaplasia

**F 28 :
Metaplasia of
normal
columnar (left)
to squamous
epithelium
(right) in a
bronchus,**



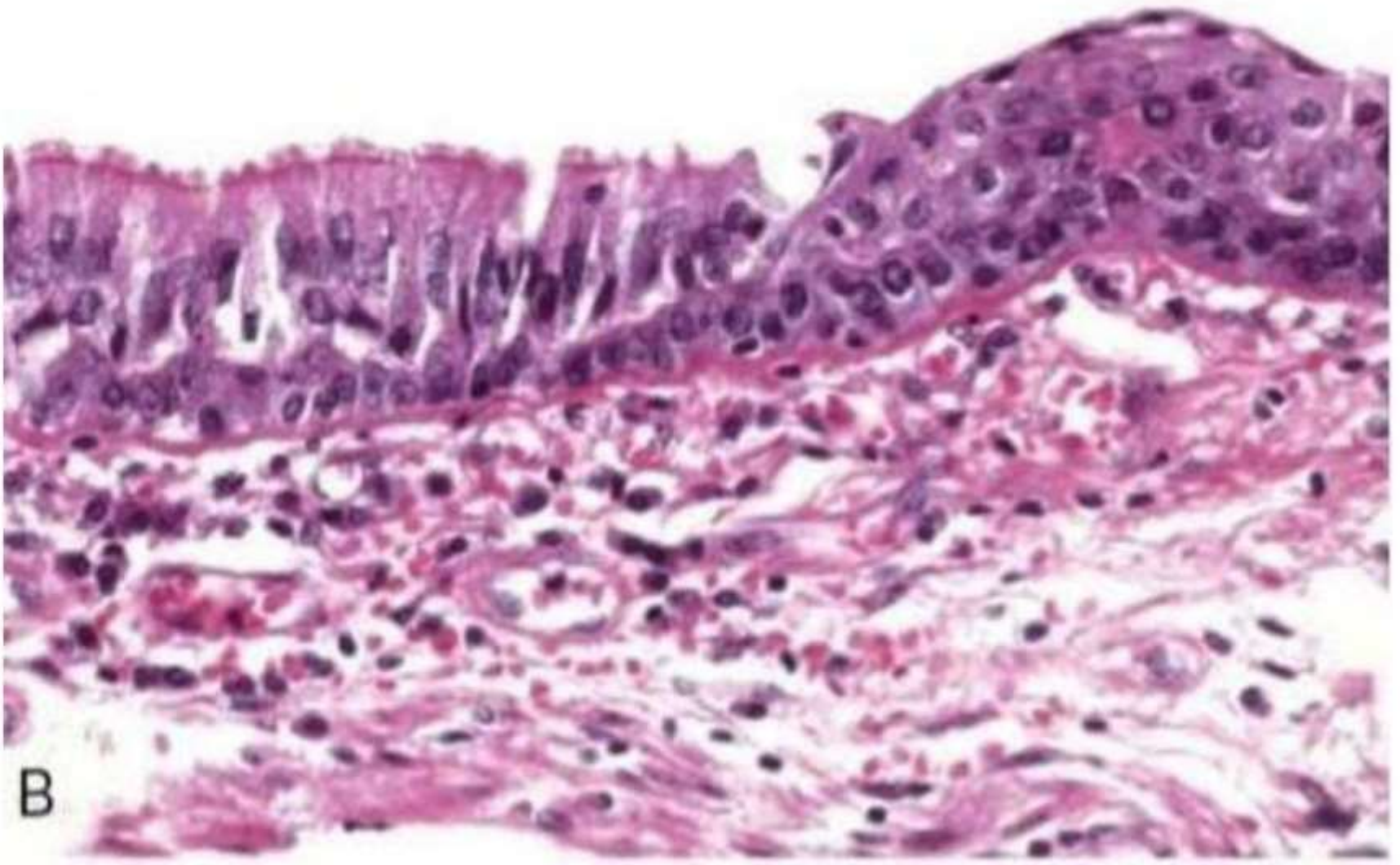


Figure 29 : Microscopic view of bronchial epithelium showing squamous metaplasia of respiratory columnar epithelium

(II) **Chronic irritation** caused by the presence of stones Or **bilharzial ova** may cause squamous metaplasia in the renal pelvis, urinary bladder & gall bladder.

(III) In chronic gastric reflux : **During regurgitation of the acidic gastric contents to the esophagus** the normal stratified squamous epithelium of the lower esophagus may undergo metaplastic transformation to gastric or intestinal -type columnar epithelium , called mucous metaplasia , also refered to as “Barrett esophagus” which may predispose to peptic ulcer or even adenocarcinoma of the esophagus.

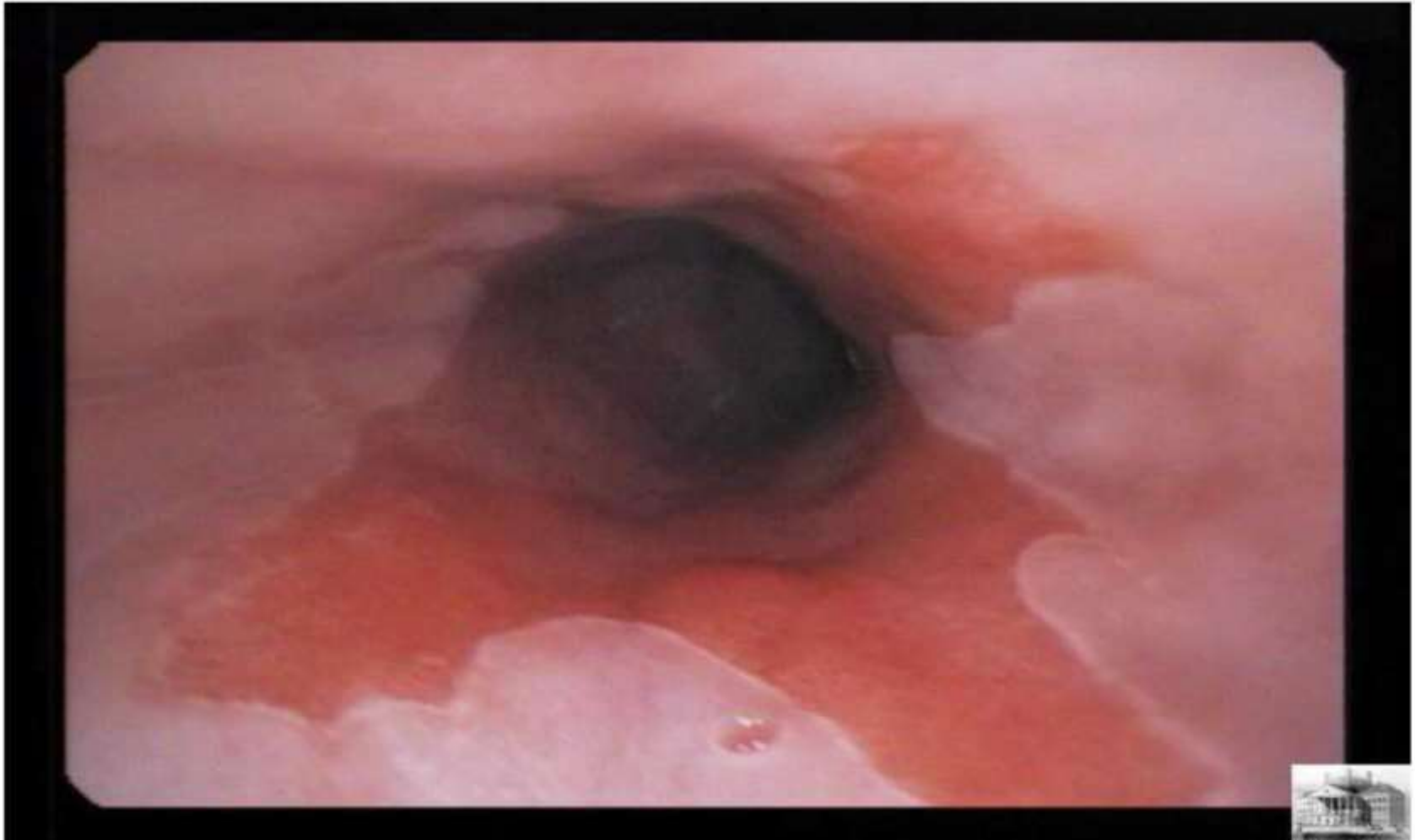


Fig. 30 - Barrett Esophagus : Endoscopic view of esophagus ,show change of the normal squamous epithelium (white color) , into Mucous secreting epithelium (red color)

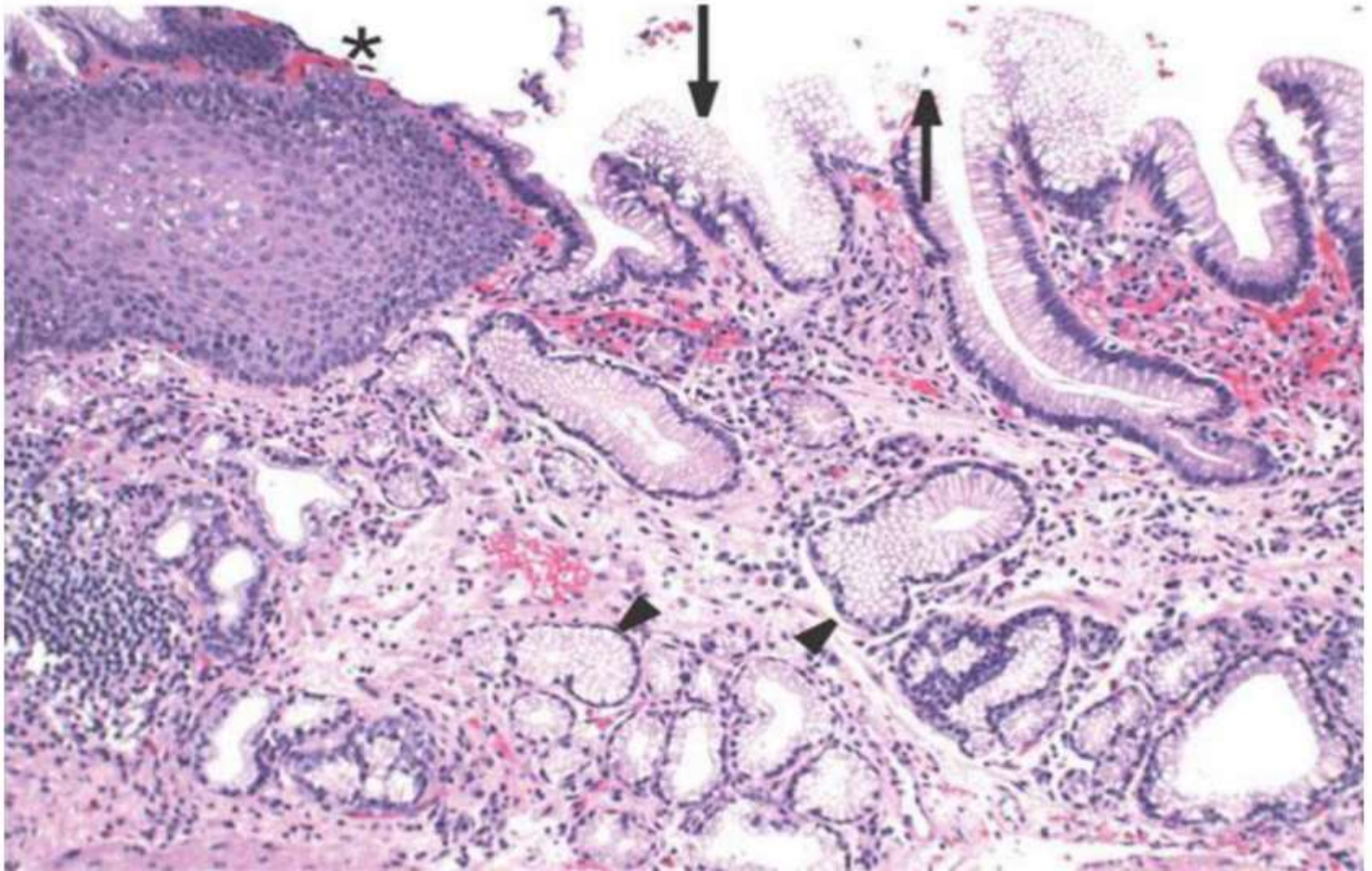


Fig. 31 - Barrett esophagus : mucous metaplasia (black arrows) of squamous epithelium (star *).



Figure 32 : Microscopic view of Barrett mucosa showing metaplasia of esophageal squamous epithelium into mucin-secreting columnar epithelium .

★ Metaplasia may also occur in **mesenchymal cells**, but less clearly as an adaptive response.

□ At sites of injury to soft tissue and skeletal or even smooth muscles bone or cartilage may form in tissues where they are normally not encountered, e.g., bone is occasionally formed in soft tissues so-called **ossification**, e.g., injury to skeletal muscle may cause a tumor-like swelling at site of injury called **myositis ossificans**



Fig 33 - Myositis ossificans : Swelling in left femur mesenchymal metaplasia in skeletal muscle after injury.



Fig. 34 : X-ray appearance of right arm showing myositis ossificans (arrow) .

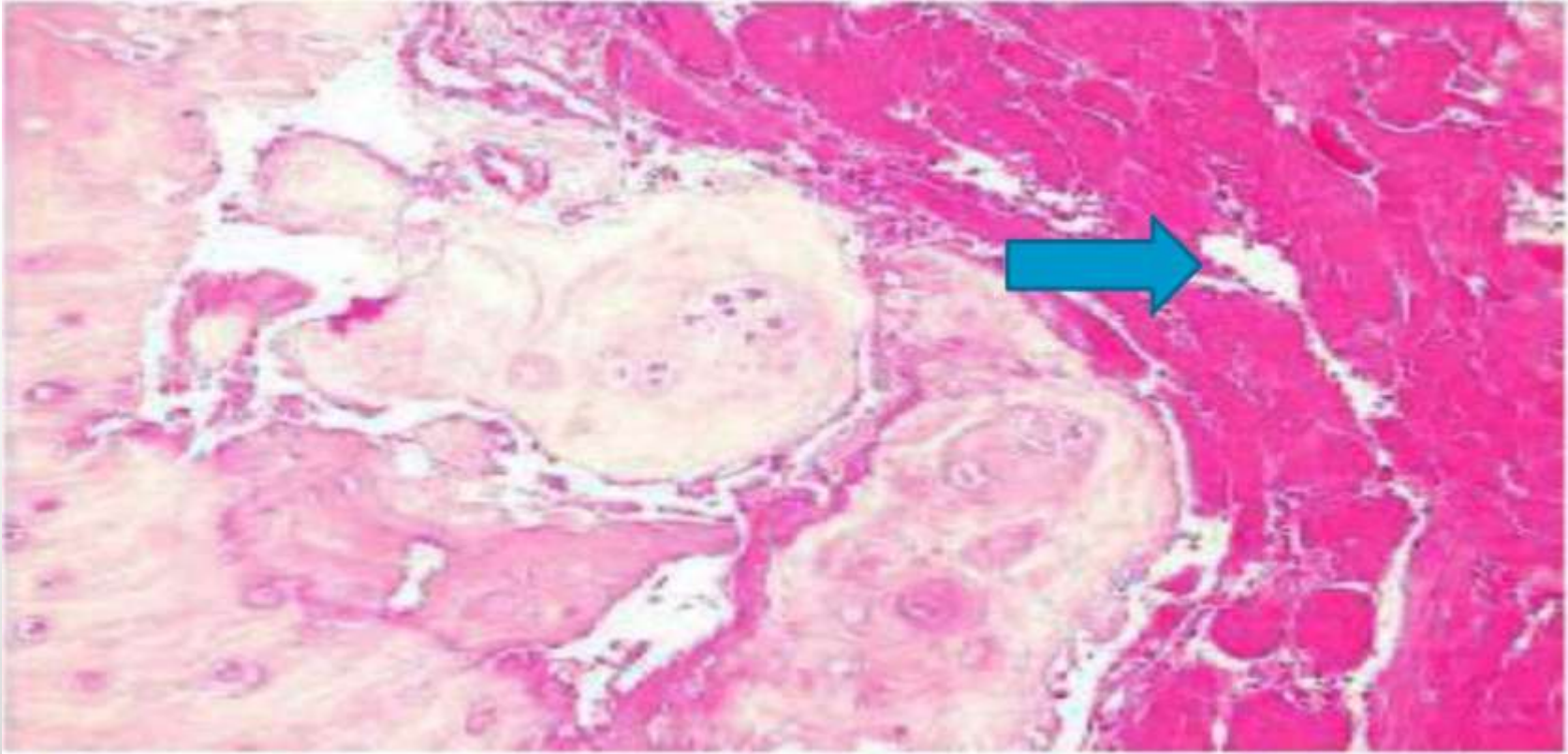


Fig. 35. Microphotograph from the pathologic examination of the surgical specimen showing: cartilaginous tissue with areas of endochondral ossification, rounded type striated muscle tissue (hematoxylin-eosin, X200)

Fig. 35 - Myositis ossificans : Mesenchymal metaplasia in skeletal muscle showing cartilage & bone (left) due to injury , normal red skeletal muscle seen (right) & arrow .

Cell injury, Overview of cell injury & cell death

- ❑ Normal cells are in state of homeostasis i.e. an equilibrium with their environment.
- ❑ Injury is defined as a set of biochemical and/or morphologic changes that occur when the state of homeostasis is affected by adverse influences.

Cell injury occurs:

- (1) when cells are so severely injured that they are no longer able to adapt.
- (2) when cells are exposed to inherently damaging agents .

Cell injury can be :

Reversible or Irreversible

What is the difference between reversible & irreversible cell injury ?

- ❑ The difference is **quantitative** .
- ❑ Reversible injury is **mild** , and following the removal of the adverse influences , the cell reverse to normal state .
- ❑ If the cell **cannot recover** , the injury is considered **irreversible** .

Normal Cell

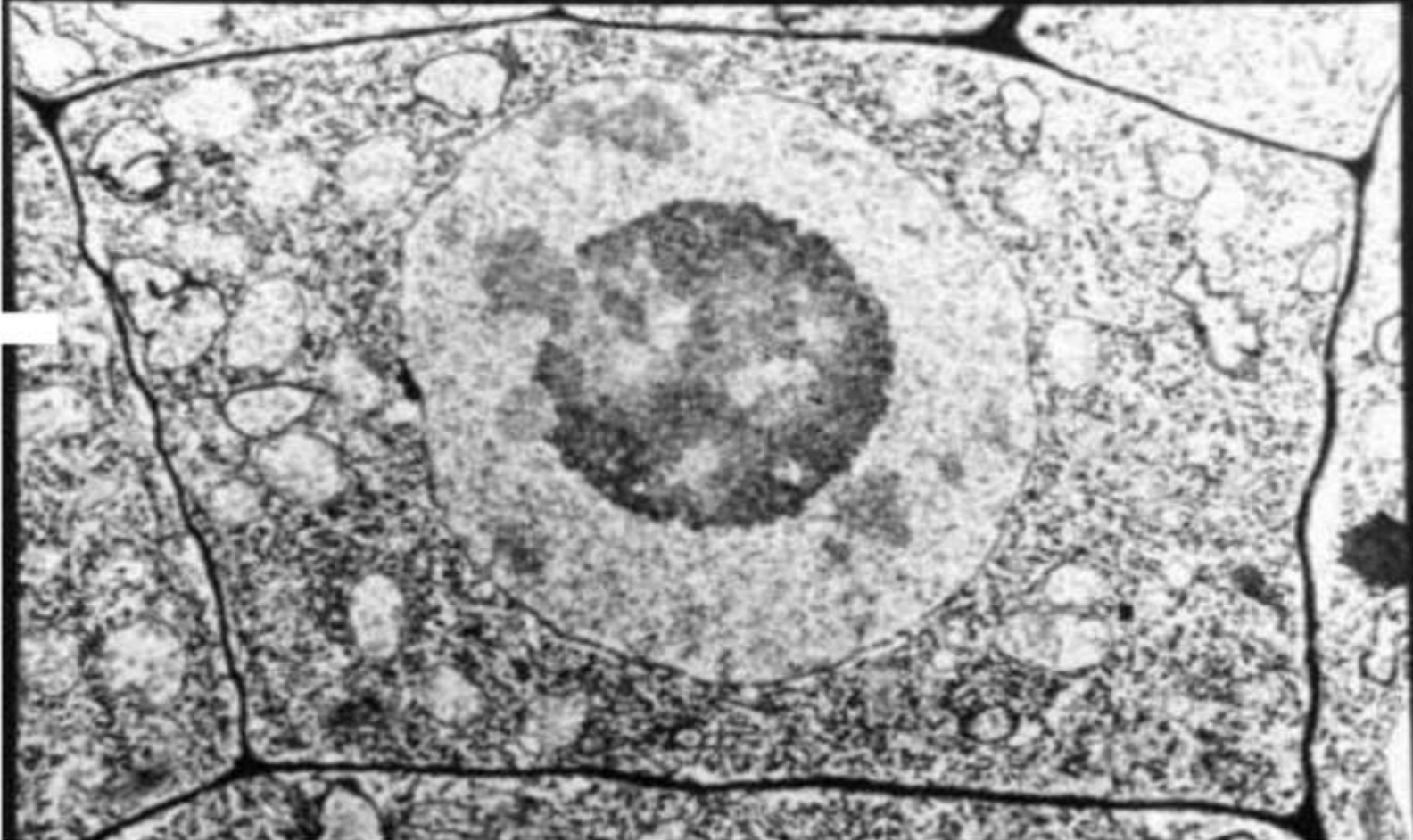


Figure 36 - Normal cell.

CAUSES OF CELL INJURY

Known causes of cell injury can be grouped into the following categories:

- (1) Oxygen Deprivation
- (2) Chemical Agents,
- (3) Infectious Agents
- (4) Immunologic Reactions,
- (5) Genetic Defects,
- (6) Nutritional Imbalances,
- (7) Physical Agents,
- (8) Aging.

(1) OXYGEN DEPRIVATION

(1) Oxygen Deprivation

Hypoxia = oxygen deficiency, interferes with aerobic oxidative respiration, & is an extremely common & important cause of cell injury & death.

Hypoxia can be caused by

(1) Ischemia which is the most common cause of hypoxia, it results from loss of blood supply to the cells due to arterial occlusion or to interference of venous drainage.

(2) Inadequate oxygenation of the blood, as in diseases of the lung most commonly seen in pneumonia (inflammation of the lung).

(3) Reduction in the oxygen-carrying capacity of the blood, i.e. anemia, or in Carbon monoxide (CO) poisoning

(CO forms a stable complex with hemoglobin that prevents oxygen binding).

(2) Chemical Agents :

- ❑ Virtually any chemical substance can cause cell injury . Potentially active agents like air pollutants , insecticides , CO , asbestos & ethanol, even oxygen at sufficiently high partial pressure can cause cell or tissue injury .
- ❑ ★ Poisons cause severe damage at the cellular level by increasing membrane permeability e.g. by mercury in mercuric chloride poisoning or cyanide, a supertoxic agent binds to mitochondrial cytochrome oxidase.

(3) **Infectious Agents** : All infectious agents can trigger cell & tissue injury .Viruses as an example

Bacterial damage to host tissues depends on;

- (a) The ability of the bacteria to adhere to & enter host cells,
- (b) To promote hypersensitivity reaction, or
- (c) Deliver toxins which are either: exo-, or endo-toxins.

(5) Genetic Defects

May result in pathologic changes as clear as the congenital malformation associated with Down syndrome (Trisomy of chromosome 21) & sickle cell anemia .

(6) Nutritional Imbalances

Nutritional deficiencies are major cause of cell injury

- * Protein —calorie insufficiency among poor populations is the most obvious example .

- * specific vitamin deficiencies (scurvy, rickets) .

- * Excesses of nutrition are also important causes of morbidity & mortality; e.g. obesity markedly increase the risk for type 2 diabetes mellitus, endometrial & breast cancers,& hypertension.

Diets rich in animal fat is strongly implicated in the development of atherosclerosis .

(7) Physical Agents :

Trauma, extremes of temperatures (e.g., burn) radiation, electric shock, & sudden changes in atmospheric pressure all have wide-ranging effects on cells.

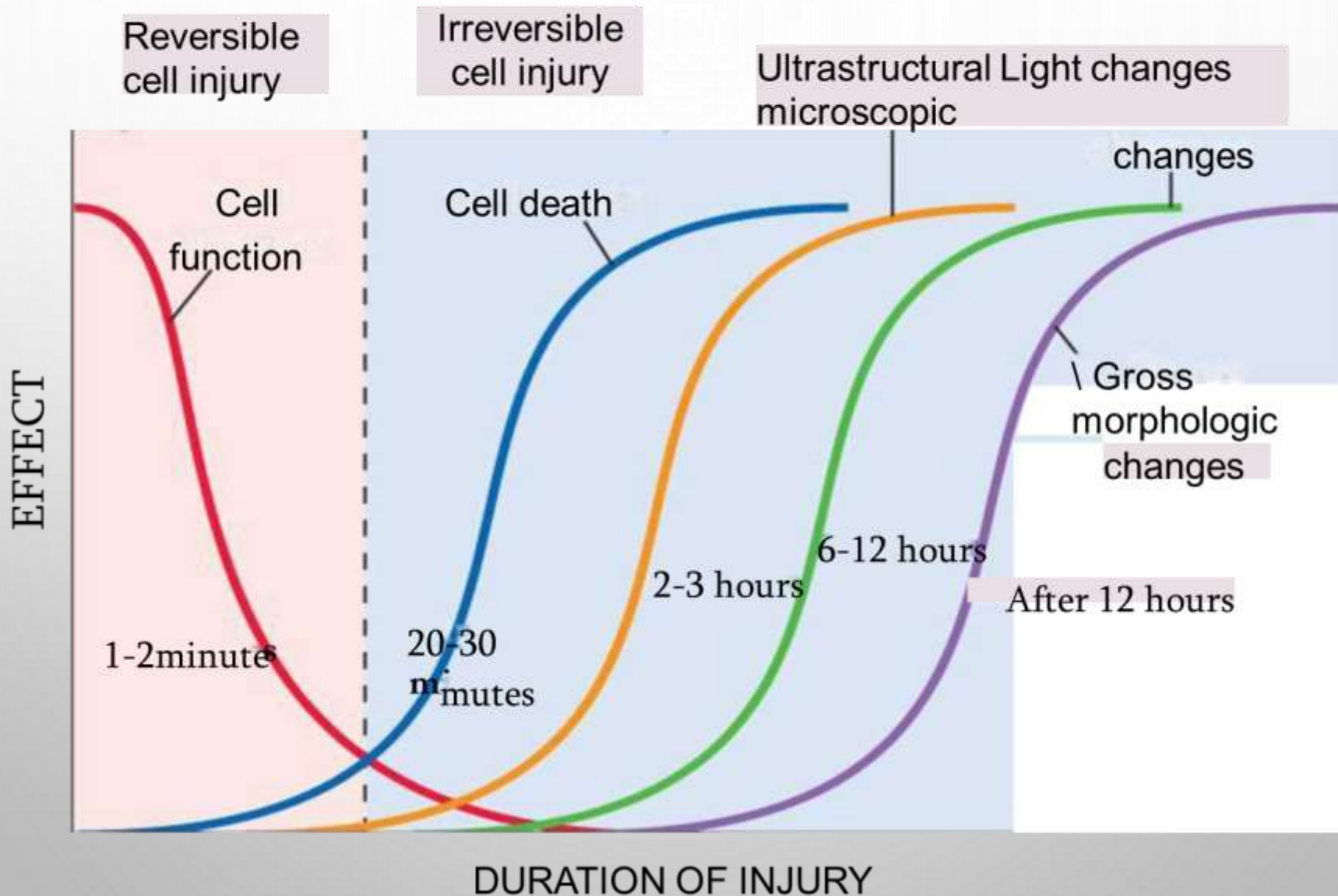
(8) Aging :

Cellular senescence leads to alterations in replicative & repair abilities of individual cells & tissues, which result in a diminished ability to respond to exogenous stimuli & injury & eventually, the death of cells.

THE MORPHOLOGY OF CELL AND TISSUE INJURY

- ❑ All stresses & noxious influences exert their effects first at the molecular or biochemical level.
- ❑ **Cellular function** may be lost first, long before the cell death, e.g. myocardial cells become non-contractile within **1-2 minutes of ischemia** occurs which is although they do not die until **20-30 minutes** the morphological changes of cell death on ultrastructural level (EM) **seen 2-3 hours later**, then at light microscopy level seen **after 6-12 hours**, & finally on gross examination level, can be seen after 12 hours of ischemia respectively.

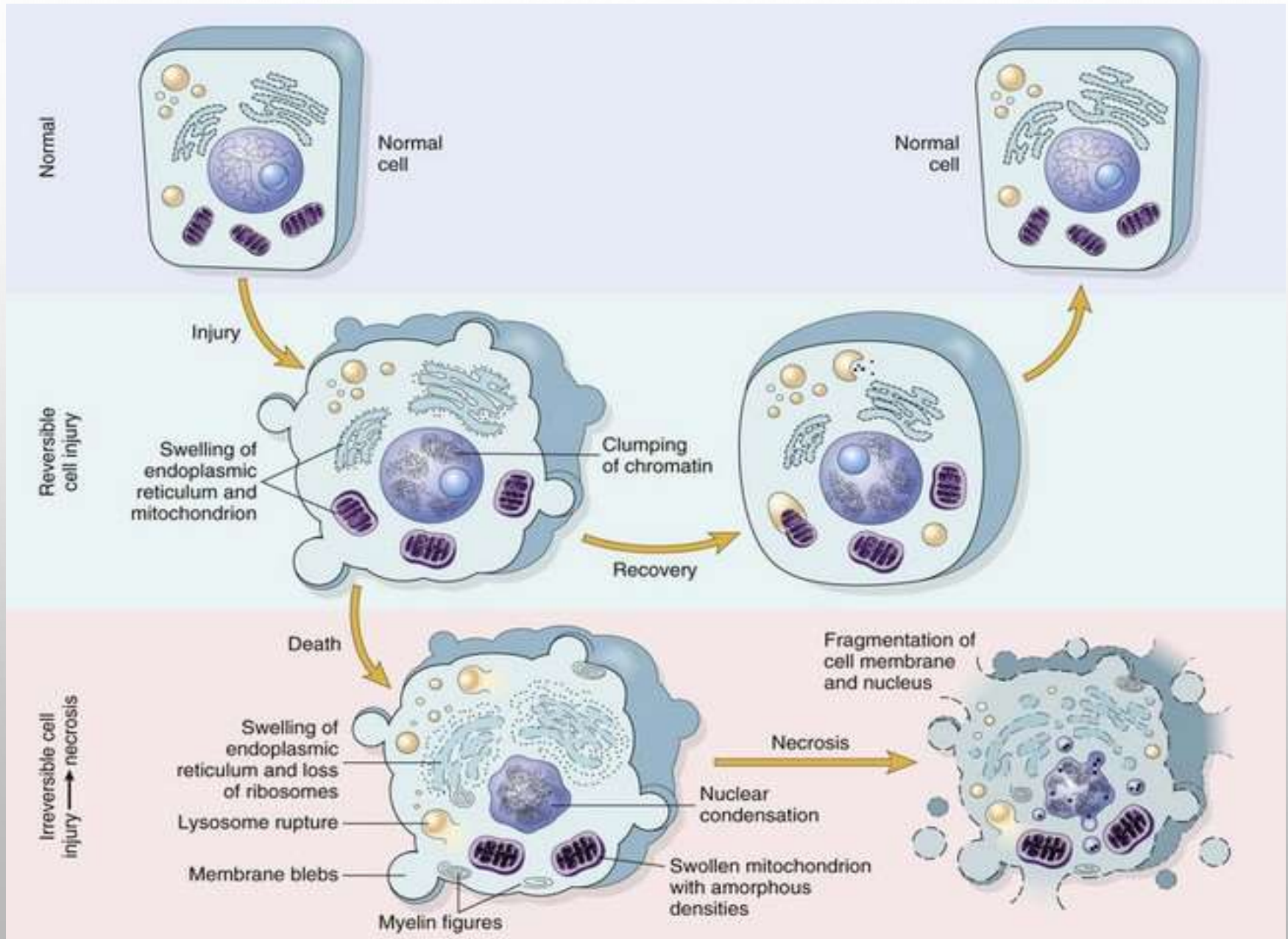
F 37 : The relationship between cellular function, cell death, & the morphologic changes of cell injury.



The ultrastructural (EM) changes of reversible cell injury

- (1) Plasma membrane alterations such as blebbing; blunting or distortion of microvilli; & loosening of intercellular attachments
- (2) Mitochondrial changes such as swelling .
- (3) Dilation of the smooth endoplasmic reticulum (ER) which is involved in metabolism of various chemicals with injury there is hypertrophy of ER as well as increased activity , later it shows detachment of ribosomes & dissociation of polysomes in rough ER .
- (4) Nuclear clumping of chromatin .

A normal cell & the changes in reversible & Normal! irreversible cell injury (necrosis).



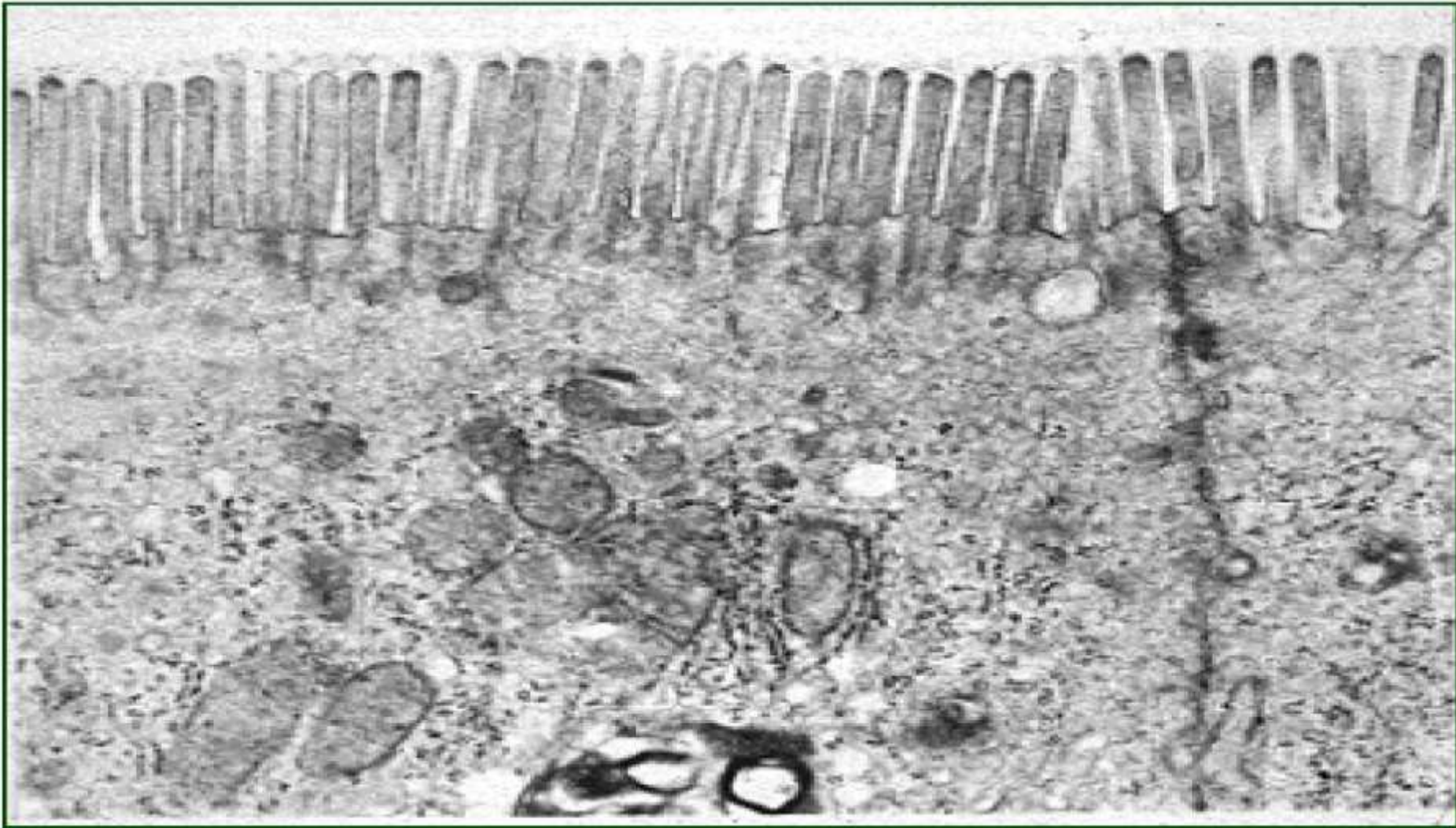


Fig. 39 : Ultra structural view of normal microvilli mitochondria ,lysosome & intercellular junction..



Fig. 40 : Ultra structural view of reversible cell injury , showing ,distortion of microvilli , blebs , swollen mitochondria & nuclear chromatin clumping lysosomes , myelin figures .

Mitochondrial Swelling

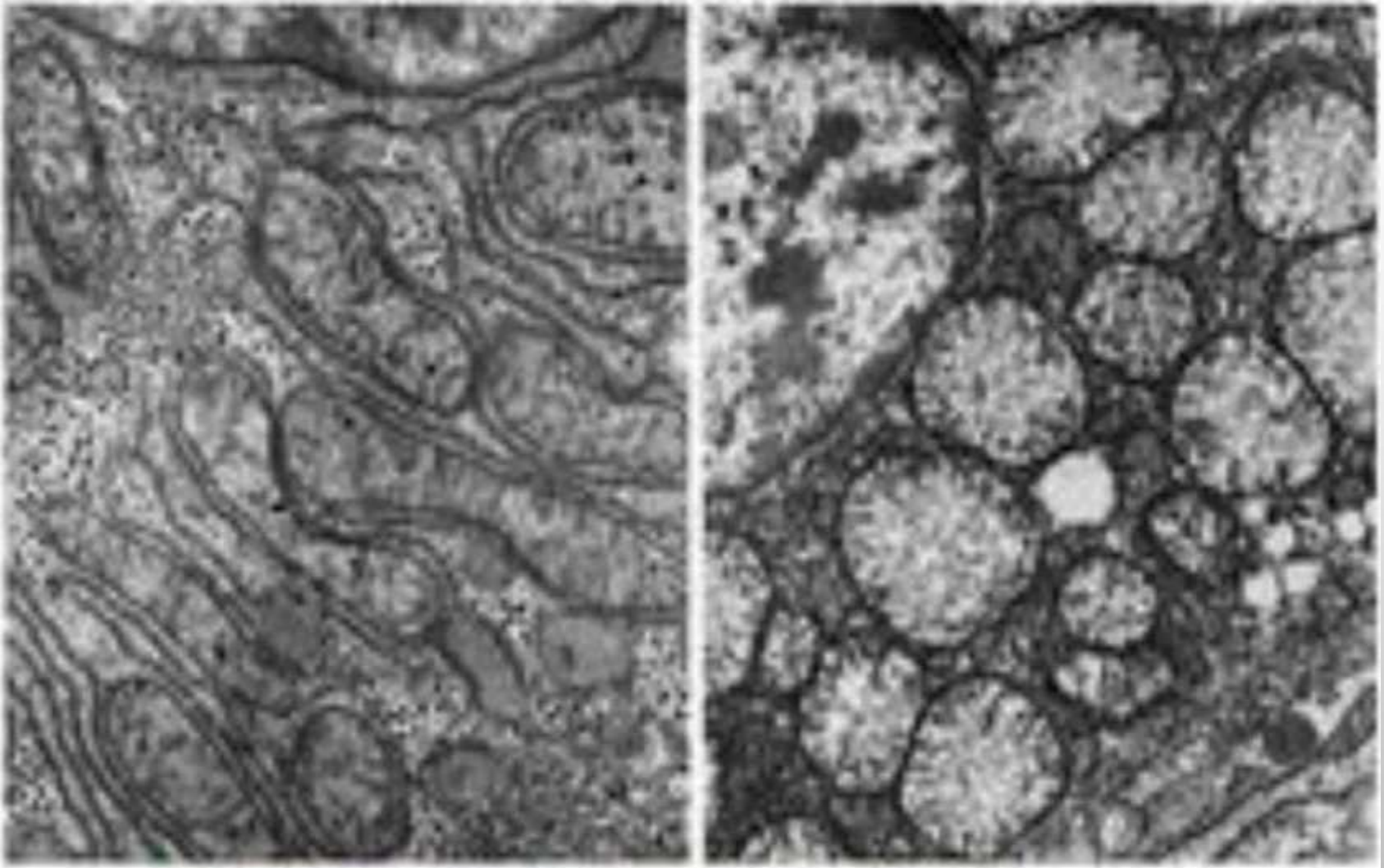


Fig. 41 : E .M . view of reversible cell injury.

❑ At light microscopy, the two patterns of morphologic change of reversible cell injury are :

❑ Cellular swelling & Fatty change

1. Cellular swelling:

Is the first manifestation of almost all forms of injury to cells ; **it is the result of failure of energy-dependent ion pumps in the plasma membrane** , leading to inability to maintain ionic & fluid homeostasis , resulting in the accumulation of Na & water within the cytoplasm .

❑ This pattern of **non-lethal** , such reversible injury is sometimes called **Hydropic change** , **Vacuolar degeneration** , or **Cloudy swelling** .

- ❖ So Hydropic degeneration is a result of ion and fluid homestasis that lead to an increase of intracellular water.
- ❖ **GROSSLY** : when all cells in an organ are affected, there is *gross* pallor , swelling, & increased weight of the organ.
- ❖ **Microscopically** : in cellular swelling, small, clear vacuoles may be seen within the cytoplasm; these represent distended & pinched off segments of endoplasmic reticulum (ER) .

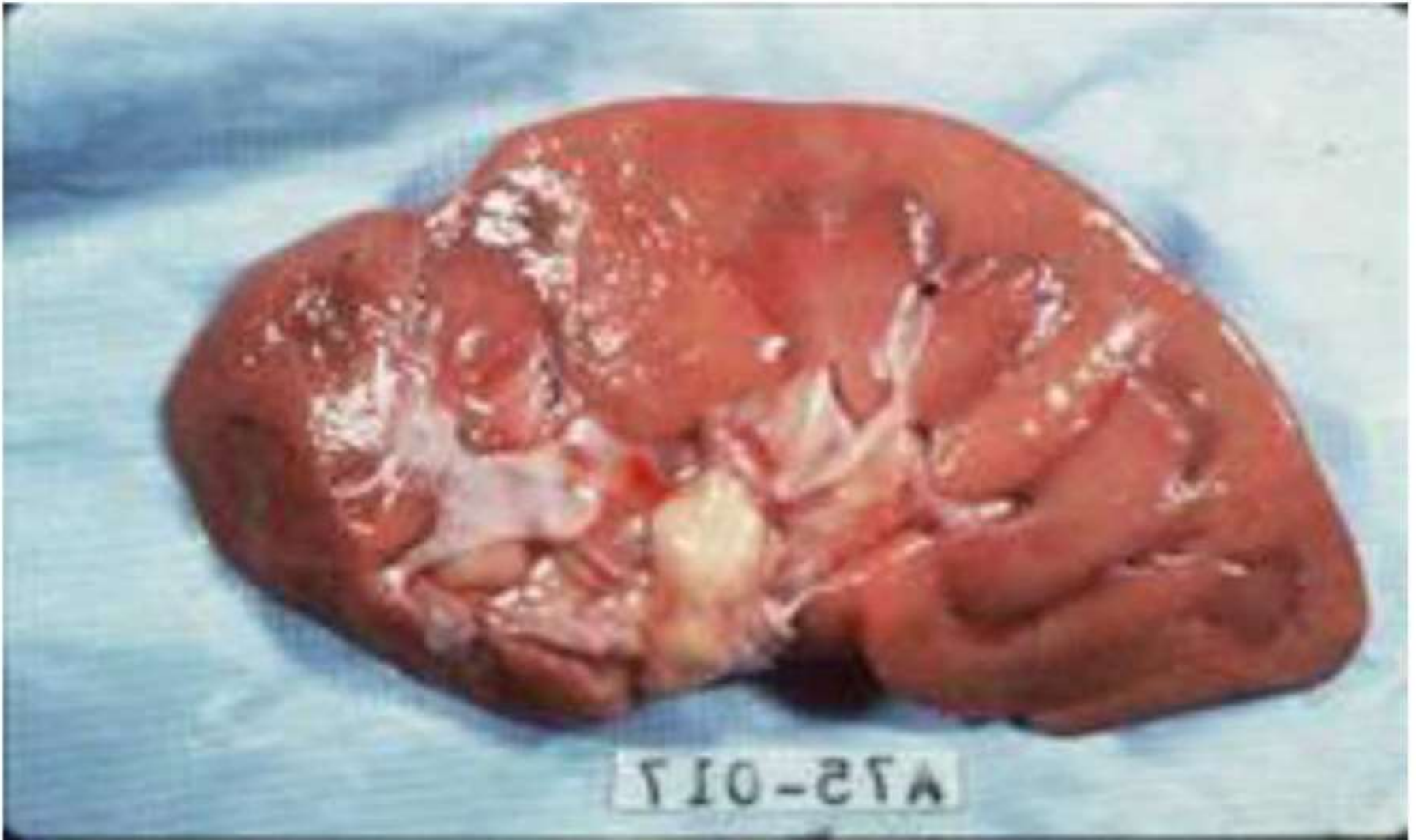
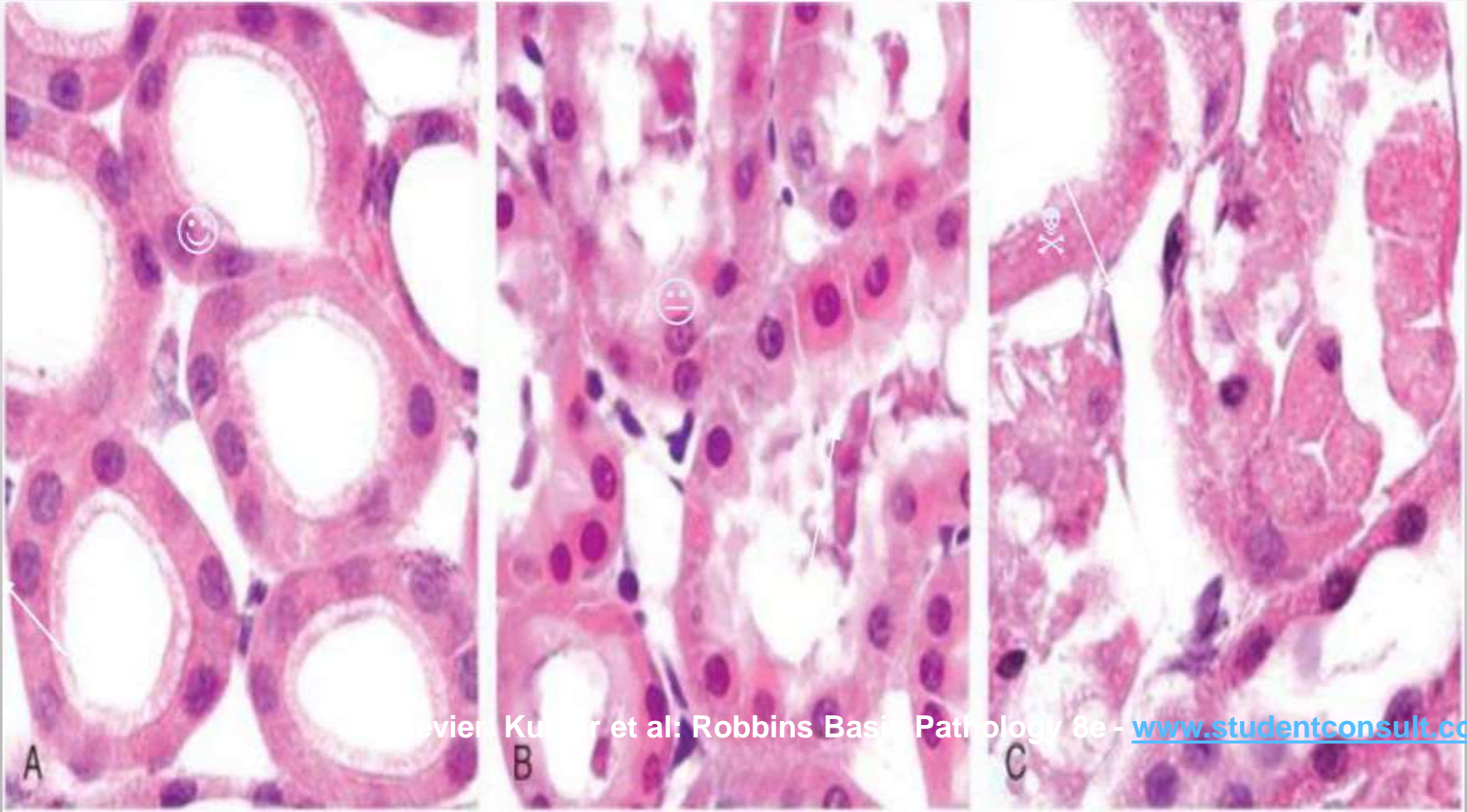


Fig. 42 - Kidney : Gross view of Hydropic degeneration, the kidney is heavy swollen and pale .

A, Normal Kidney tubules with viable epithelium.

B, Early reversible ischemic injury showing surface blebs, increased eosinophilia of cytoplasm, & swelling of occasional cells.

C, Necrotic epithelial cells with loss of nuclei & fragmentation of cells & leakage of contents.



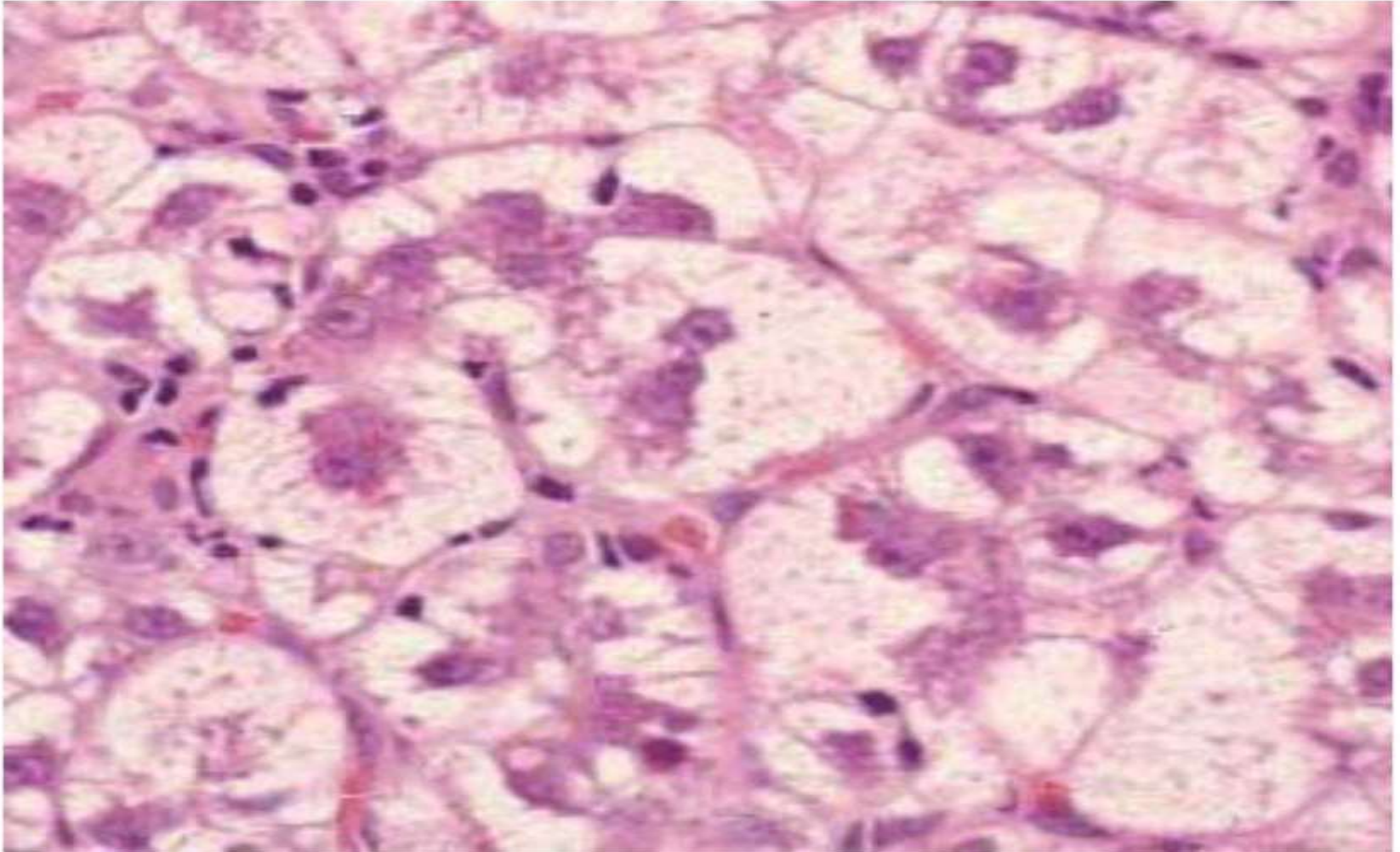
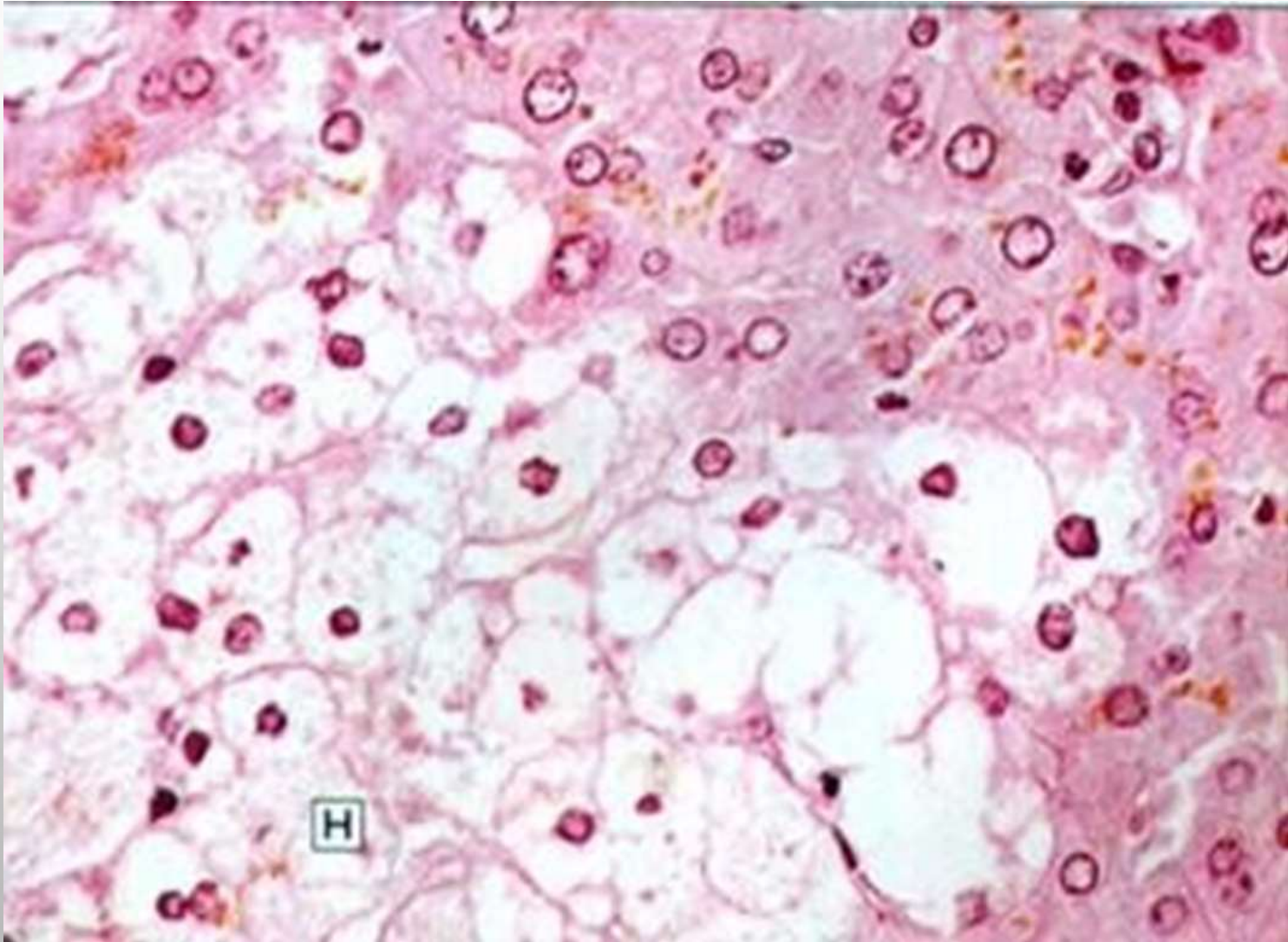


Figure: Liver biopsy showing hydropic degeneration. Clear watery vacuoles seen within the cytoplasm.

**HYDROPIK DEGENERATION OF THE LIVER.
CLEAR WATERY VACUOLES SEEN WITHIN THE CYTOPLASM.**



2.Fatty change :

Occurring in hypoxic, toxic, or metabolic injury, is manifested by the appearance of *vacuoles* in the cytoplasm, principally encountered in cells participating in fat metabolism (e.g., hepatocytes (liver) & myocardial cells (Heart)).

Grossly :

In fatty change affecting the liver , it is usually enlarged , heavy may weigh 3-6 kilograms (Normal weight 1.5 Kg) and tends to be bright yellow , soft , and greasy organ .

Fatty change is also reversible.

Microscopically :

- ❑ The hepatocytes contain minute membrane-bound inclusions (liposomes) closely applied to endoplasmic reticulum called microvesicular , gradually with further fat deposition these vacuoles fuse to produce a single large vacuole filling the cytoplasm and pushing the nucleus to the periphery of the cells so called signet ring appearance .
- ❑ As in conditions of starvation & also seen in alcoholic or viral hepatitis ,or other diseases .

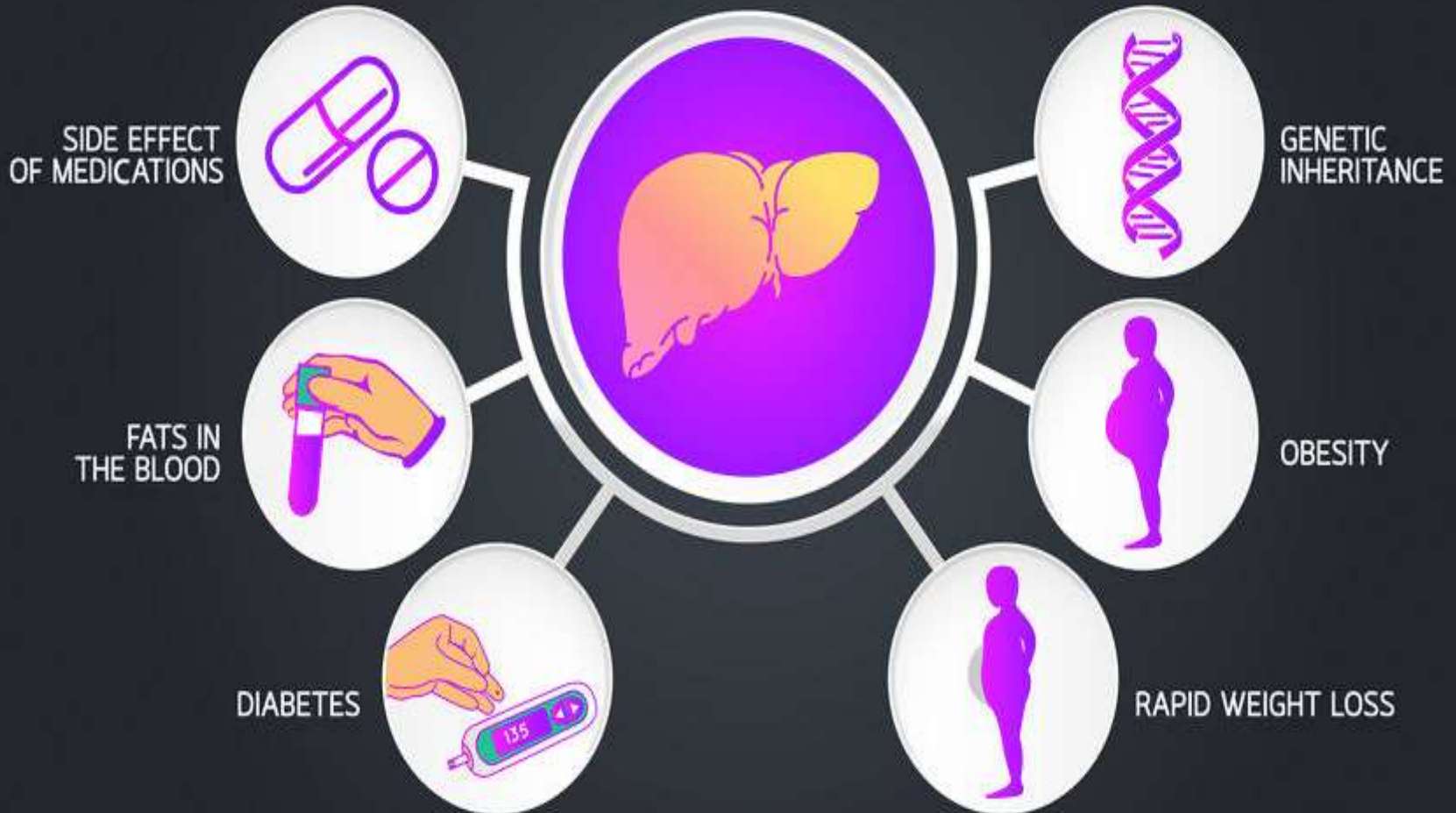
Fatty change: liver. The patient was chronic alcoholic.

The presence of large quantities of neutral fat within the liver cells result in a uniform yellow appearance of the liver section.



5-4 Fatty change: liver

CAUSES OF FATTY LIVER



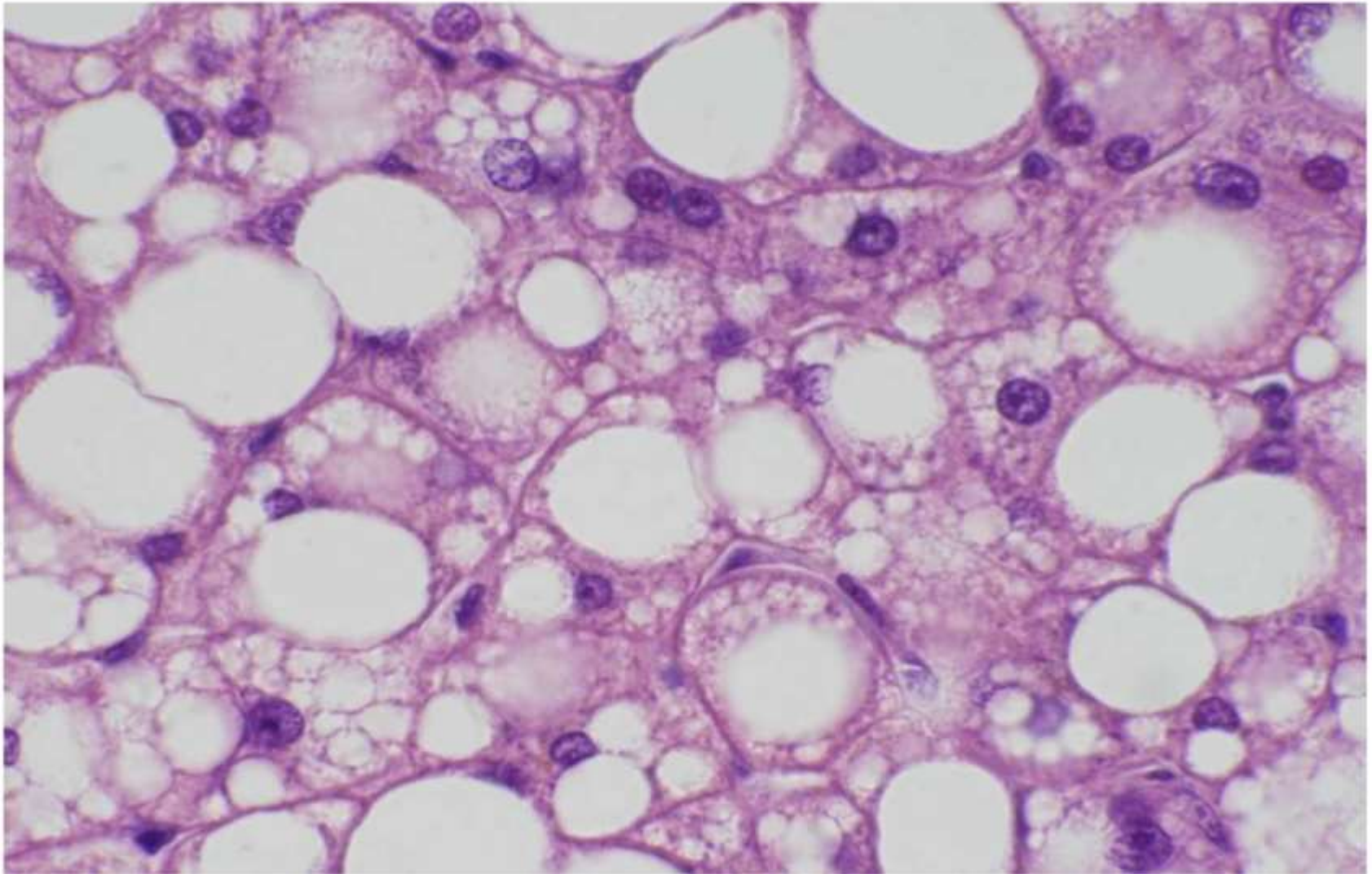
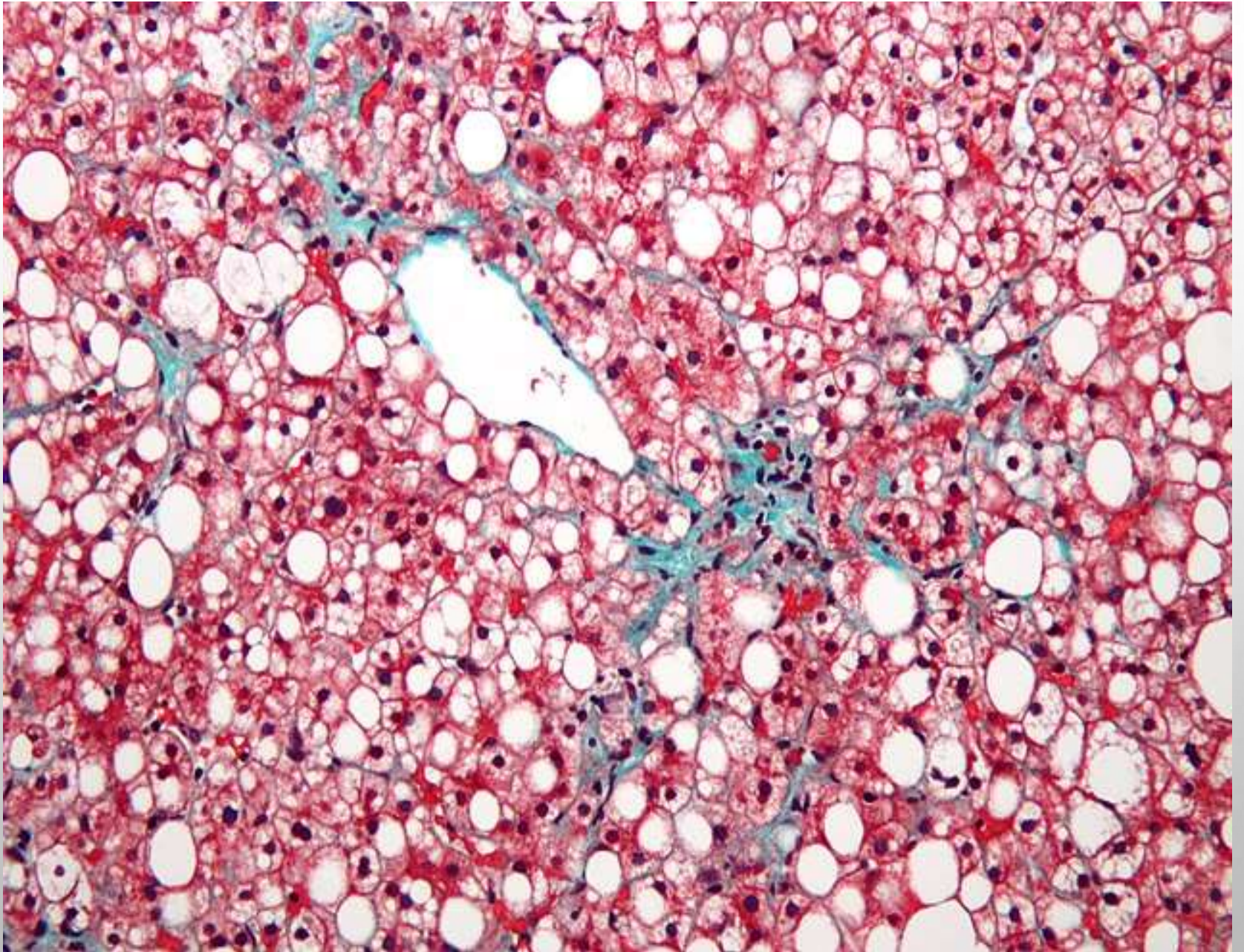


Fig. 46 : Microscopic view of liver showing fatty change. Small & large cytoplasmic vacuoles pushing nucleous to the periphery .



Irreversible cell injury(NECROSIS)

- ❖ **Necrosis refers to a series of changes that accompany cell death in living tissue .**
- ❖ **These changes resulting from the degradative action of enzymes on lethally injured cells derived either from the :**
 - (1) **lysosomes of the dying cells themselves , or from the**
 - (2) **lysosomes of the leukocytes (WBCs) that are recruited as part of the inflammatory reaction to the dead cells .**

□ Necrotic cells are **unable to maintain membrane integrity** & the subsequent **leakage of intracellular proteins** through the damaged cell membrane, into the circulation provides a means of detecting tissue-specific necrosis **using blood or serum samples**.

Example:

- ❖ In myocardial infarction (M.I) the serum contains a unique isoform of the **enzyme creatine kinases** & of the **contractile protein troponin** , which are released from necrotic myocytes .
- ❖ These leakage processes require hours to develop .
- ❖ While in hepatitis i.e. inflammation of liver the enzymes **alkaline phosphatase** , and **transaminases (GPT and GOT)** increase in the serum after necrosis of the hepatocytes (liver cells) .

❑ **By EM irreversible cell injury** i.e. necrotic cells are characterized by :

- (1) Lysosomes rupture .
- (2) Fragmentation of plasma & organelle membranes .
- (3) The cytoplasm may contains phospholipids & the appearance of phospholipids-rich amorphous densities derived from damaged cellular membranes , called Myelin figures .
- (4) Nuclear condensation or dissolution .
- (5) Endoplasmic Reticulum swelling with loss of ribosomes
- (6) Mitochondrial dilatation with the appearance of large amorphous densities .

MORPHOLOGY OF irreversible cell injury (NECROSIS)

- In light microscopic examination dead (necrotic) cells show:
 - (1) **Increased cytoplasmic eosinophilia** (pink staining from the eosin dye used in staining tissue with hematoxyline & eosin stain (H&E) due to binding of eosin to denatured cytoplasmic proteins and decreased RNA .
 - (2) The cell may have a more **glassy homogenous appearance** than viable cells, due mainly to **the loss of glycogen particles**.
 - (3) When enzymes have degraded the organelles, the cytoplasm becomes vacuolated & appears moth- eaten .
 - (4) Dead cells may be replaced by large, whorled phospholipid masses, called myelin figures, derived from damaged cellular membranes .

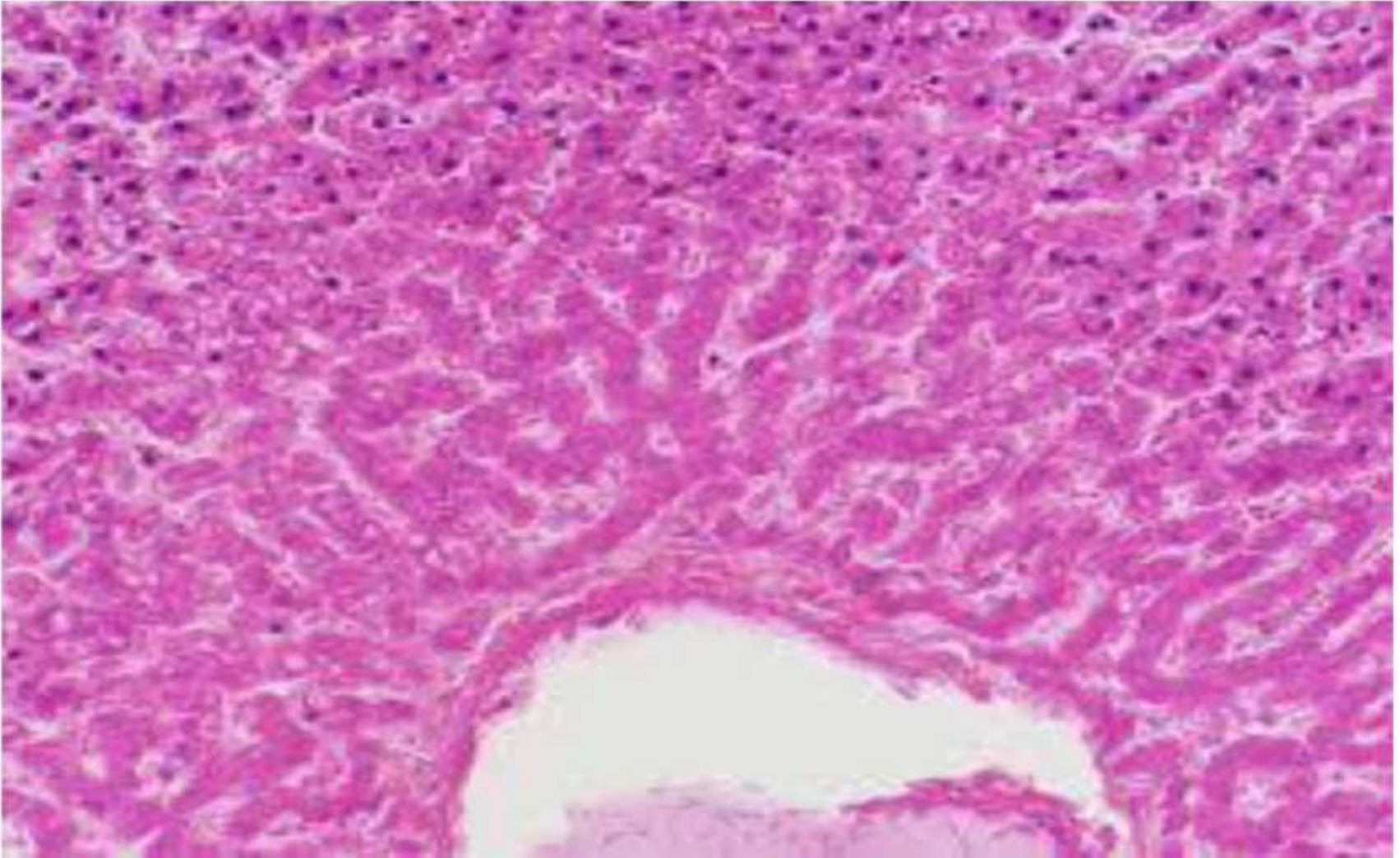
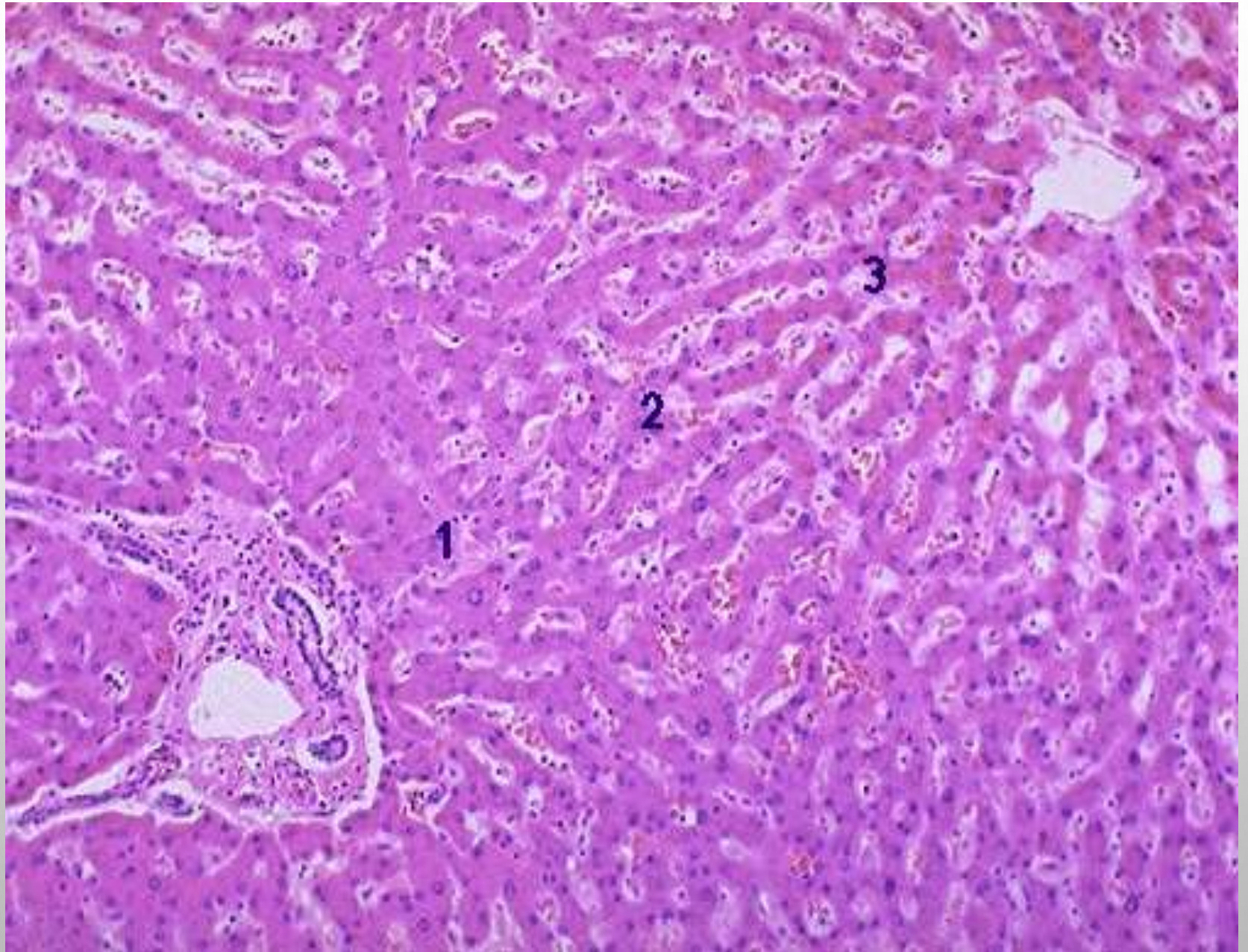


Figure 48 - Necrotic hepatocytes look homogenously eosinophilic no nuclei seen , compared to a viable hepatocytes at periphery .



Nuclear changes:

Breakdown of DNA & chromatin causes:

- (a) **Pyknosis** : nuclear shrinkage & condensation & increased basophilia ; the DNA condenses into a solid shrunken mass, or
- (b) **Karyorrhexis** : fragmentation of the pyknotic nucleus .
- (c) **karyolysis** : in which the basophilia (blue staining from the Hematoxylin dye of the chromatin may fade all the nuclear chromatin disappear secondary to deoxyribonuclease (DNase) activity

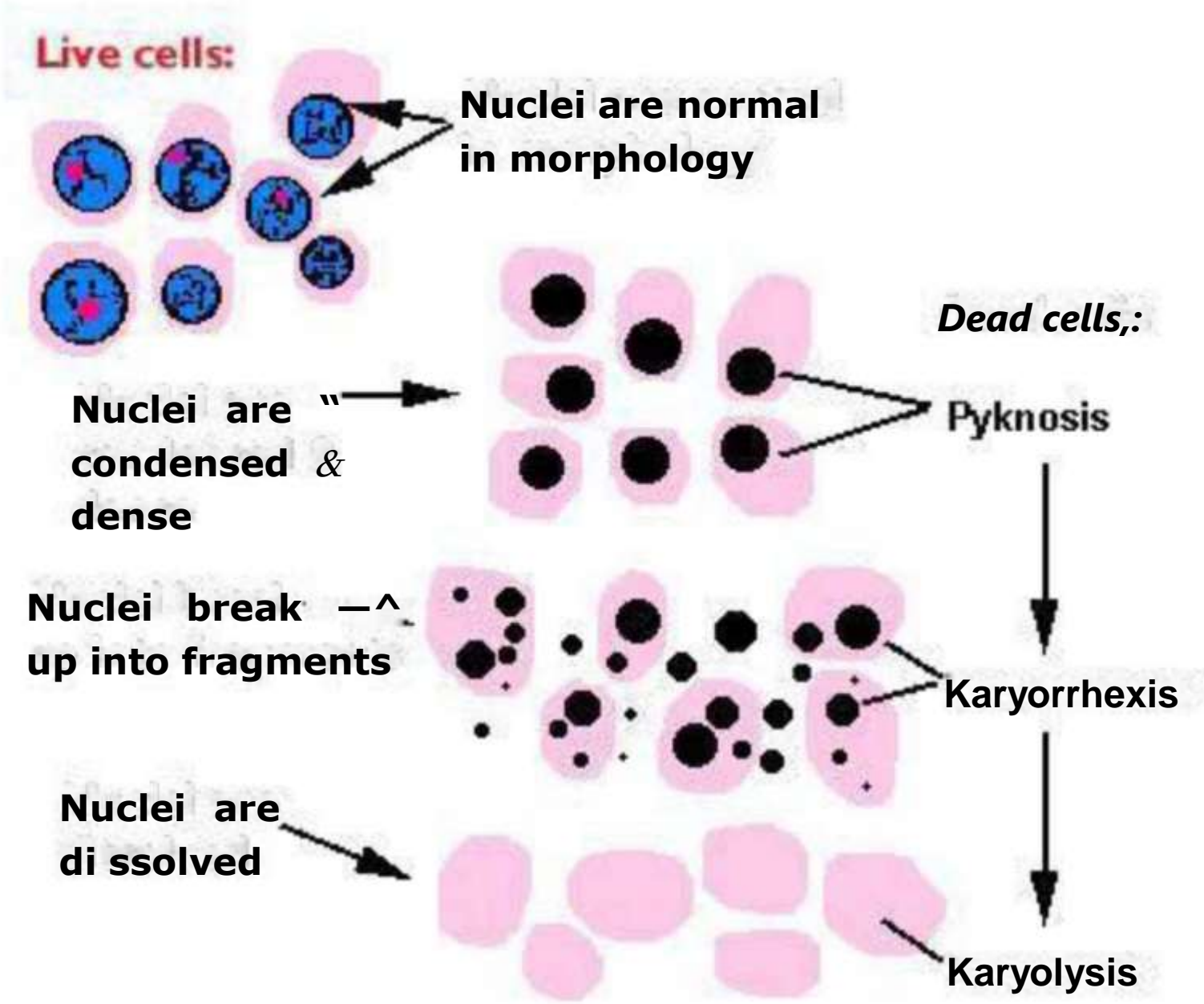
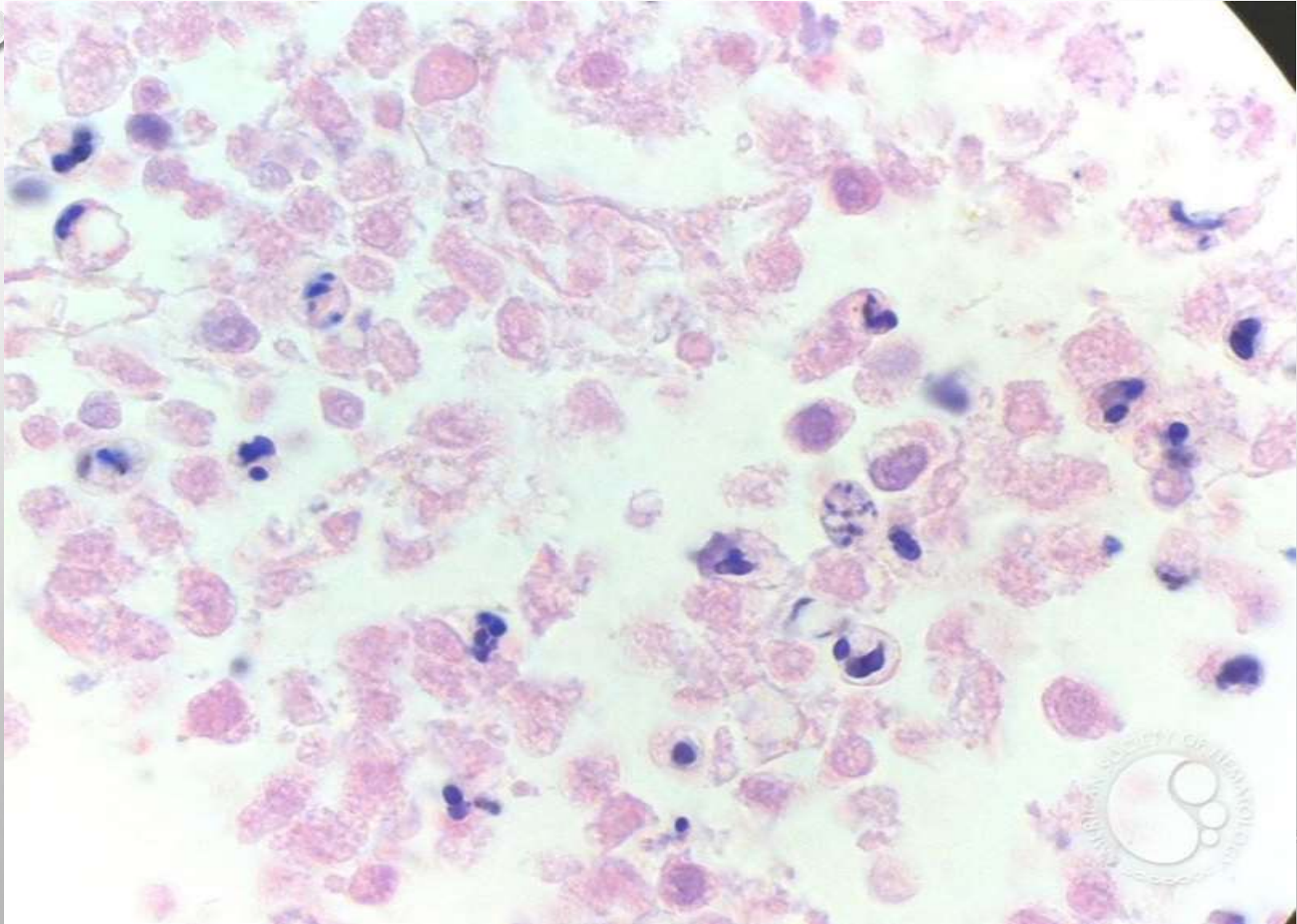
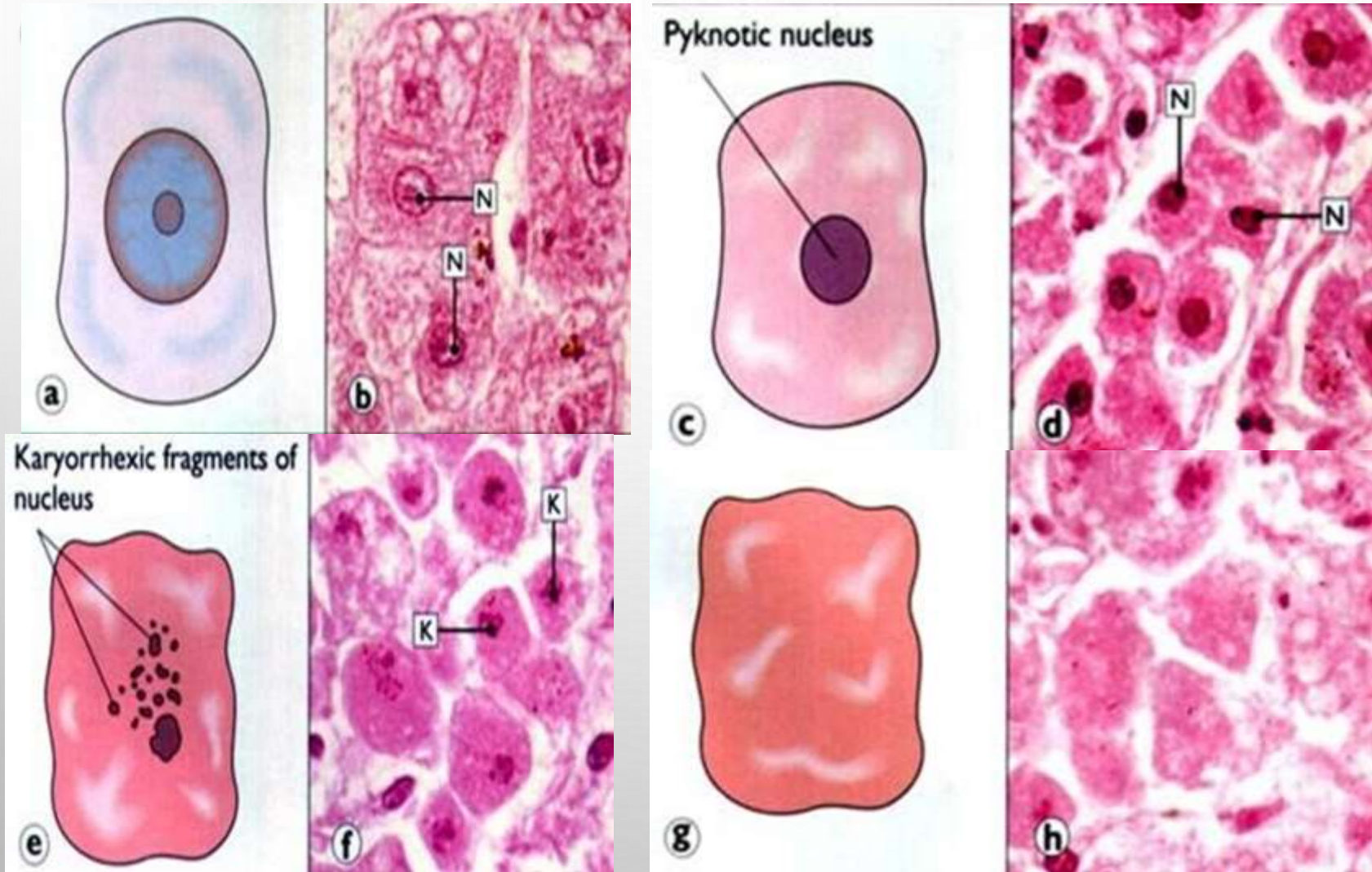


Figure 49 : Nuclear changes in injury.

NECROTIC CELLS ,SHOWING NUCLEAR PYKNOSISKARYORRHEXIS &
KARYOLYSIS



NUCLEAR CHANGES IN IRREVERSIBLE INJURY



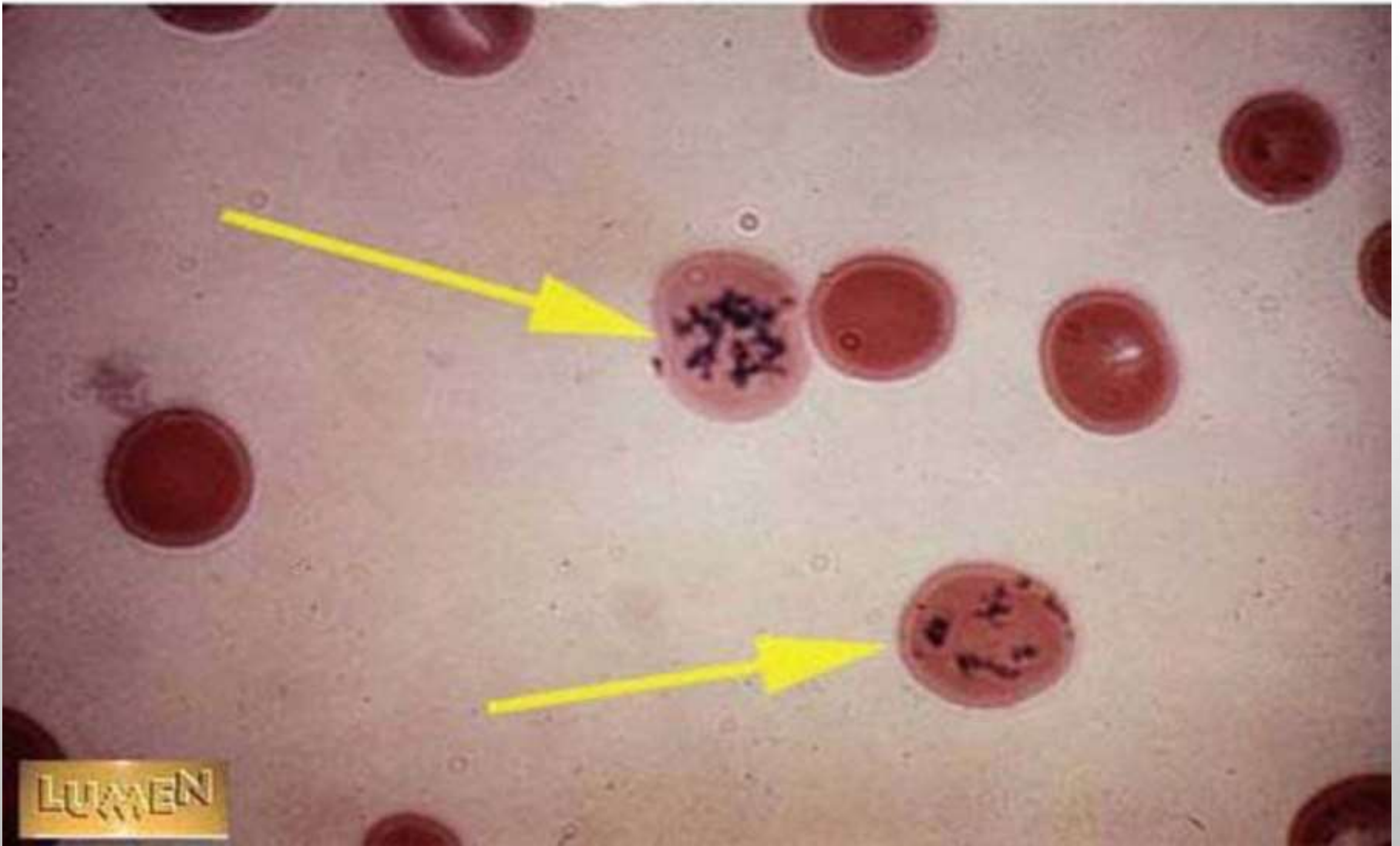


Fig 50 - Karyorrhexis i.e. nuclear chromatin fragmentation

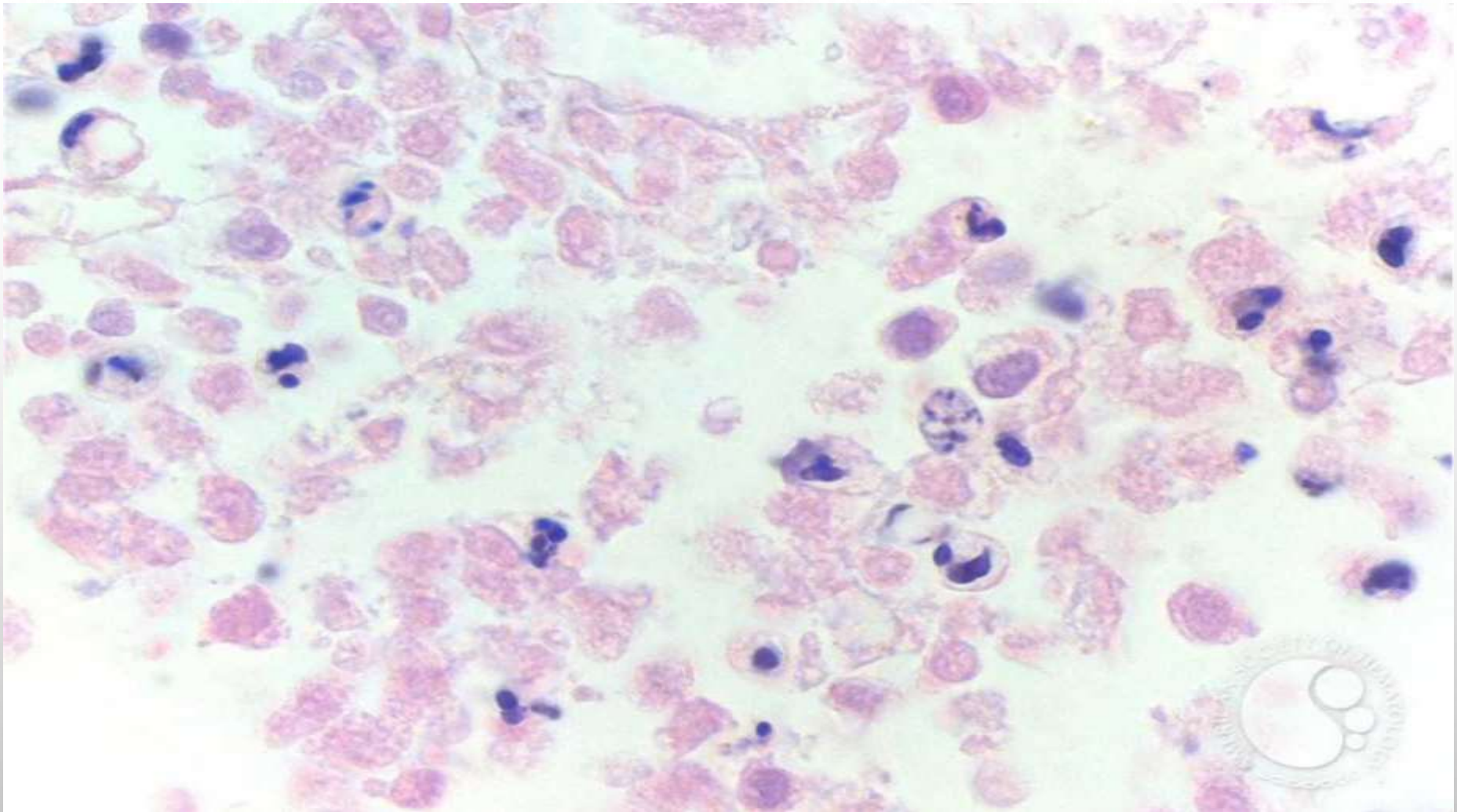


Fig. Necrotic cells ,showing nuclear pyknosis karyorrhexis & karyolysis .

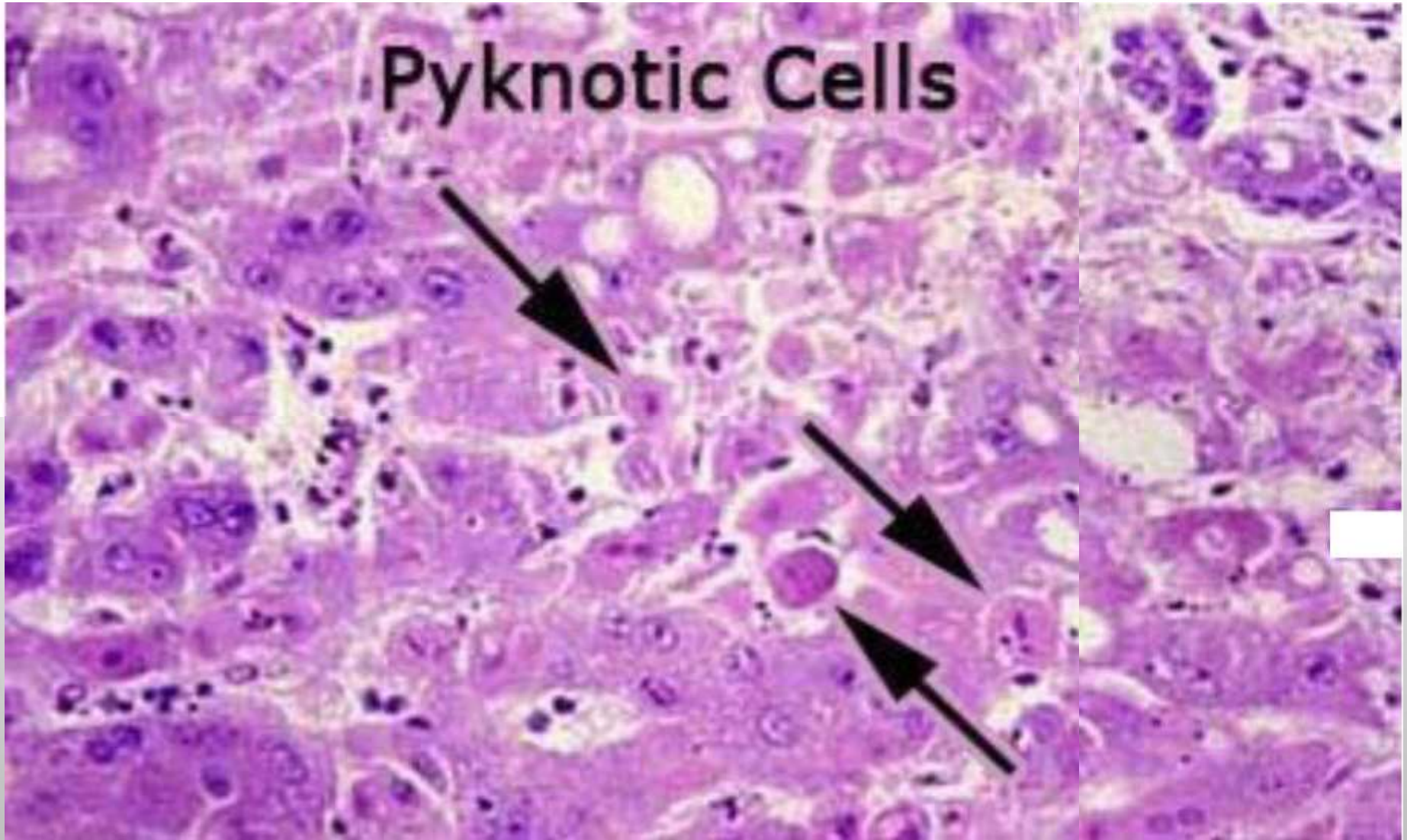


Figure: Microscopic view of liver biopsy ,sowing pyknosis karyorrhexis & karyolysis .

Patterns of Tissue Necrosis

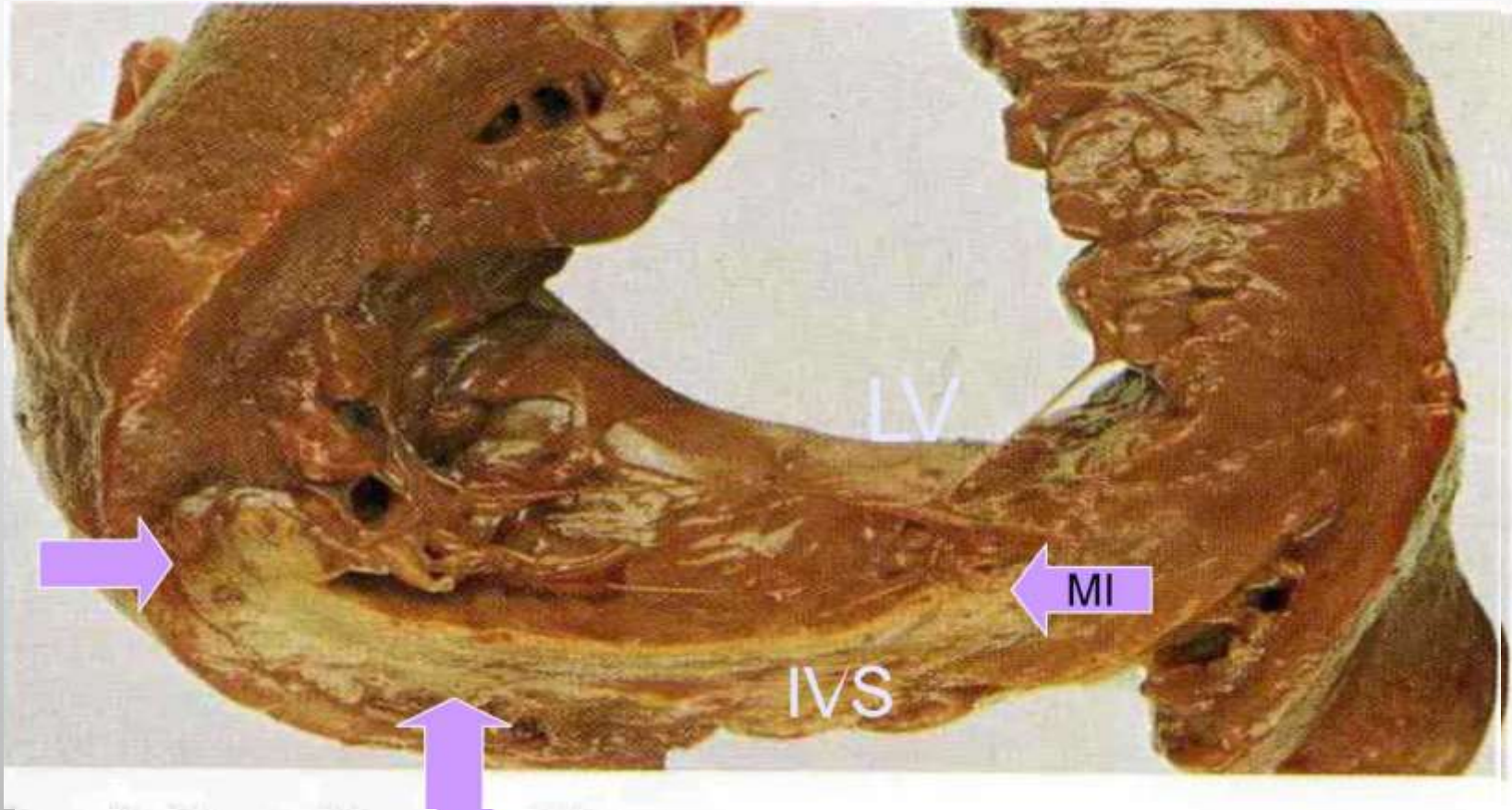
Grossly, necrotic tissue may show either:

- (1) Coagulative necrosis.
- (2) Liquefactive necrosis.
- (3) Caseous necrosis.
- (4) fat necrosis .
- (5) fibrinoid necrosis.
- (6) Gangrenous necrosis.

(1) Coagulative necrosis

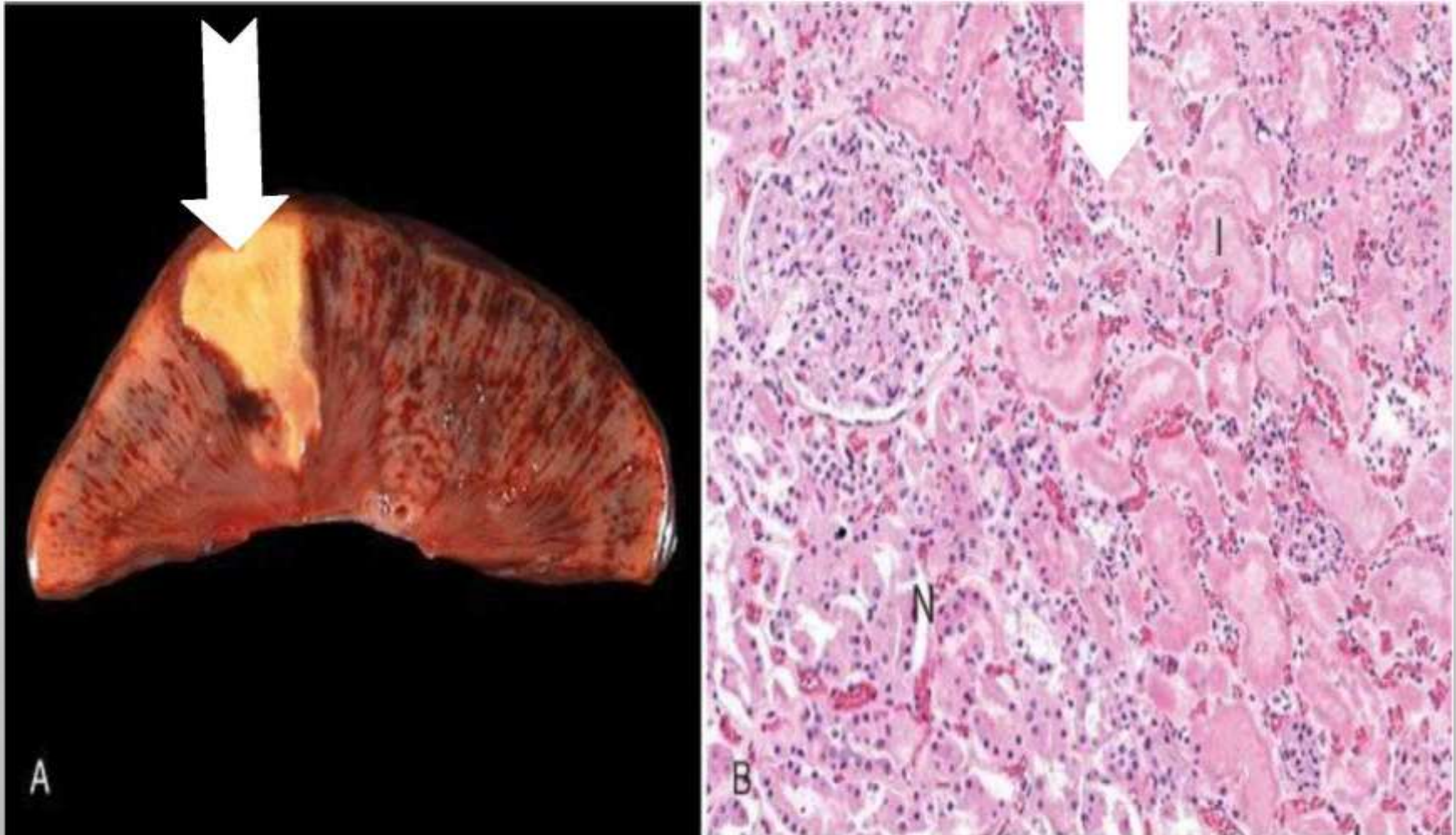
- ❑ The most common type of necrosis, characterized by **preservation of tissue architecture** in which there is **dominant protein denaturation** and the injury also **inhibits the enzyme activity** thus **blocks the proteolysis of dead cells** , as a result , **eosinophilic ,anucleated** cells may persist for days or weeks.
- ❑ This type of necrosis seen in all solid organs like heart, kidney , spleen, and adrenals . etc.
- ❑ Best example of coagulative necrosis is **Myocardial infarction (MI)** in which the necrotic myocytes appears acidophilic, with **loss of nuclei & striations**. Ultimately, the necrotic cells are removed by fragmentation & phagocytosis by WBC.

Fig : Coronal section of the Left Ventricle from a 44y old female patient had MI 8 days before her death . showing yellow-white MI of the left ventricle (*bottom, arrow*) .



6.70 Infarct of myocardium

Coagulative necrosis. A, Wedge-shaped (▼) kidney infarct (yellow) with preservation of the outlines. B, H of the edge of the infarct, within normal kidney (left) & necrotic cells in the infarct (right) . The necrotic cells show preserved outlines with loss of nuclei & an inflammatory infiltrate is present.



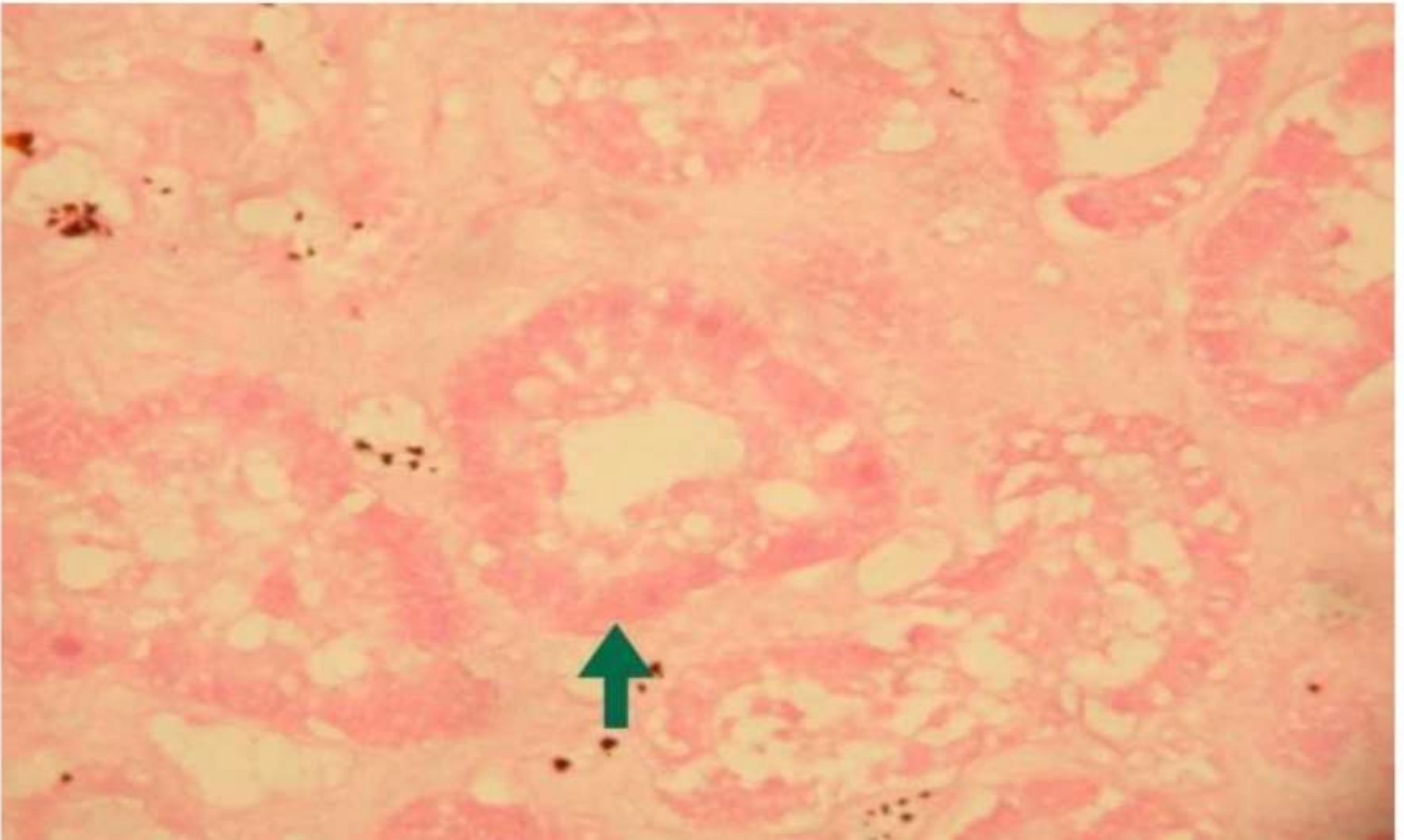


Fig. : Microscopic view of coagulative necrosis in renal infarct ,showing preserved architecture of renal tubules (arrow) showing nuclear karyolysis.

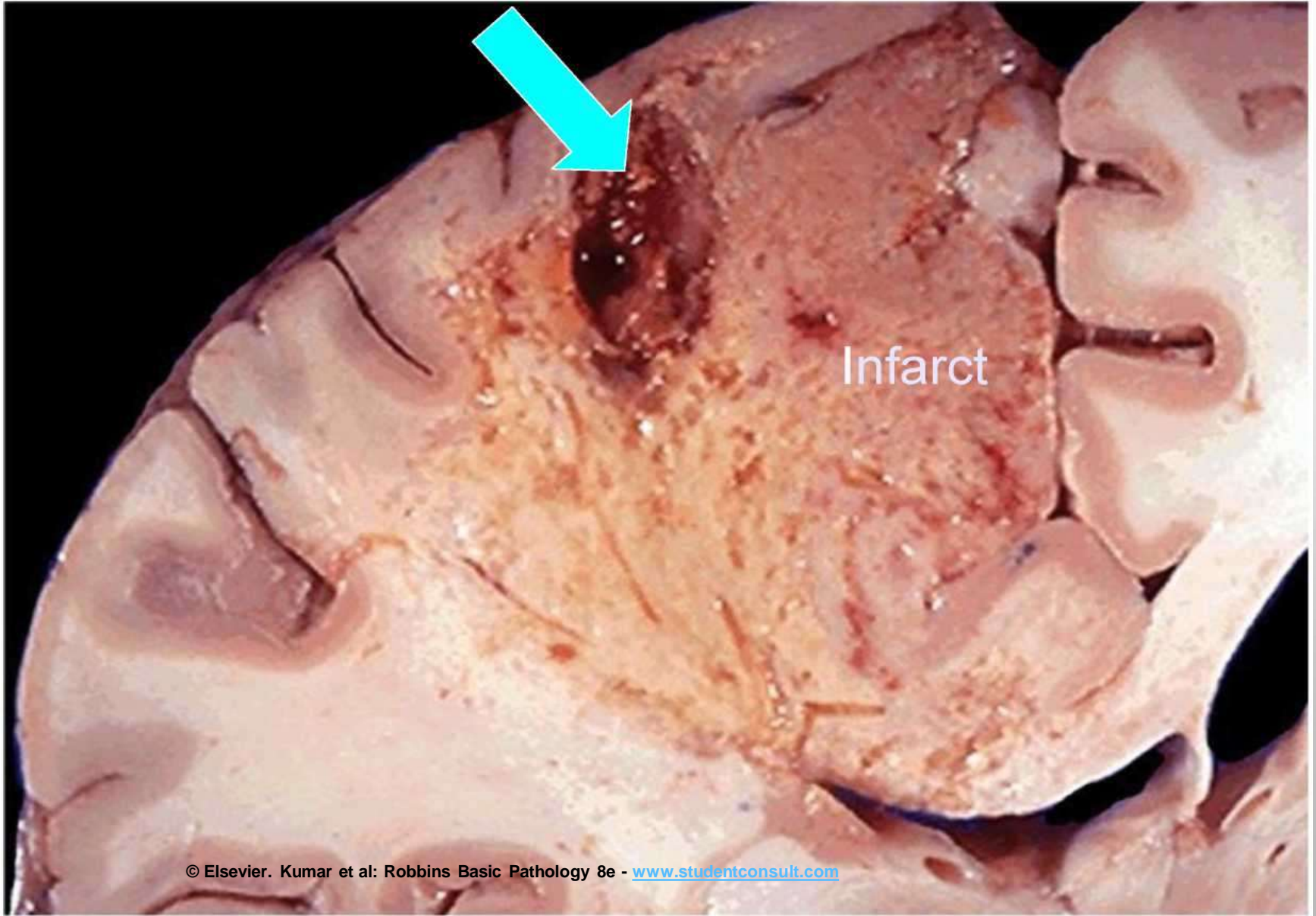


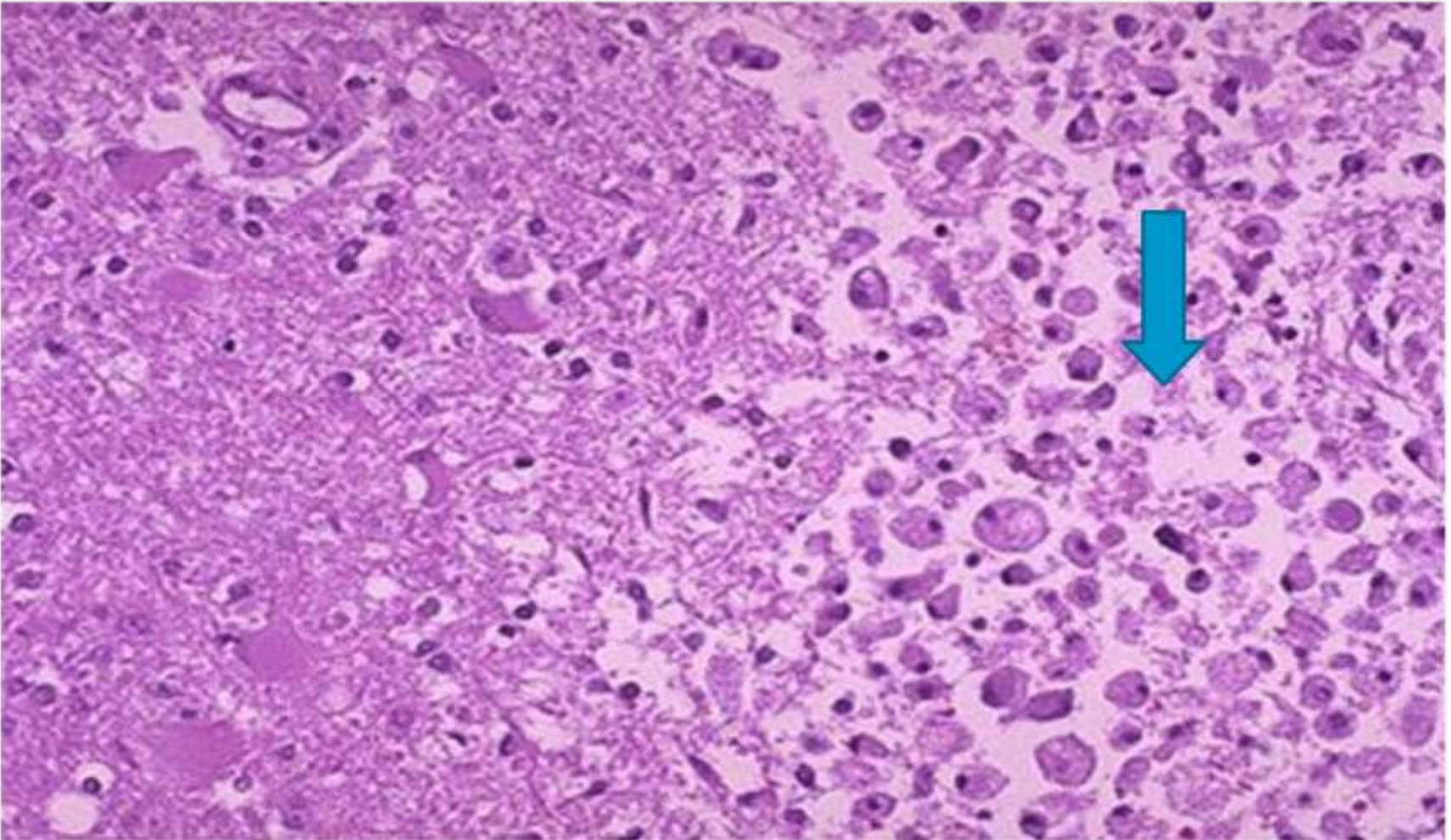
Fig. : Gross appearance of spleen , multiple splenic infarcts example of coagulative necrosis.

(2) Liquefactive necrosis :

- ❑ Characterized by **softening** of the necrotic tissue to the point at which it transforms into a **paste-like mush** or **watery debris** . It implies complete digestion of dead cells with its transformation of it into a **viscous liquid & loss of the general tissue architecture** . The digested tissue is removed by phagocytic cells .
- ❑ Liquefactive necrosis is characteristic of 2 lesions:
 - (I) **Brain Infarction &**
 - (II) **Abscess** : Resulting from pyogenic bacterial or fungal infections .
- ❑ Liquefaction of tissues occurs because of the **action of hydrolytic enzymes** as in brain infarct , or the effect of **lysosomes of inflammatory cells invading the tissue as in abscess** , in which the dead tissue with inflammatory cells are transformed into thick yellow creamy material called pus .

Figure : Liquefactive necrosis. Brain. Cerebral infarct, showing dissolution of the brain tissue.





Microscopic appearance of cerebral infarct : showing normal brain tissue (left)and totally necrotic brain tissue (liquifactive necrosis) (arrow) being replaced by macrophages edema fluid & necrotic debris .

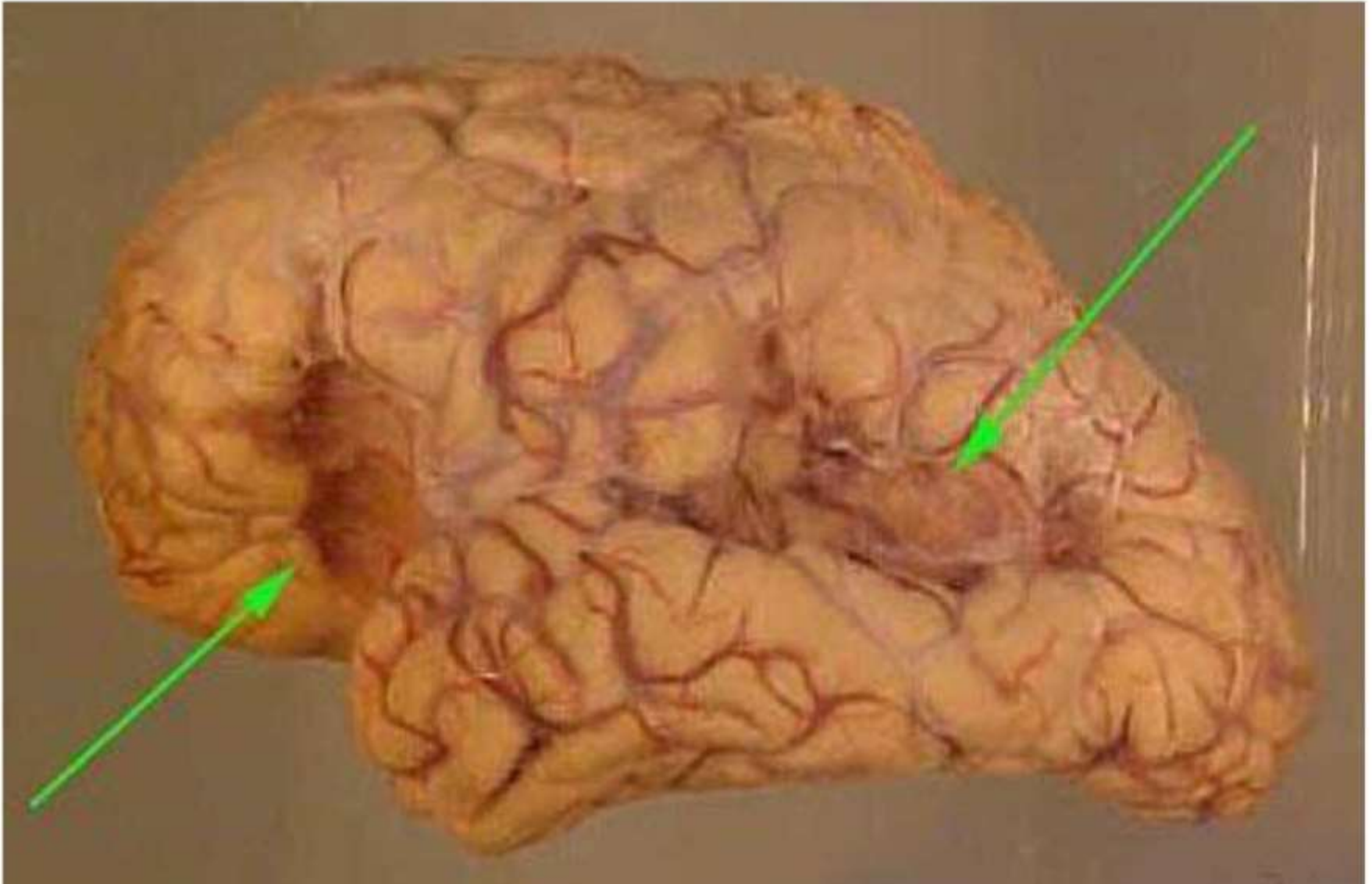
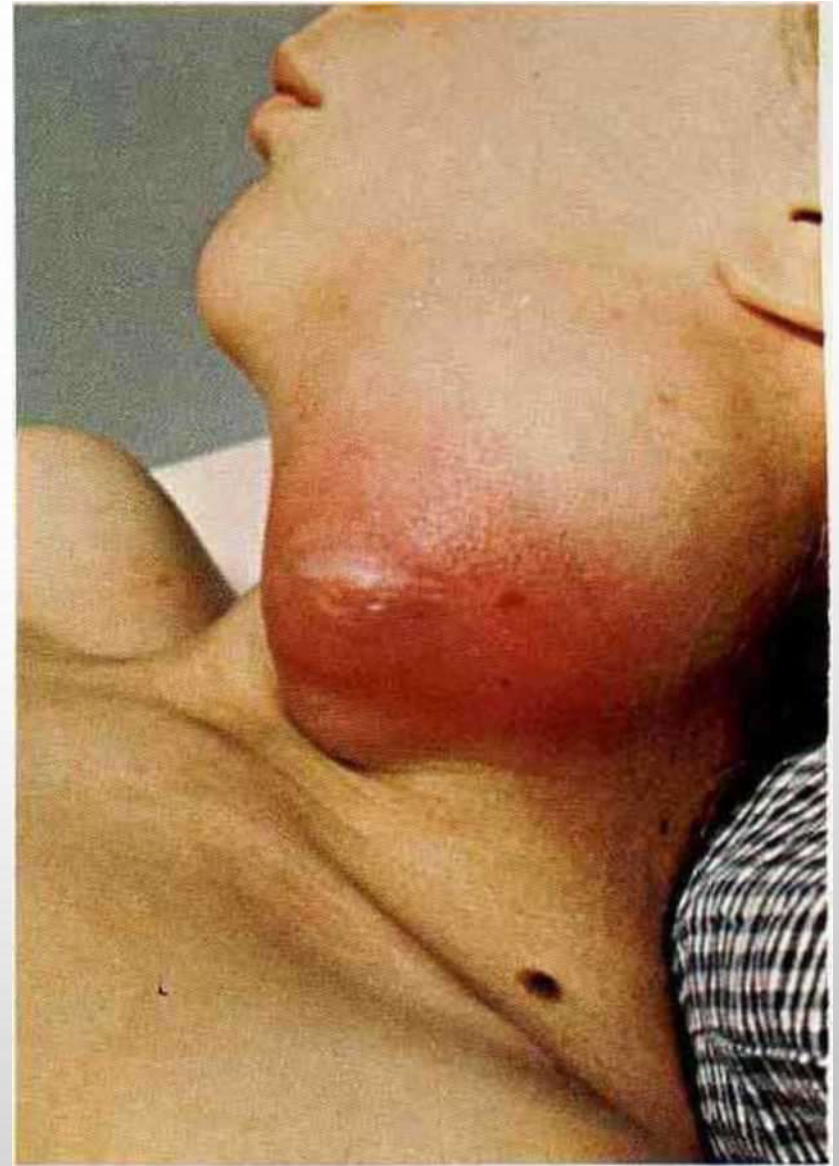
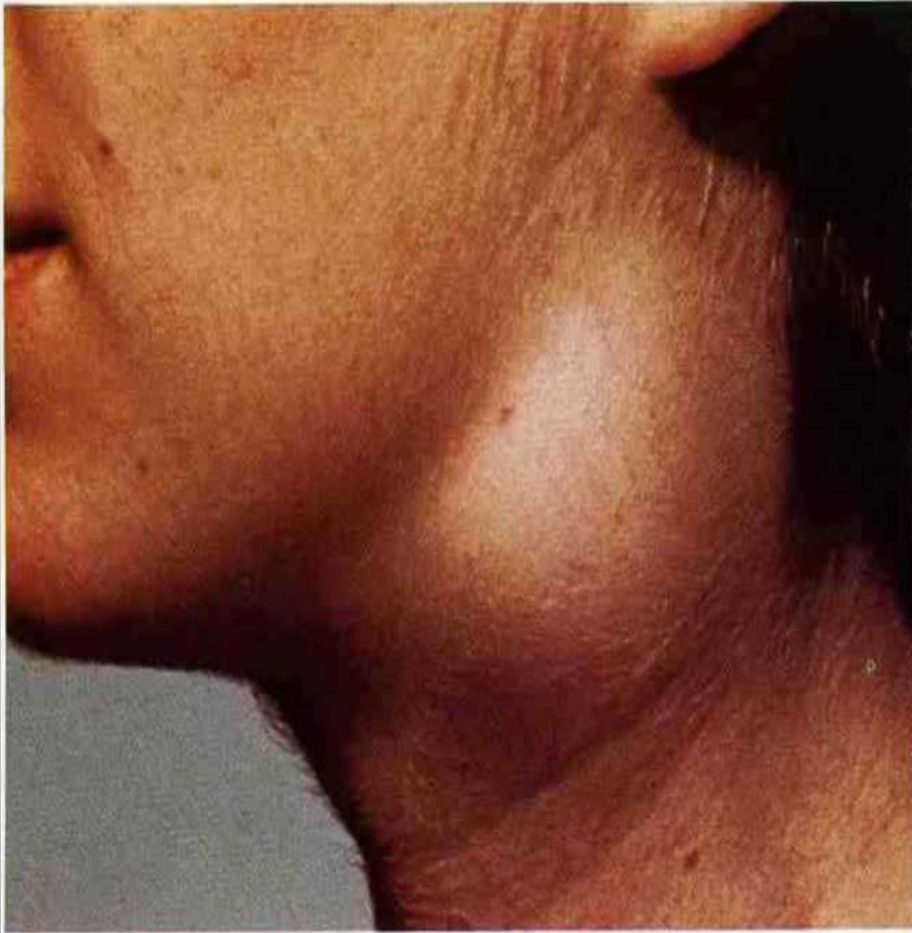
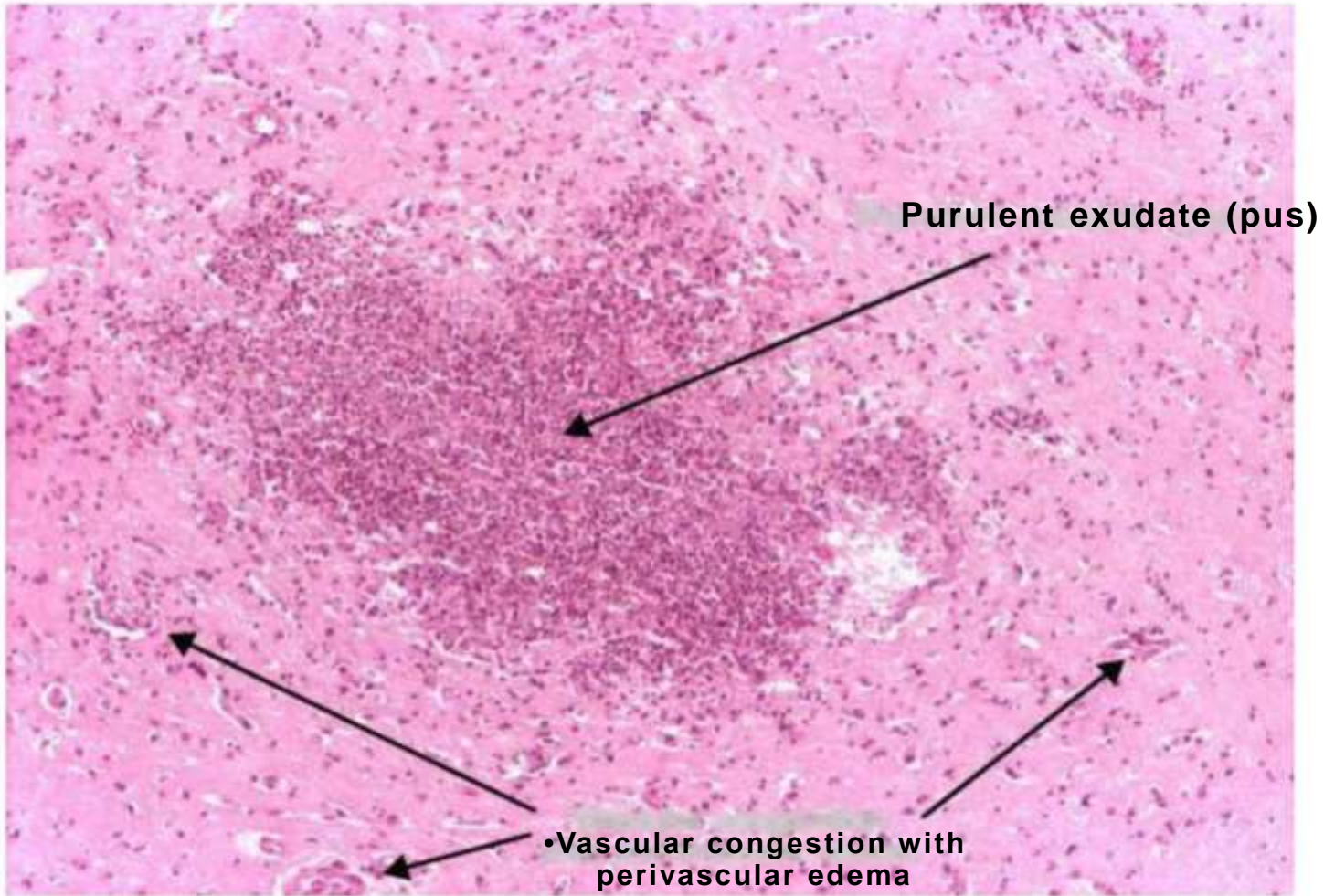


Fig. - Brain : Old cerebral infarcts showing advanced liquefactive necrosis leaving empty cystic lesions(arrows).

Figure - Inflamed cyst, in the neck with suppuration & abscess formation filled with pus, showing redness & swelling.



Suppuration and abscess formation: branchial cvst

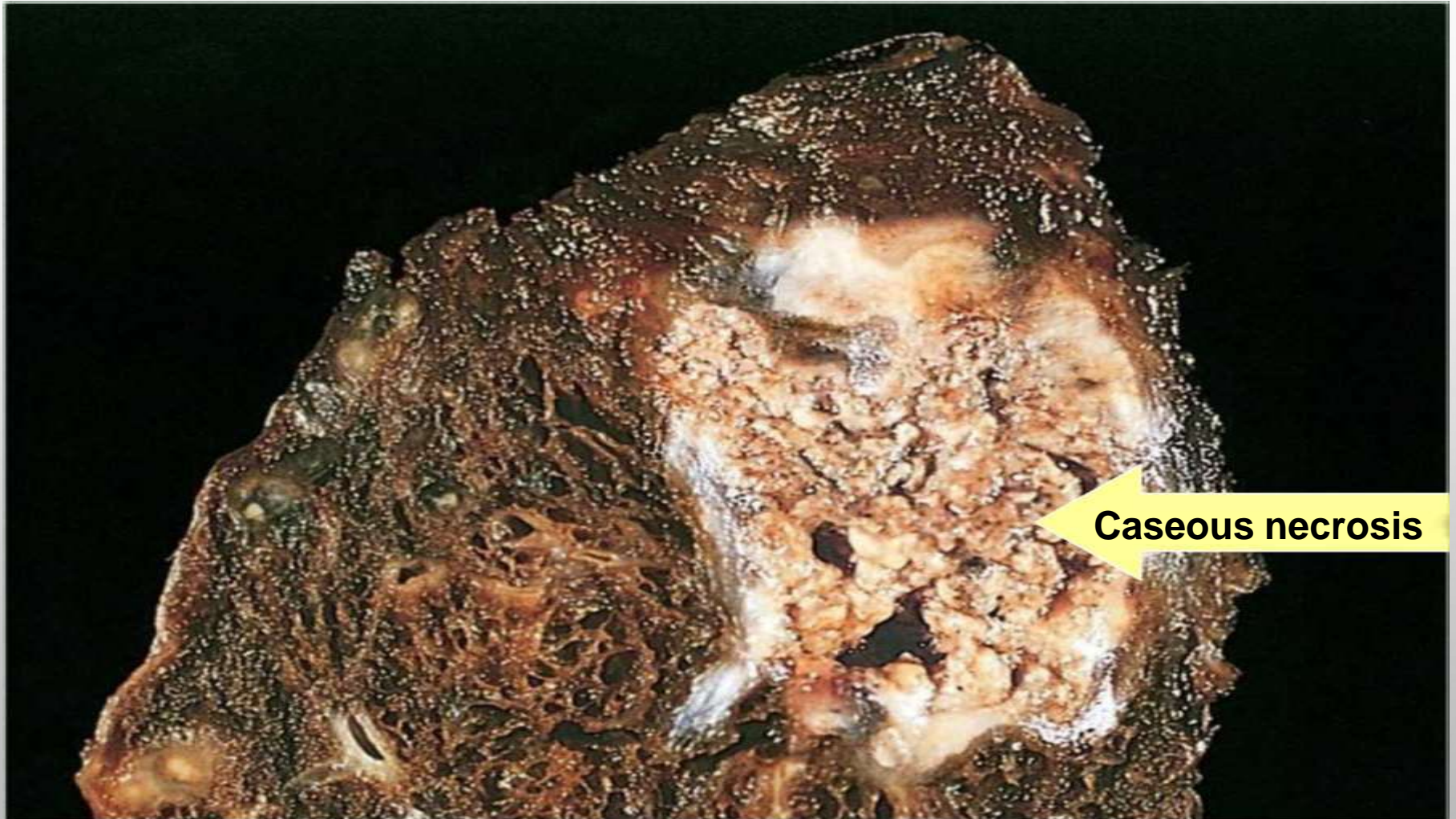


Brain abscess .Microscopic view

(3) Caseous necrosis

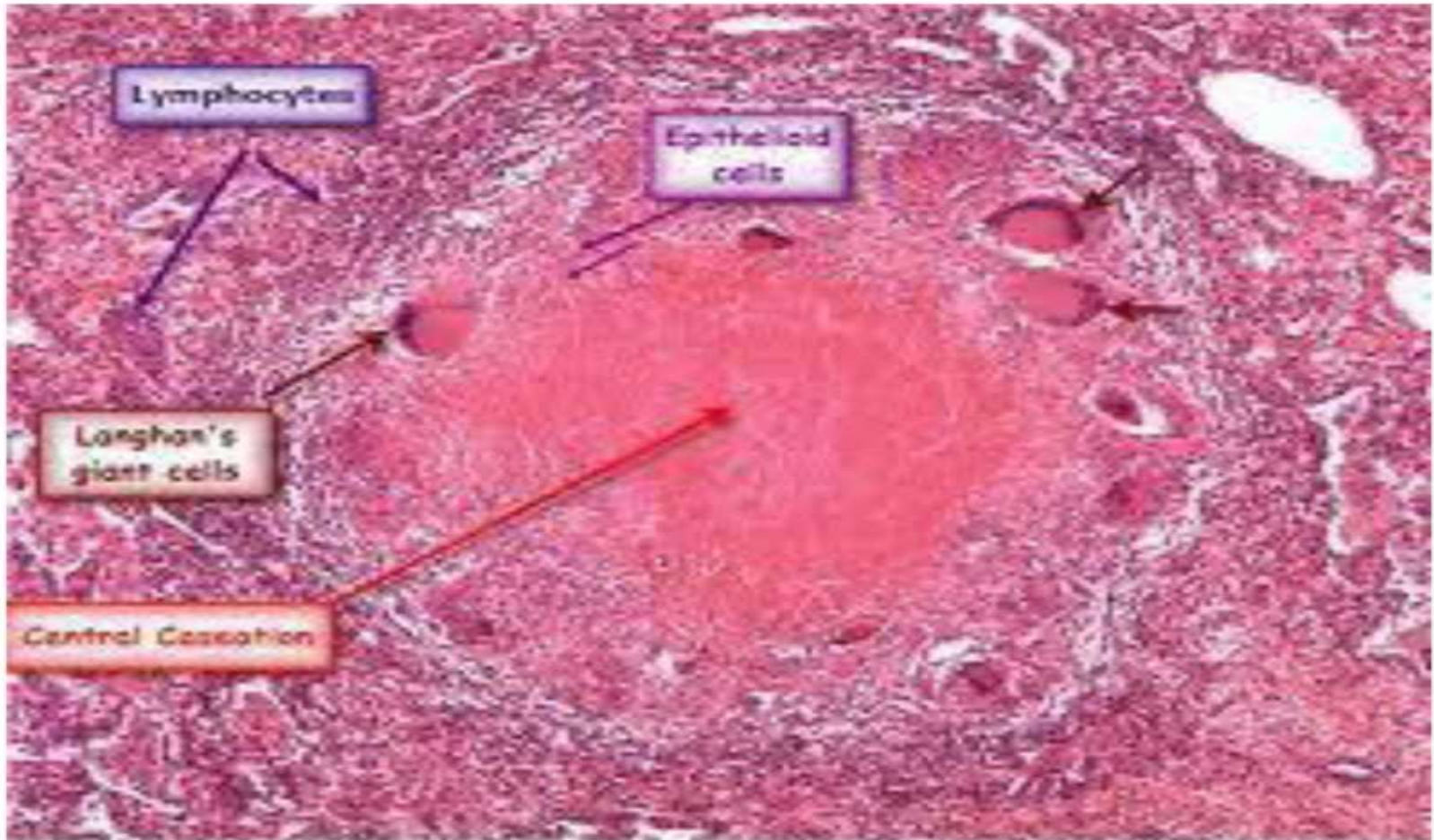
- ❑ “Caseous” term is derived from the cheesy yellowish white gross appearance of the necrotic area , necrotic material is soft greasy . It is a form of necrosis mostly seen in foci of tuberculous infection (TB)
- ❑ Macroscopically : The necrotic focus composed of structureless, amorphous granular debris (fragments) with complete loss of tissue architecture, as in liquefactive necrosis.
- ❑ Microscopically : the caseous necrosis is often enclosed by rim of epithelioid cells (altered macrophages) & the whole lesion is called (granuloma) which is a special type of inflammatory reaction , with multinucleate giant cells called Langhan’s giant cells .

Figure 59 - Caseous necrosis. Large tuberculous (TB) lesion in the lung apex, containing yellowish – white & cheesy debris of caseous necrosis.



This is more extensive **caseous necrosis**, with confluent cheesy tan granulomas in the upper portion of this lung in a patient with tuberculosis. The tissue destruction is so extensive that there are areas of **cavitation (cystic spaces) being formed as the necrotic (mainly liquefied) debris drains out via the bronchi.**





Labeled by: www.scienceway.com

Figure - Microscopic appearance of caseous necrosis (Tuberculosis)

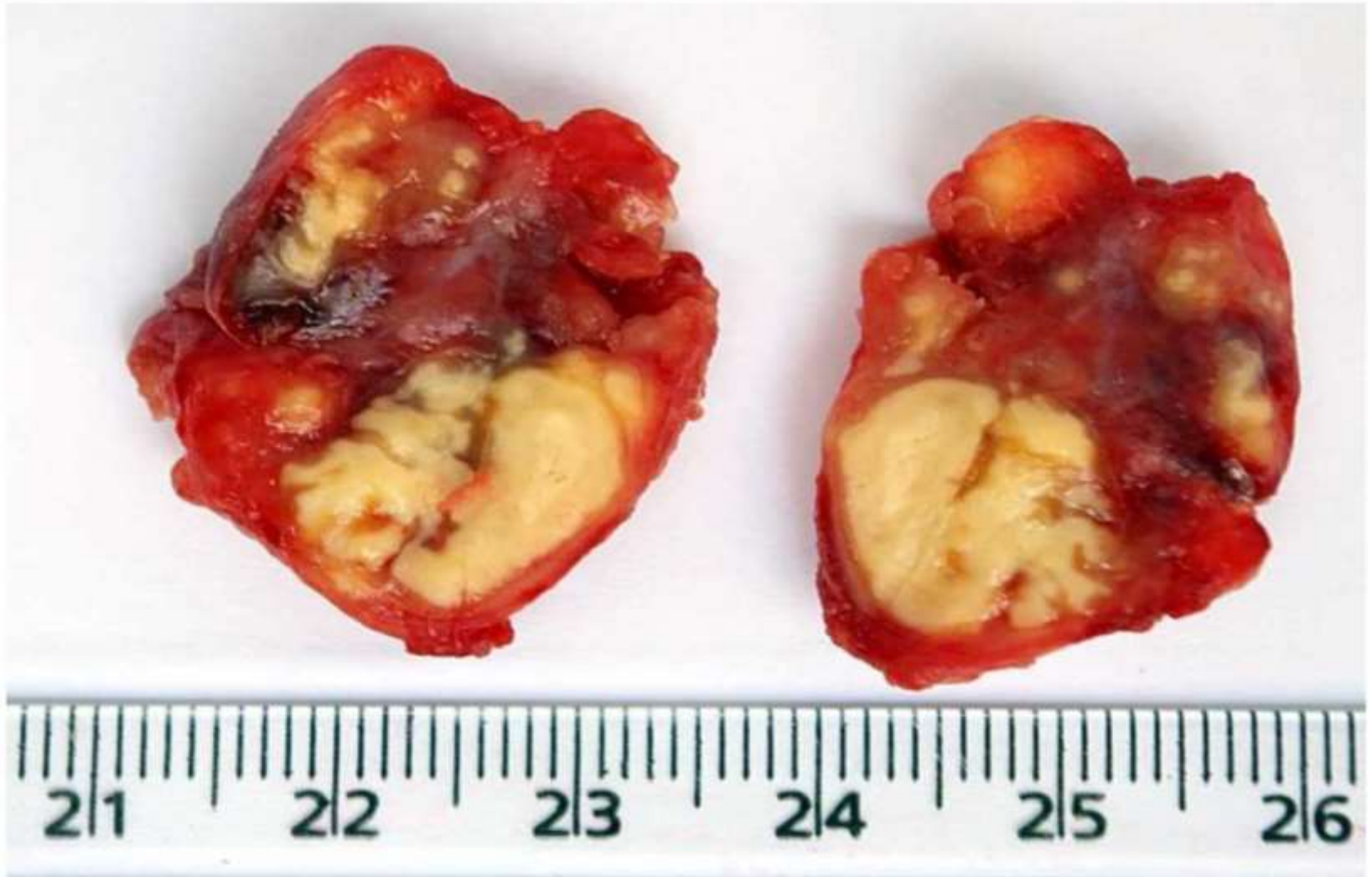


Fig. - Lymph node in Tuberculous lymphadenitis showing caseous necrosis which is a soft yellowish white cheesy material

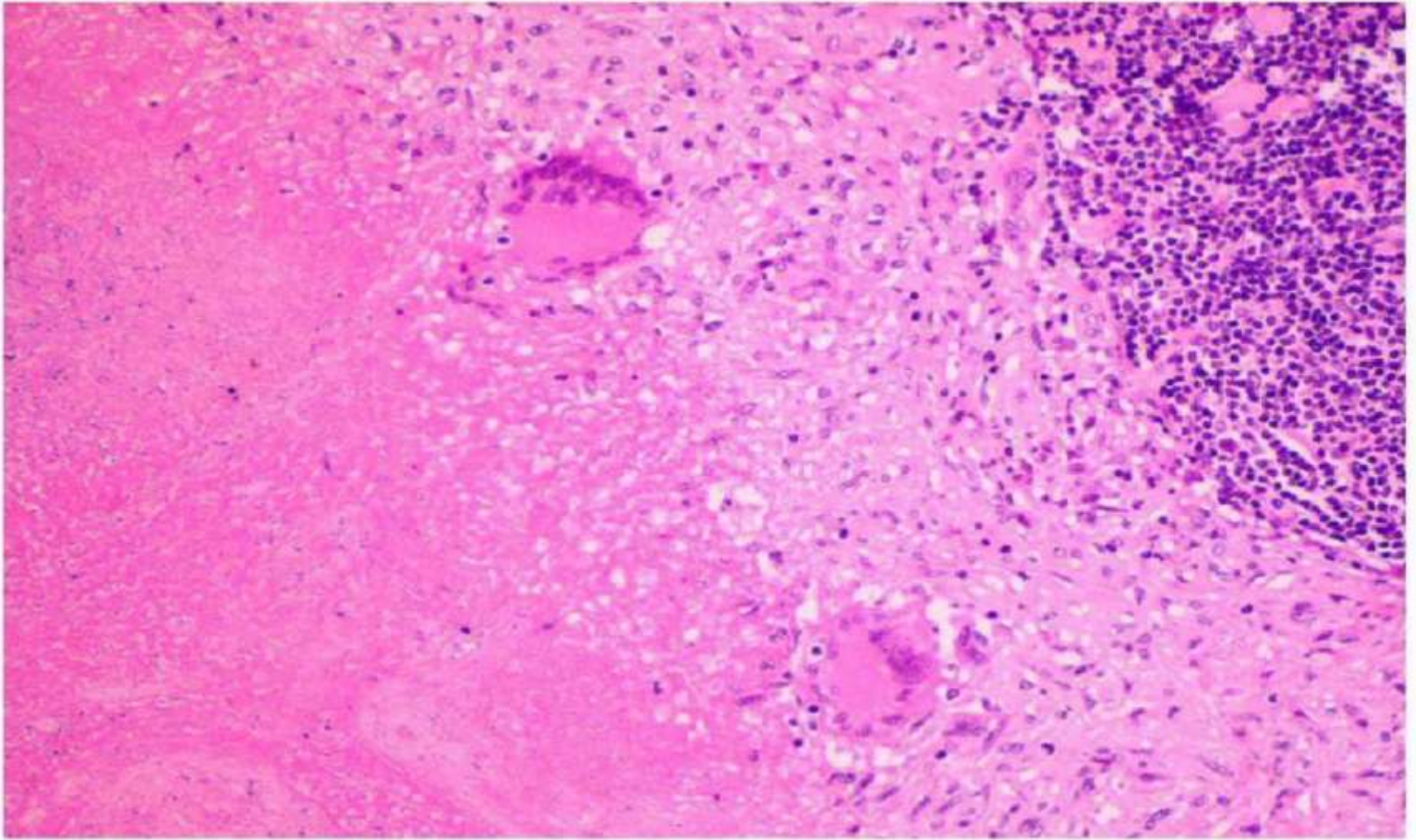


Fig. - Tuberculous Lymphadenitis: microscopic appearance showing caseous necrosis with two Langhans' giant cells fibrous tissue & lymphocytes (right) .

(4) Fat necrosis

- ❑ Describes focal areas of **enzymatic fat destruction**, typically resulting from release of **activated pancreatic lipases** into the substance of **the pancreas & the peritoneal cavity**.
This typically involves fat cells in & around the pancreas , in omentum & subcutaneous tissues .
- ❑ In **acute pancreatitis**, in which **pancreatic enzymes** that have leaked out of the acinar cells & ducts liquefying the membranes of fat cells in the peritoneum, & **lipases split the triglycerides esters contained within fat cells**. The accumulated fat globules can be identified by special stain called **Oil red O or Sudan IV** done on fresh frozen tissues staining fat as red intracytoplasmic globules .
- ❑ The released **fatty acids** combine with calcium (from the blood) to produce typical gross **chalk white areas** .
- ❑ Fat necrosis is also seen following trauma to **fat as in breast or subcutaneous tissue** .

Greater Omentum

Liver-

Stomach —

Gallbladder

Transverse
colon

underneath

Greater——'
"3-omentum

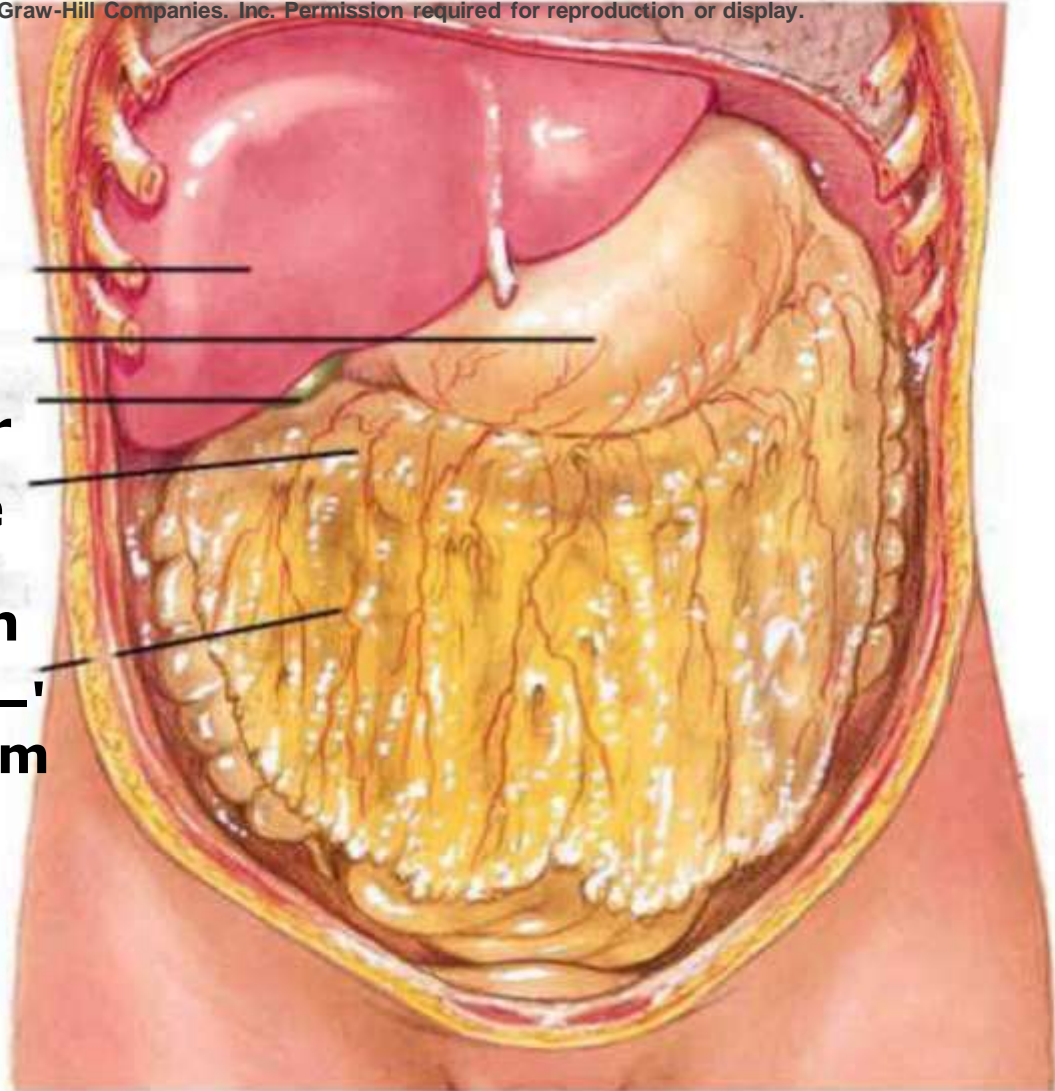


Figure - Normal greater omentum.

Figure - Fat necrosis in acute pancreatitis . Omentum.



- Acute hemorrhagic pancreatitis :

Omentum being studded with small yellow-white foci of fat necrosis.



5.44 Acute haemorrhagic pancreatitis: omentum

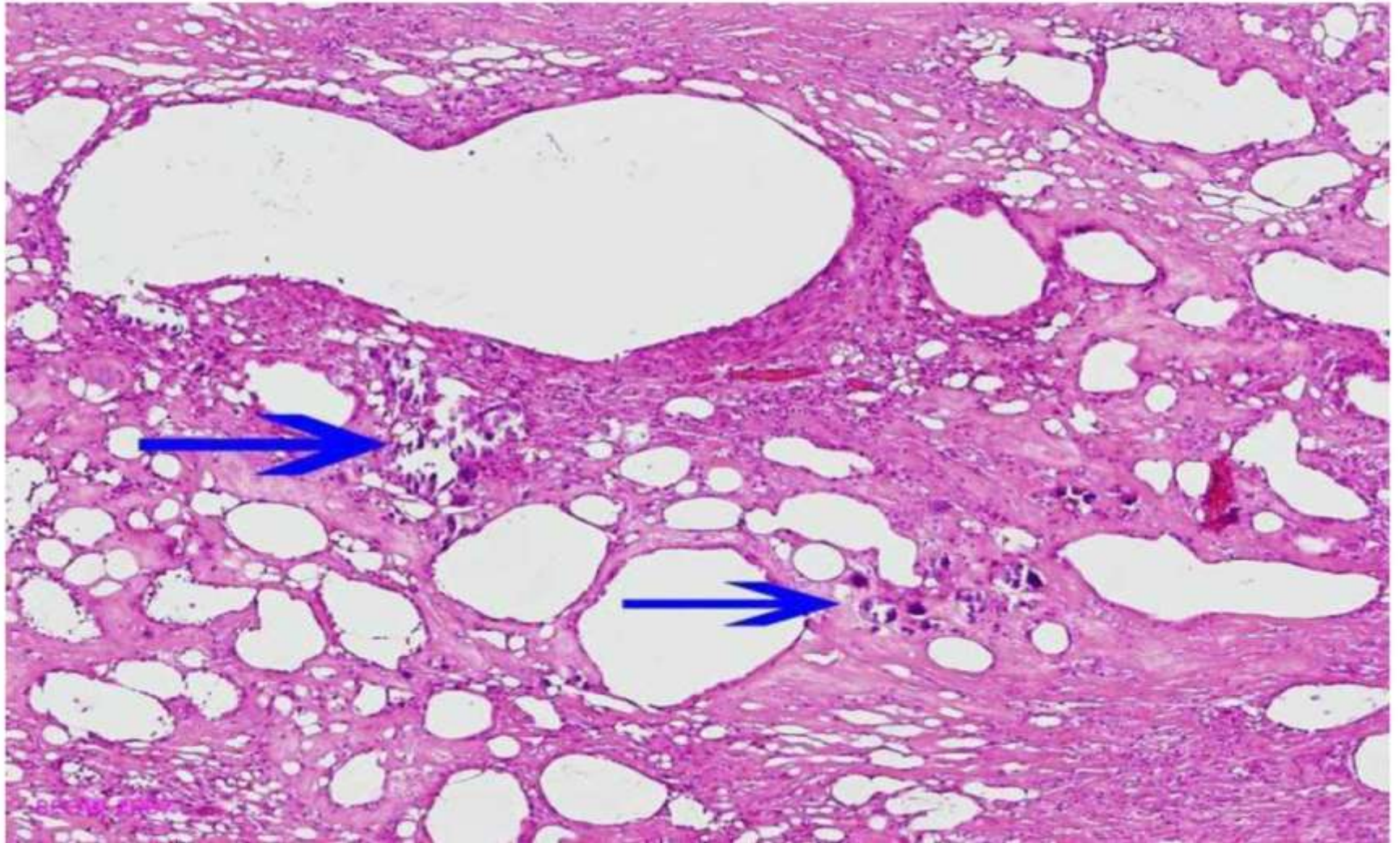


Figure : Microscopic picture of fat necrosis , showing fat vacuoles ,(white areas) & spotty calcification of necrotic tissue (stained purple- blue).



Fig . : Hand of a child showing fat necrosis following a trauma.

Fig. 69 : Fat necrosis: Skin. Trauma may cause necrosis of adipose tissue (e.g in the breast, or skin). The released neutral fat is then ingested by macrophages, resulting in the pale pink appearance of their cytoplasm in the H&E stain (thin arrow) X135.

Right : Oil red O or Sudan IV stain fat globules red on frozen section .

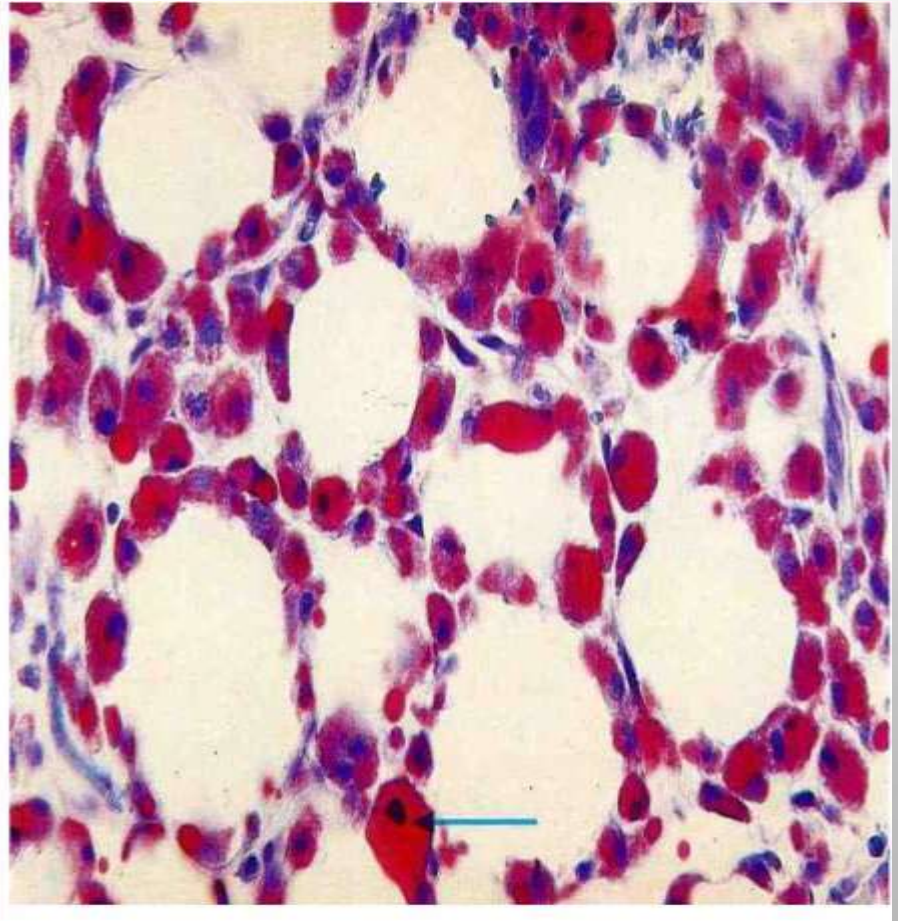
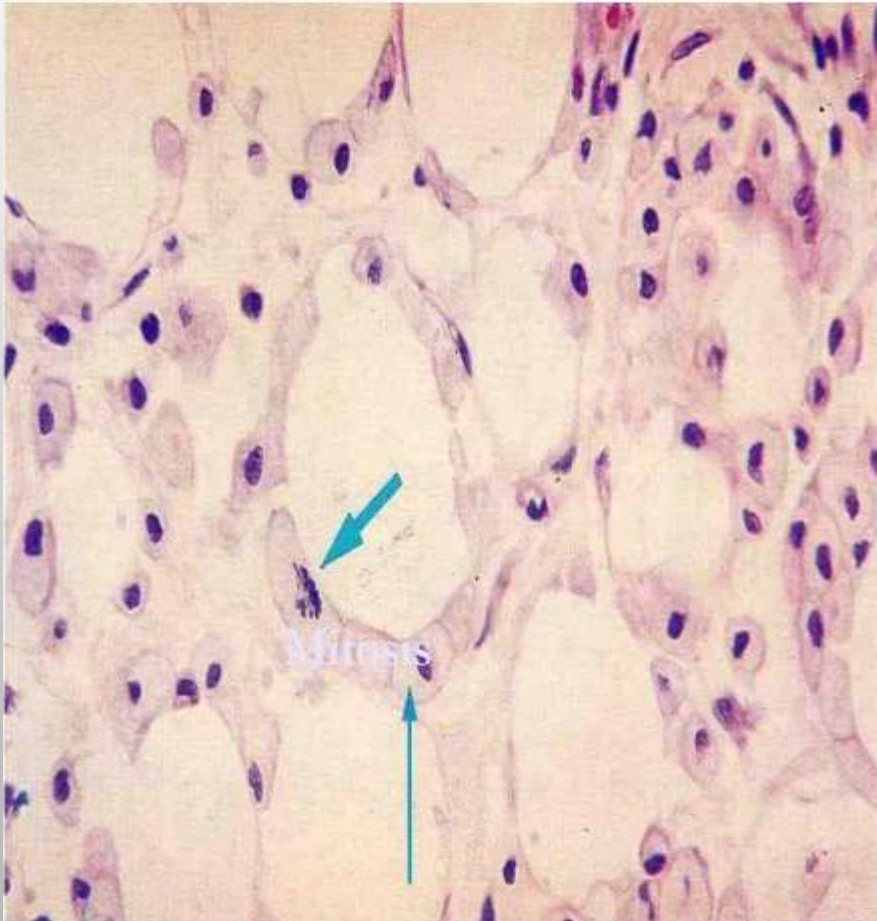




Fig. - Mammographic appearance of normal breast (right) and a breast showing focal fat necrosis (left).

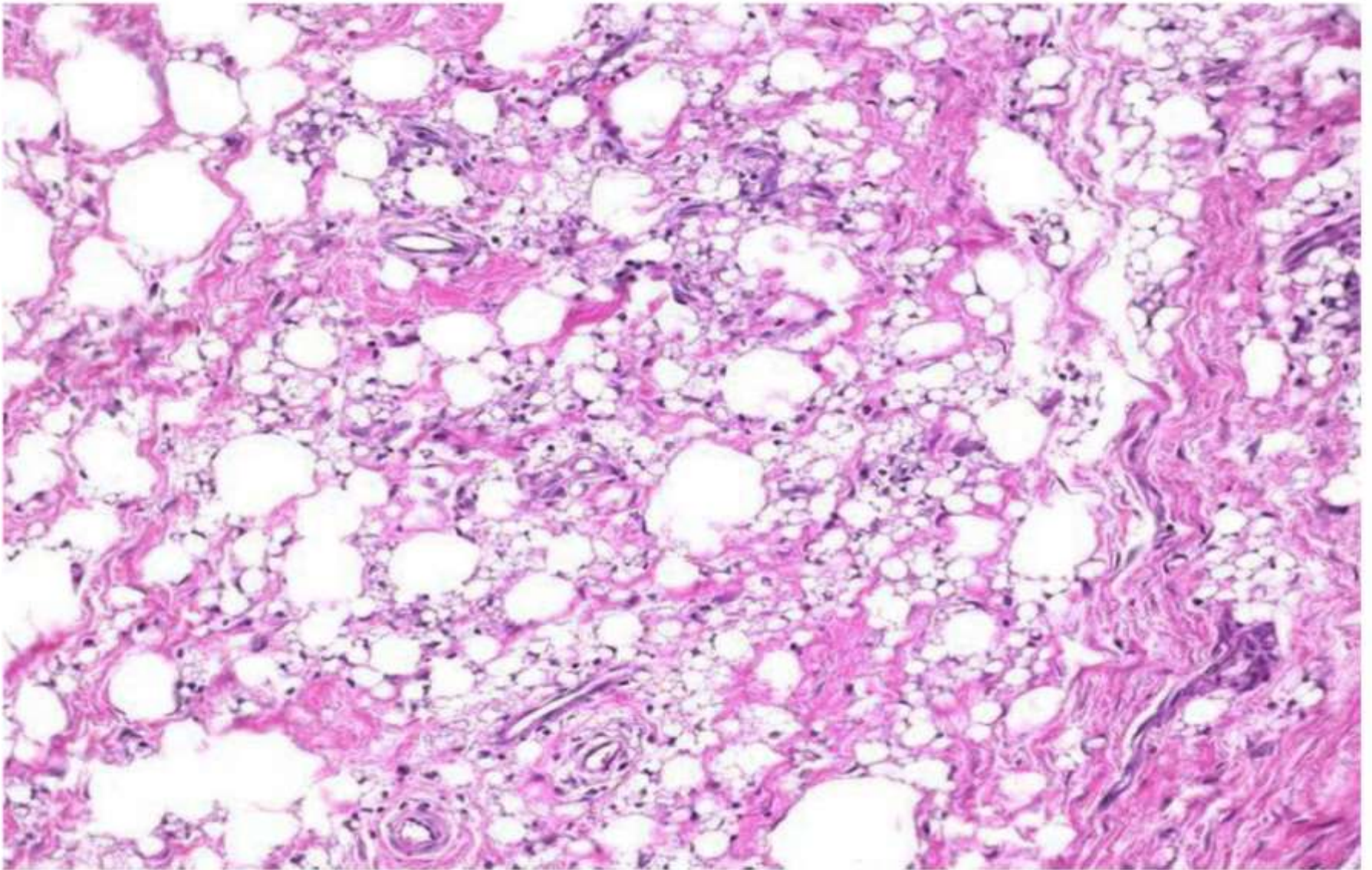


Figure - Fat necrosis in subcutaneous tissue.

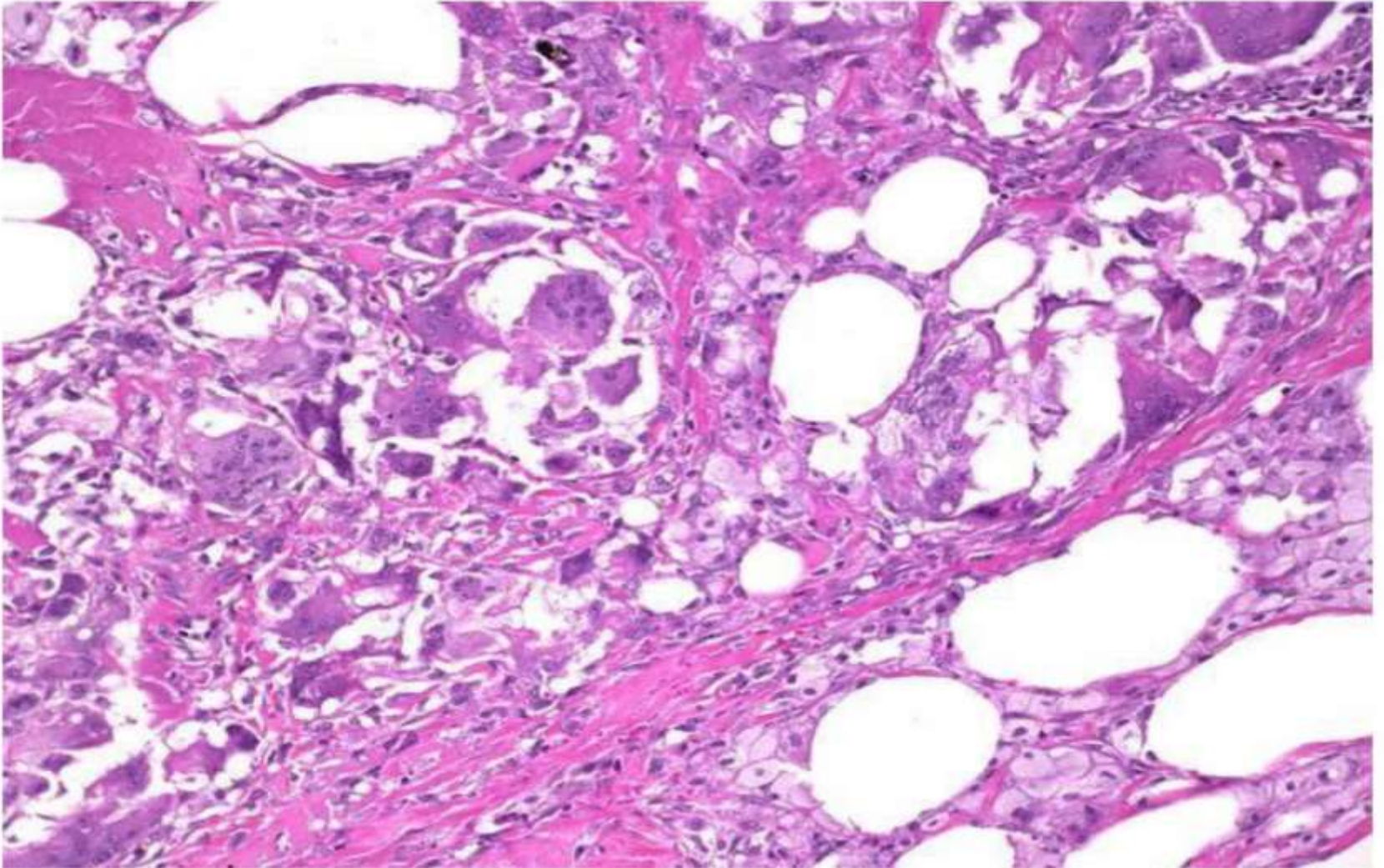


Fig. : Microscopic view of fat necrosis with foreign-body giant cell reaction surrounding fat globules .

(5) Fibrinoid necrosis

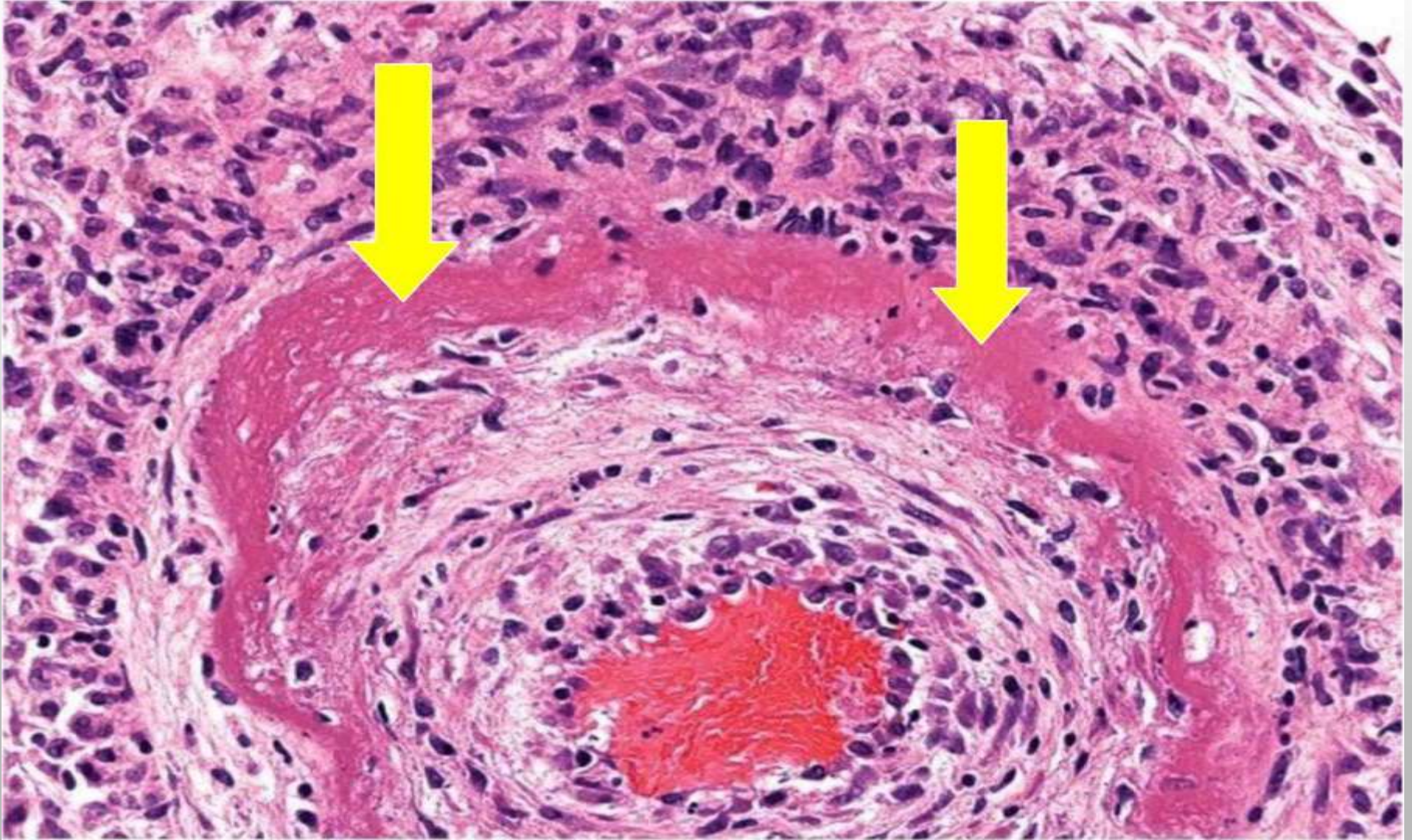
□ Special form of necrosis limited to **small blood vessels**, the walls of necrotic blood vessel including renal glomeruli become impregnated with fibrin usually seen in:

(1) in arterioles in malignant hypertension &

(2) in *immune reactions* involving Blood Vessels

(e.g., polyarteritis nodosa) in which: *antigens & antibodies complexes* are deposited in the wall of arteries which together with + *fibrin* that has leaked out of vessels = result in *a bright pink & homogeneously red amorphous appearance* called “fibrinoid” i.e. fibrin-like necrosis .

Figure - Fibrinoid necrosis in an artery in a patient with polyarteritis nodosa. The wall of the artery shows a circumferential bright pink area of necrosis with protein deposition & inflammation (dark nuclei of neutrophils).



(6) Gangrenous necrosis

- ❑ It is not a distinctive pattern of necrosis it is a clinical term refers to infarction + superimposed infection by saprophytic bacterial i.e. (bacteria which cannot live on living tissue , only survive on dead necrotic tissues) .
- ❑ The term is first applied to describe a limb that lost its blood supply with resultant coagulative necrosis which later on becomes liquified by the action of saprophytic bacteria .

Gangrenous Necrosis

Gangrene can be :

Dry gangrene (Diabetes mellitus)

Wet gangrene (Intestine)

Gas gangrene (Caused by Clostridia

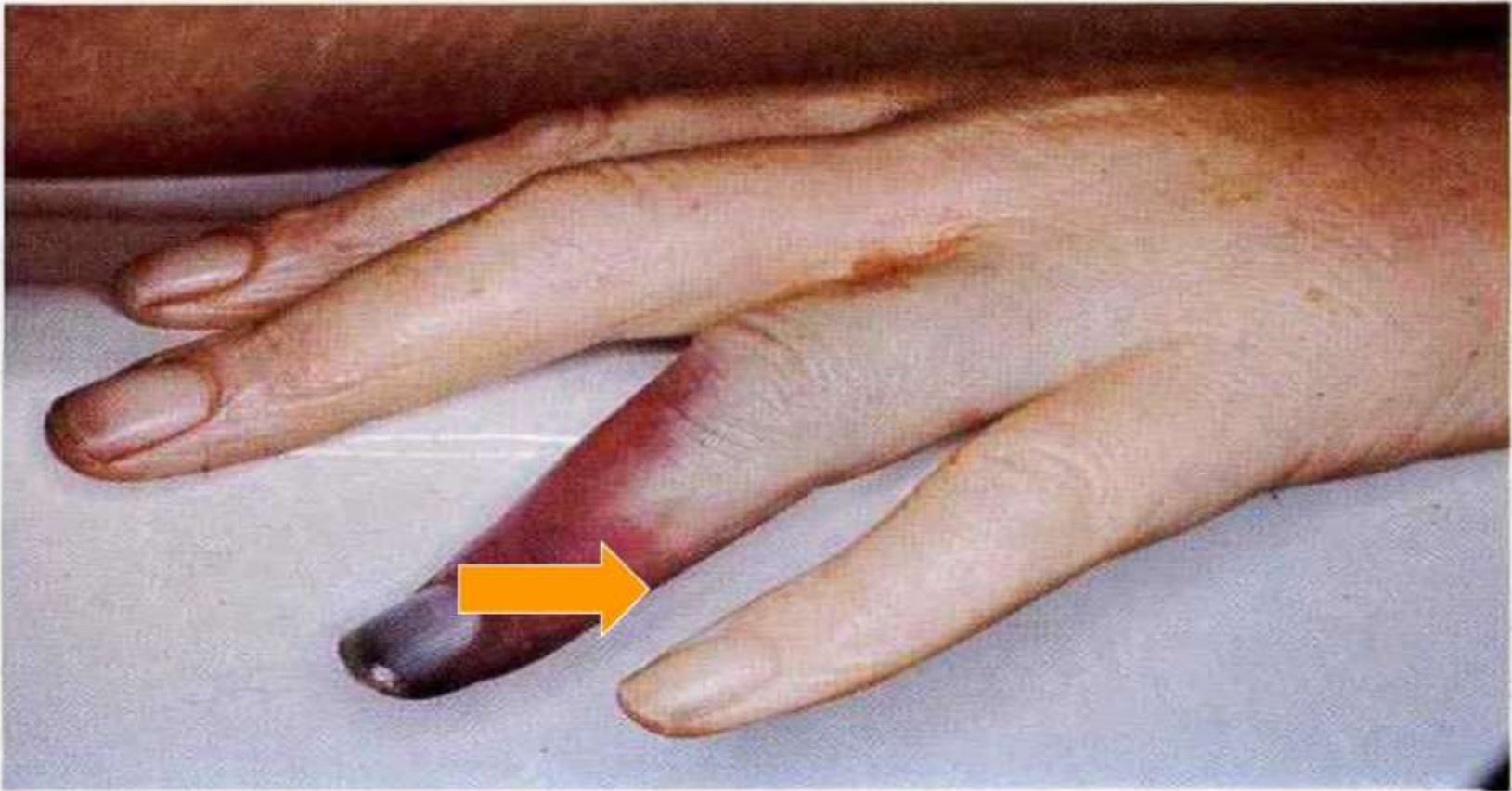
Welchii infection of ischemic tissue .)

Figure - Digital gangrene.

Gangrene of the top of the third ('ring⁵) finger.

The nail is bluish-black & the skin is dusky purplish-red.

The gangrene was due to thrombosis of the digital arteries .



14.16 Digital gangrene

Figure 75 - Gangrenous infarction of small intestine as a result of volvulus, i.e., twisting of a loop of intestine upon itself, a process which obstructs the intestine & interferes with its blood supply. The gangrenous small intestinal coils are greatly distended & blue-black.



4.28 Volvulus: small intestine

This is gangrene of the lower extremity. In this case the term "wet" gangrene is more applicable because of the liquefactive component from superimposed infection in addition to the coagulative necrosis from loss of blood supply. This patient had diabetes mellitus with severe peripheral vascular disease.



MECHANISMS OF CELL INJURY:

(1) **The cellular response to injurious stimuli depends on:**

- A. - Type of injury,
- B. - Duration of injury,
- C. Severity of Injury

- ❖ **Low doses of toxins or a short duration of ischemia may lead to reversible injury.**
- ❖ **Exposure to larger toxin doses, even in short intervals may result in irreversible injury.**
- ❖ **Slow ischemic injury as in atherosclerosis of renal arteries will not result in renal infarct formation , while sudden thrombotic occlusion of renal arteries leads to infarction .**

(2) The consequences of an injurious stimulus depend on:

- ❖ Type of the injured cell
 - ❖ Nutritional status (or hormonal) of the injured cell
 - ❖ Adaptability of the injured cell
 - ❖ Genetic makeup of the injured cell.
- ❑ The same injury has vastly different outcomes depending on the cell type ; **striated skeletal muscle in the leg withstand** complete ischemia for **2 to 3 hours** without necrosis , whereas, liver cell **after 2 hours** , cardiac muscle dies **20 to 30 minutes** after coronary occlusion, neuron dies **after 3-5 minutes**.

❑ The nutritional (or hormonal) status

can also be important; a glycogen filled liver cell will tolerate ischemia much better than one that has just burnt its last glucose molecule, like brain cells .

❑ Genetically determined diversity in metabolic pathways

: -

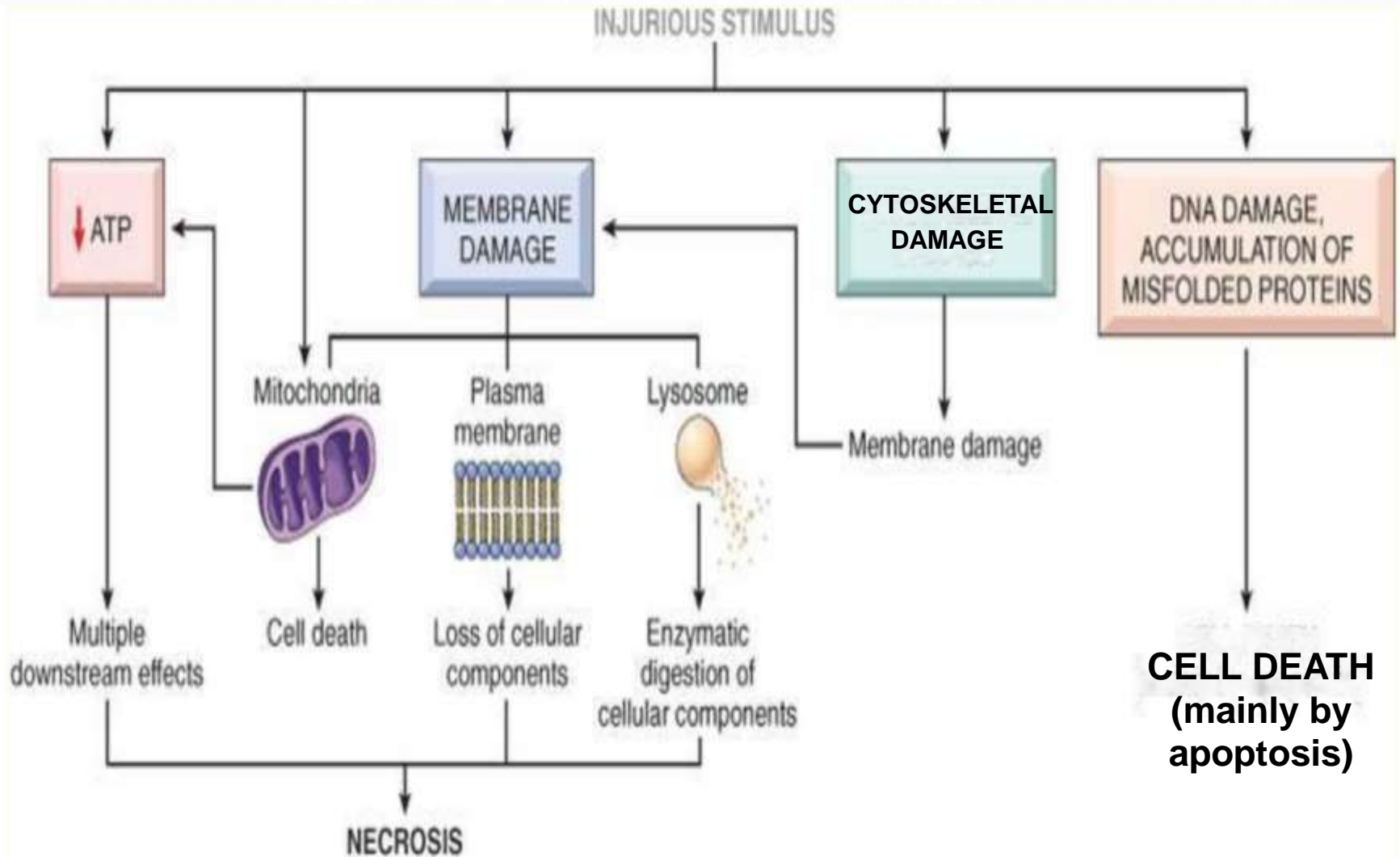
For instance, when exposed to the same dose of a toxin, individuals who inherit variants in genes encoding cytochrome P-450 may catabolize the toxin at different rates, leading to different outcomes . (e.g., carcinogenic effect of smoking).

Cell injury results from functional & biochemical abnormalities in one or more of five essential cellular components .

The most important targets of injurious stimuli are the:

- 1. Mitochondria the sites of ATP generation,**
- 2. Cell membranes which maintain the ionic & homeostasis of the cell.**
- 3. Protein synthesis**
- 4. Cytoskeleton**
- 5. Genetic apparatus of the cell.**

F 76: Sites of damage in cell injury



- **Damage to Mitochondria**

- ❖ Normally: mitochondria are the cell's mini factories that produce a life —sustaining energy in the form of ATP.
- ❖ Mitochondria can be damaged by :
 - * Decreased O₂ i.e. **hypoxia** (commonest type is ischemic hypoxia)
 - * Increased **cytosolic Ca²⁺**
 - * **ROS** : (Reactive Oxygen Species) Free radicals.
 - * Radiation .

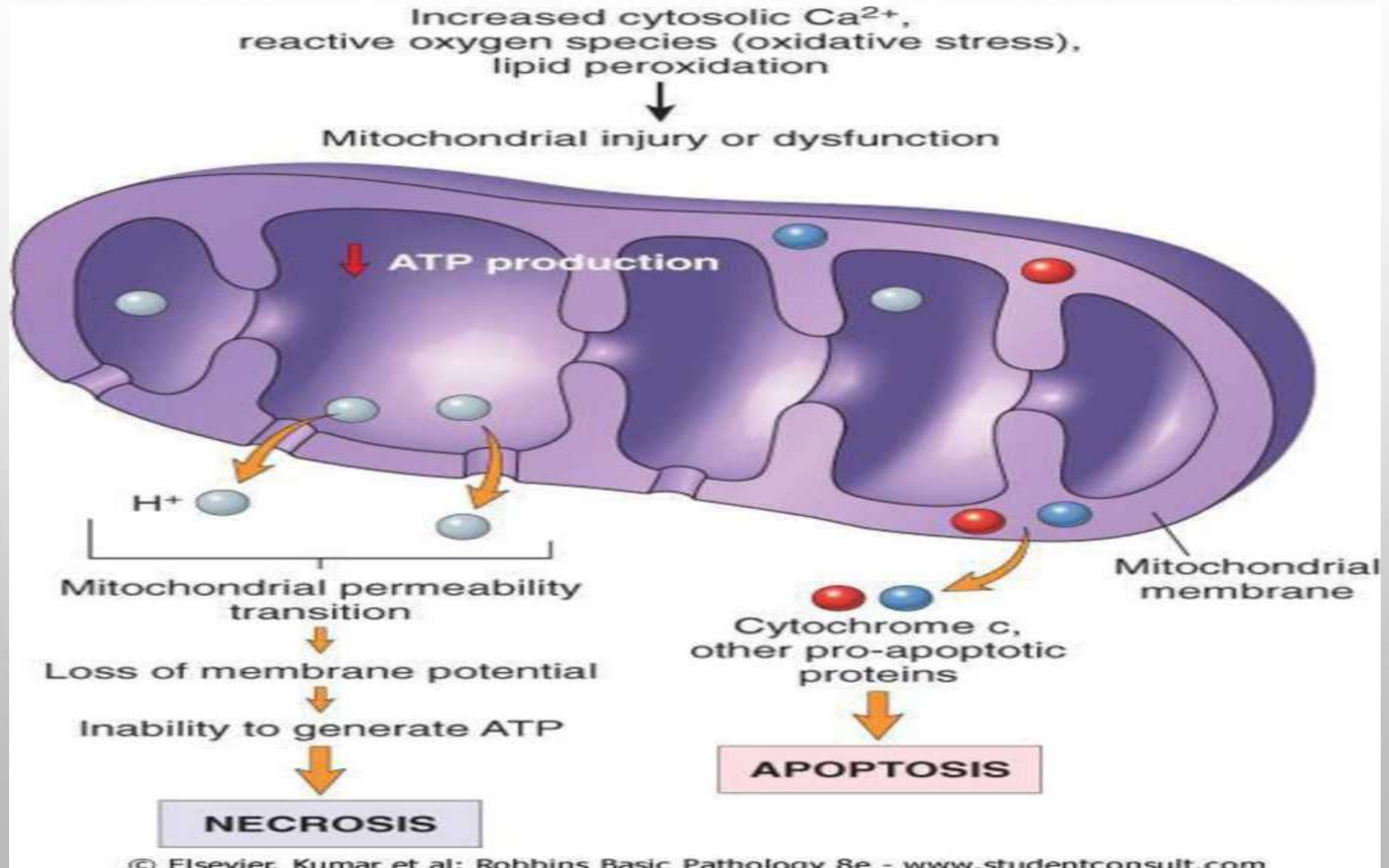
❖ **The major consequences of mitochondria damage**
1-Failure of oxidative phosphorylation leads to progressive depletion of ATP, leading to cell necrosis .

2- Abnormal oxidative phosphorylation also leads to formation of reactive oxygen species (ROS) , which have a harmful effects on the cell.

3-formation of a high-conductive channel in the mitochondrial membrane, called
“mitochondrial permeability transition pore”

- **Opening of the high-conductance channel** in the mitochondria leads to two major consequences :
 - (I) loss of mitochondrial membrane potential & pH changes resulting in failure of oxidative phosphorylation and **progressive depletion of ATP & cell death by necrosis** .
 - (II) **leakage of cytochrome C protein**_into the cytosol_ activate the apoptotic pathways (BAX & BAK), resulting in cell death by apoptosis.

Consequences of mitochondrial dysfunction, culminating in cell death by necrosis or apoptosis.



- **Depletion of ATP**

ATP, the energy store of cells, is produced mainly by:
(1) in the presence of **oxygen**, through the oxidative phosphorylation of adenosine diphosphate (ADP) .

(2) **in the absence** of oxygen (anoxia), by using glucose derived from the circulation or from the hydrolysis of intracellular glycogen (**anaerobic glycolysis**) .

The compensatory increase in **anaerobic glycolysis** in an attempt to maintain the cell's energy sources , leads to :

- (1) intracellular glycogen depletion
- (2) lactic acid accumulation, leading to
- (3) decrease intracellular pH (acidosis) &
- (4) Decrease activity of many cellular enzymes .

Depletion of ATP to **less than (5% to 10%)** of normal levels, has widespread effects on many critical cellular systems .

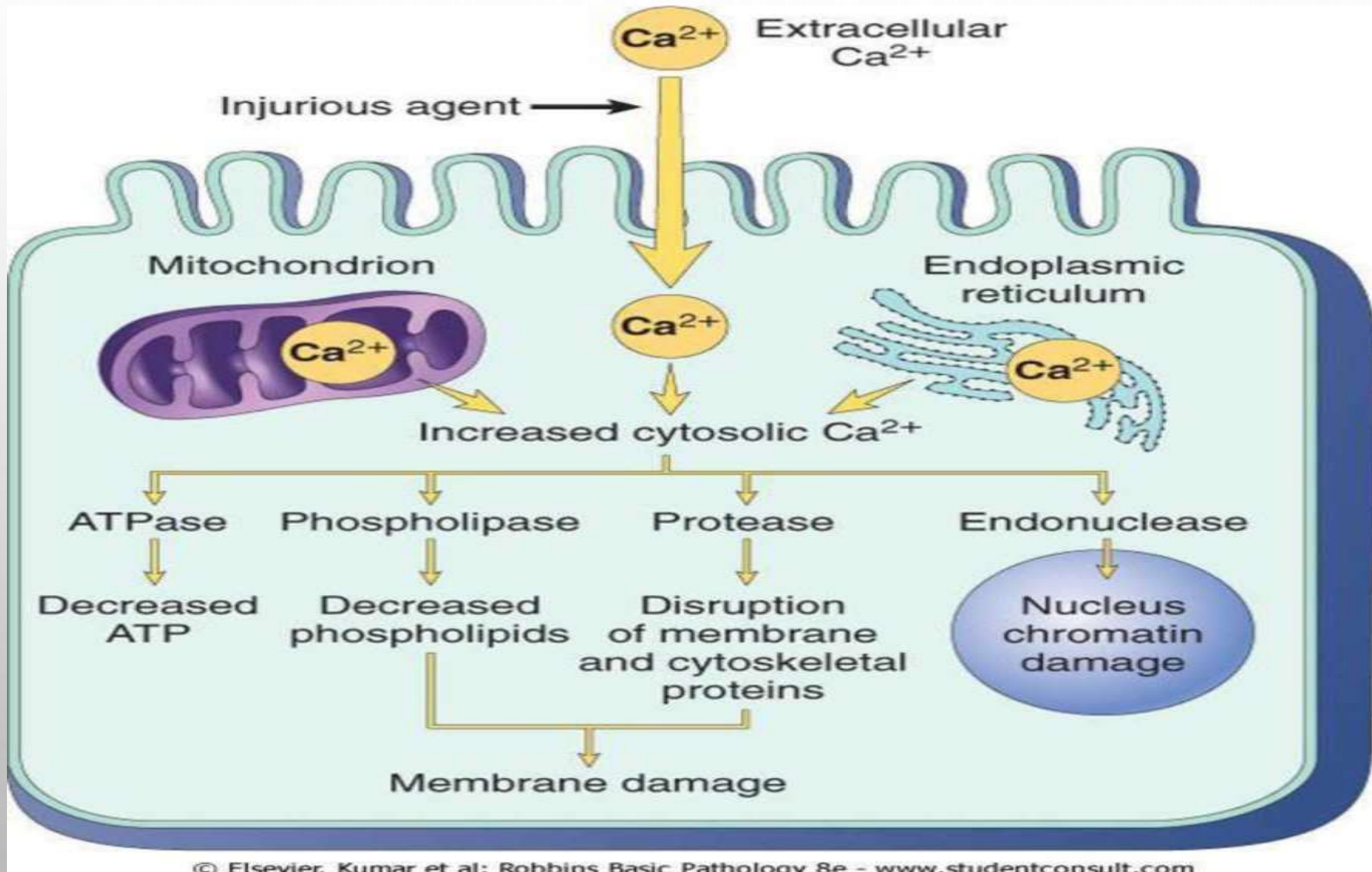
1- Activity of the **energy-dependent plasma membrane transport is impaired**: sodium pump is reduced, resulting in intracellular accumulation of **sodium & efflux of potassium**, with net **iso-osmotic gain of water**, causing (**cellular swelling**) & **ER dilation**) .

Ultimately, there is **irreversible damage to mitochondrial & lysosomal membranes**, & the cell **undergo necrosis**.

2- **Prolonged depletion of ATP** causes detachment of ribosomes from the RER & dissociation of polysomes into monosomes, with a consequent **reduction in protein synthesis** & lipoprotein production leads to **fatty change** .

- **Increased cytosolic Ca²⁺** activates number of enzymes, with potentially damaging cellular effects, including :
 1. Phospholipases, cause membrane damage,
 2. Endonucleases fragmenting the DNA & chromatin material,
 3. ATPases, accelerating ATP depletion, &
 4. Proteases which break down of both membrane & cytoskeletal protein .
- Increased cytosolic Ca²⁺ levels also result in the **induction of apoptosis by:**
 - (a) Direct activation of caspases
 - (b) By increase mitochondrial permeability.

Sources & consequences of increased cytosolic calcium in cell injury.



- **Accumulation of Oxygen- Derived Free Radicals (FR) (Oxidative Stress)**
 - ❑ FR are chemical species with a single unpaired electron in it's outer orbital .
 - ❑ Cells generate energy by reducing molecular oxygen to water , during this process small amounts of partially reduced reactive oxygen forms are produced as a byproducts of mitochondrial respiration during the reduction-oxidation reaction , these are called reactive oxygen species (ROS) or free radicals .
 - ❑ FR chemical states are extremely unstable, & readily react with organic or inorganic chemicals; they strongly attack & degrade nucleic acids & membrane molecules.
 - ❑ Normally, ROS which are produced in cells during mitochondrial respiration & energy generation, they are degraded & removed by **cellular defense systems**.

Generation of FR occurs through :

(1) The reduction-oxidation (redox) reactions that occur during normal mitochondrial metabolism

Normally during respiration, O_2 is reduced in mitochondria by the addition of four electrons of H to generate water. In the process, small amounts of toxic intermediate species are generated by partial reduction of oxygen ; these ROS include: Superoxide(O_2^-) + Hydrogen peroxide (H_2O_2) + Hydroxyl radical OH.

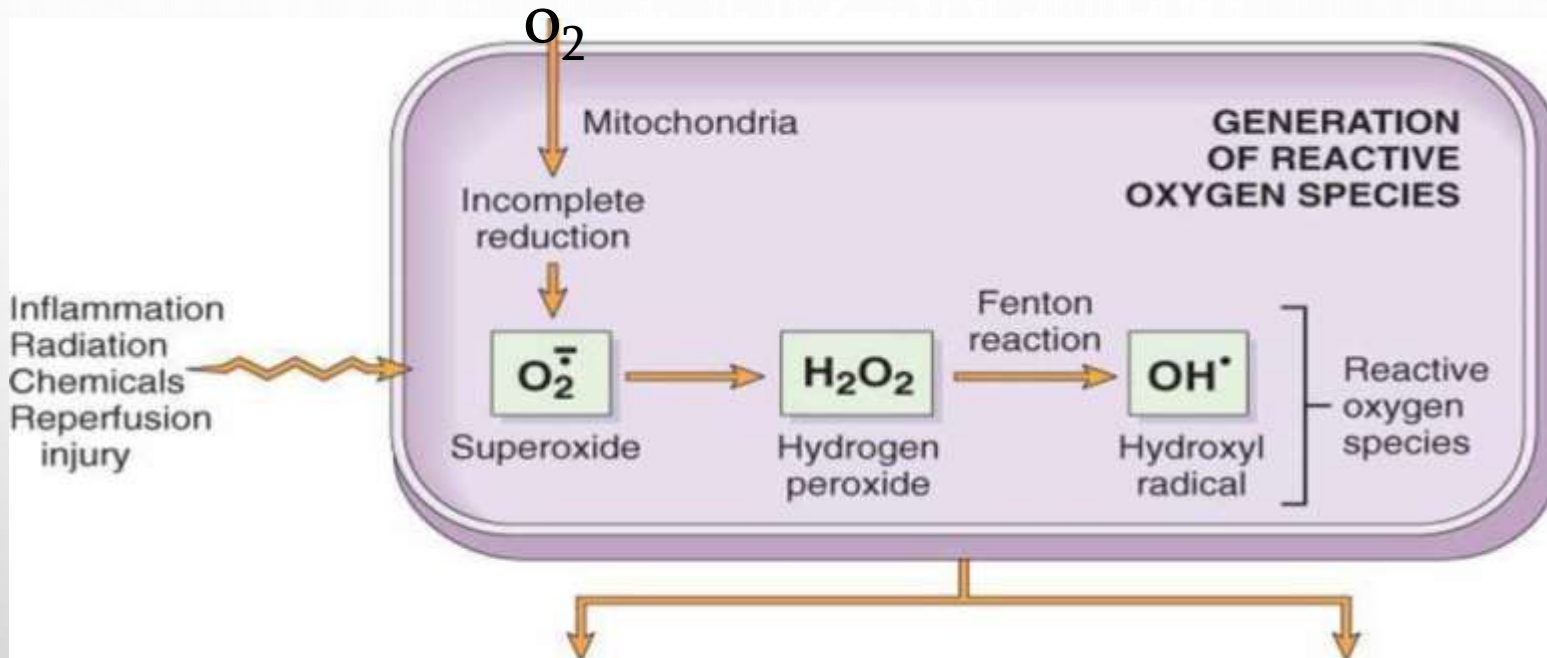
(2) The absorption of radiant energy (e.g., X -rays ultraviolet light). Ionizing radiation can hydrolyze water into hydroxyl (OH^\cdot) & hydrogen (H^+) FR.

(3) The metabolism of exogenous chemicals (e.g., CCL4 into toxic FR CCL3) .

(4) Inflammation, because FR are produced by leukocytes (WBC) that infiltrate tissues.

(5) Nitric oxide (NO), normally synthesized by many cell types e.g., endothelial cells of blood vessels .

F 85 : The role of reactive oxygen species (ROS) in cell injury.



PATHOLOGIC EFFECTS OF ROS: CELL INJURY AND DEATH

ROS react with:

- Fatty acids \rightarrow oxidation \rightarrow generation of lipid peroxidases \rightarrow disruption of plasma membrane, organelles
- Proteins \rightarrow oxidation \rightarrow loss of enzymatic activity, abnormal folding
- DNA \rightarrow oxidation \rightarrow mutations, breaks

REMOVAL OF FREE RADICALS

Antioxidant mechanisms:

- SOD (in mitochondria) converts $O_2 \rightarrow H_2O_2$
- Glutathione peroxidase (in mitochondria) converts $OH^\cdot \rightarrow H_2O_2$
- Catalase (in peroxisomes) converts $H_2O_2 \rightarrow H_2O + O_2$

FR cause cell injury by three reactions:

1. Lipid peroxidation of membranes : Causing injury to cell membrane .
2. Cross-linking of proteins : FR promote sulfhydryl mediated protein cross-linking causing defective protein synthesis ,or loss of enzymatic activity .
3. DNA fragmentation:
FR reactions with thymine in nuclear & mitochondrial DNA produce single-strand breaks.
Such DNA damage has been implicated in both cell killing & the malignant (cancer) transformation of cells.

- **Defects in Membrane Permeability**

- Membrane damage is a constant feature of most forms of cell injury i. e. necrosis (except apoptosis).

- **Damage to DNA & Proteins**

- Cells have mechanisms that repair DNA_damage, but if this damage is irreparable, the cell initiates its suicide program by apoptosis, through (P53)gene

Subcellular Responses to Injury

Are distinctive alterations involving only subcellular organelles.

(I) Autophagy: Autophagy is lysosomal digestion of the cell's own components; it is a survival mechanism in times of nutrient deprivation, the starved cell lives by eating its own contents.

- Autophagy is opposite to Hetrophagy, in autophagy, intracellular organelles & portions of cytosol are first , sequestered from the cytoplasm in an autophagic vacuole formed from of the rough endoplasmic reticulum (RER) then the vacuole fuses with lysosomes to form an autophagolysosome , then the cellular components are digested by lysosomal enzymes cell ingestion of substances .

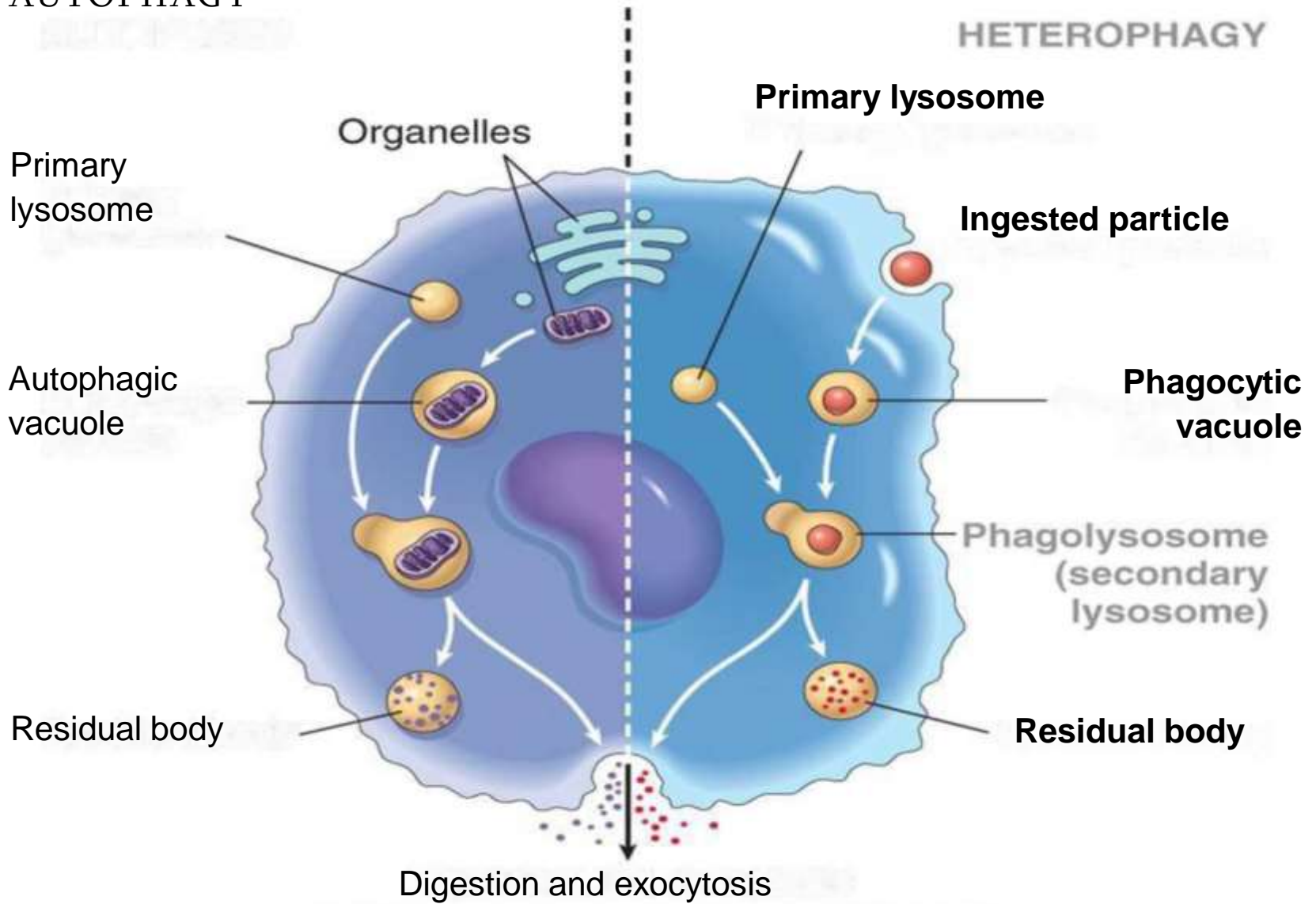
- Autophagy may also signal...cell death by apoptosis a way of telling a stressed or starved cell that it can no longer cope by living on its own organelles.

(2)Heterophagy :

- Refers to ingestion of particles from outside the cell .
Example : carbon particles inhaled from the atmosphere, or inoculated pigment in tattoos can be phagocytosed by (Heterophagy) they may persist in phagolysosomes of macrophages for decades or more.

AUTOPHAGY

HETEROPHAGY



<g> Elsevier. Kumar et al: Robbins Basic Pathology 8e - www.studentconsult.com

Figure 79 : Autophagy & Heterophagy

(II) Induction (Hypertrophy) of Smooth Endoplasmic Reticulum (SER) :

The SER is involved in the metabolism of various chemicals, & cells exposed to these chemicals show hypertrophy of the ER as an adaptive response that may have important functional consequences.

- ❑ For example, **barbiturates** are metabolized in the liver by the cytochrome P-450 mixed-function oxidase system found in the SER.
- ❑ Prolonged use of barbiturates leads to a state of tolerance, with decrease in the effects of the drug & the need to use increase doses.
- ❑ This adaptation is due to hypertrophy of the SER of the hepatocytes & increase P-450 enzymatic activity.

❑ Although P-450-mediated modification is often thought of as detoxification , many compounds are rendered more injurious by this process .

for example, carbon tetrachloride (CCL4) which is converted in the liver to toxic free radical CCL3 causes membrane phospholipid peroxidation with rapid breakdown of ER . Within 2 hours, there is swelling of the SER & dissociation of ribosomes from it followed by fatty change & cell death.

(III) Mitochondrial Alterations :

- Mitochondrial dysfunction plays an important role in acute cell injury & death.
- In some nonlethal pathologic conditions, however, there may be alterations in the number, size, shape, & presumably function of mitochondria.
- **In cellular hypertrophy, there is an increase in the number of mitochondria in cells ; conversely, mitochondria decreased in number during cellular atrophy(probably via autophagy).**
- Mitochondria may assume extremely large & abnormal shapes (megamitochondria), as seen in hepatocytes **in alcoholic liver disease & in nutritional deficiencies.**
- **In NASH, megamitochondria (giant mitochondria) are most frequently observed within hepatocytes with microvesicular steatosis as round or needle-shaped eosinophilic intracytoplasmic inclusions.**
- **NASH(Non-Alcoholic steatohepatitis)**

Apoptosis :

❑ Is a form of cell death based on sequential activation of “death genes ”and suicide pathway enzymes . Also called “programmed cell death “ .

❑ This is one method the body uses to get rid of unneeded or abnormal cells. The process of apoptosis may be blocked in cancer cells

❑ **Apoptosis** is initiated by two most important pathways :

1 **Extrinsic pathway** : which is activated by the activation of the so called death receptors on the surface of cell membrane .

Ligands for such receptors are proteins such as tumor necrosis factor or Fas ligand .

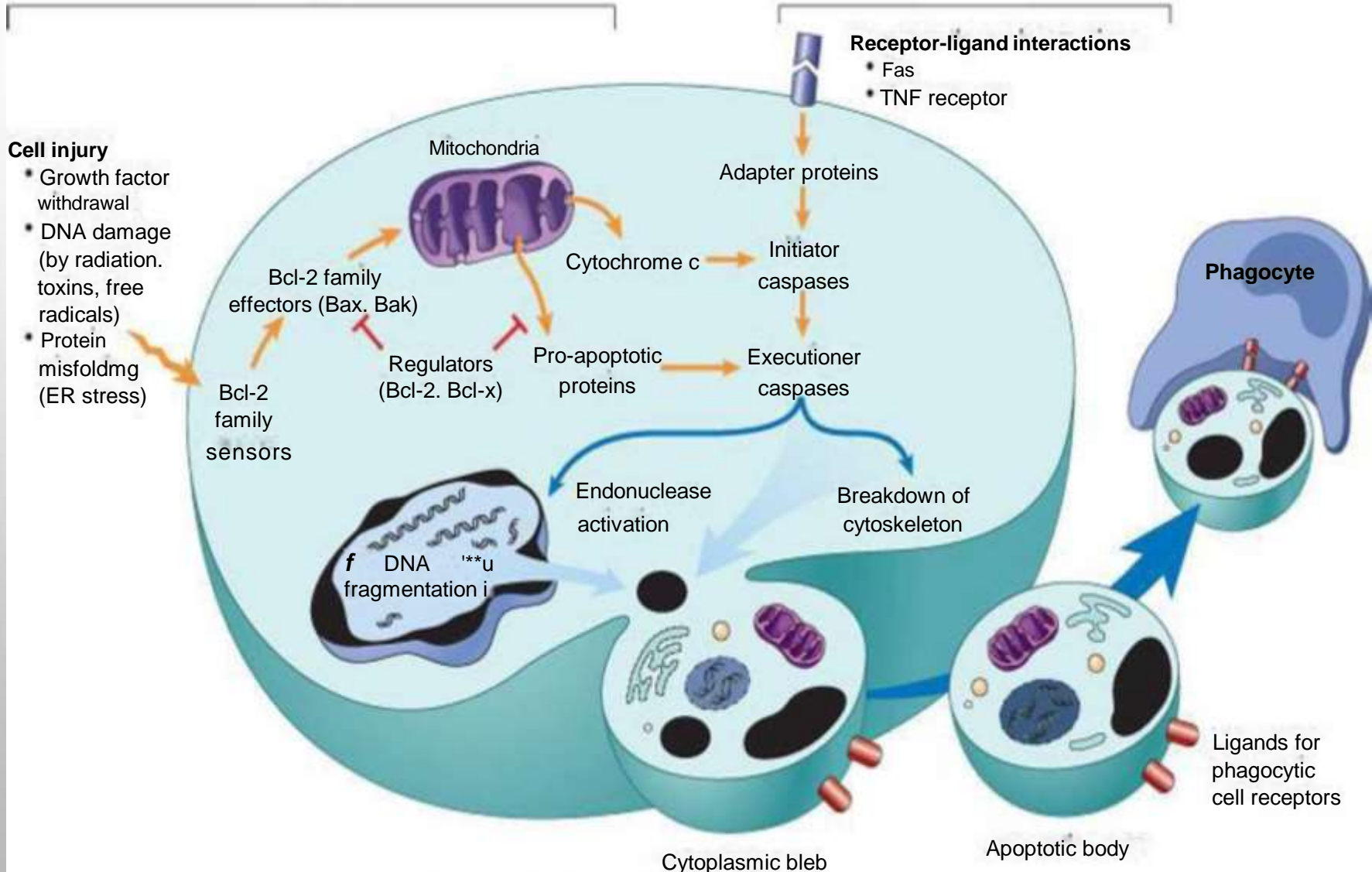
2 **Intrinsic mitochondrial pathway** : which is initiated by increased mitochondrial permeability & the release of pro-apoptotic molecules such as cytochrome C that acts on initiator caspases which are capable of degrading the cell’s own nuclear DNA & nuclear & cytoplasmic proteins .

- **APOPTOSIS CAN BE TRIGGERED BY BOTH INTERNAL AND EXTERNAL FACTORS. INTERNAL FACTORS INCLUDE MISFOLDED PROTEINS AND DEREGULATED SIGNALING, WHILE NUTRIENT LOSS, RADIATION, HEAT, AND ACTIVATION OF CELL SURFACE RECEPTORS, SUCH AS TNF AND FAS3, ARE EXTERNAL TRIGGERS.**
- **APOPTOSIS IS INITIATED BY THE INTERACTION OF PRO-APOPTOTIC AND ANTI-APOPTOTIC MEMBERS OF THE BCL-2 FAMILY, INCLUDING BAD AND BCL-2. THIS LEADS TO THE ACTIVATION OF CASPASE PROTEINS, A FAMILY OF PROTEOLYTIC ENZYMES, WHICH ACTIVATE OTHER PROTEINS THAT DISMANTLE THE CYTOSKELETON, ORGANELLES, AND DEGRADE DNA. THIS CONTROLLED PROCESS ALLOWS ADJACENT TISSUE TO SUFFER MINIMAL DAMAGE**

86 :Mechanisms of Apoptosis

MITOCHONDRIAL (INTRINSIC)
PATHWAY

DEATH RECEPTOR (EXTRINSIC)
PATHWAY



- Apoptosis is mediated by **proteolytic enzymes called caspases**, which trigger cell death by cleaving specific proteins in the cytoplasm and nucleus. **Caspases** exist in all cells as inactive precursors, or **procaspases**, which are usually activated by cleavage by other caspases, producing a proteolytic caspase cascade.
- The initiation of apoptosis is tightly regulated by activation mechanisms, because once apoptosis has begun, it inevitably leads to the death of the cell

Apoptosis differs from necrosis which is characterized by:

- (1) loss of membrane integrity
- (2) enzyme digestion of cell
- (3) leakage of cellular contents, with inflammation
- (4) always pathologic,

while apoptosis is usually physiologic, & rarely pathologic .

However, apoptosis & necrosis sometimes coexist.

CAUSES OF APOPTOSIS

Apoptosis occurs normally, as a physiological phenomenon, but pathologically, when irreparable DNA damaged cells are eliminated by apoptosis.

Apoptosis in Physiologic Situations

serves to eliminate cells that are no longer needed & maintain a steady number of various cell populations in tissues.

It is important in the following physiologic situations :

1.The programmed destruction of cells during embryogenesis, including implantation, organogenesis, developmental involution, metamorphosis.

2. **Hormone deprivation** : resulting in involution of hormone-dependent tissue such as
- (a) **endometrial cell breakdown during the menstrual cycle.**
 - (b) **regression of the lactating breast after weaning.**
3. **Cell loss in proliferating cell populations, as in intestinal crypt epithelia, so as to maintain a constant number.**
4. **Death of cells** that have served their useful purpose such as neutrophils in an **acute inflammation**, & lymphocytes at the end of an immune response.
- Apoptosis occurs in these cells because they are deprived of necessary survival signals, such as growth factors.

Apoptosis in Pathologic Conditions

Apoptosis eliminates cells that are genetically altered or injured beyond repair without eliciting host reaction.

(I) DNA damage: Radiation, Cytotoxic anticancer drugs, extreme of temperature, & hypoxia can damage DNA, either directly or via production of FR.

- ❑ If the damage is irreparable, cell triggers apoptosis .
- ❑ Inducing apoptosis of cancer cells is a desired effect of chemotherapeutic agents, many of which work by damaging DNA .

2. Accumulation of misfolded proteins

Improperly folded proteins may arise because of mutations in the genes encoding these proteins, or damage caused by FR.

3. Cell injury in certain viral infections, in which loss of infected cells is largely due to apoptotic death that may be induced either by

- (1) by the virus itself (as in adenovirus & HIV infections), or
- (2) by the host immune response (as in viral hepatitis) .

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

Morphology of apoptosis:

- ❖ Apoptotic cells appear as: a **rounded or oval masses**, with intensely eosinophilic cytoplasm.
- ❖ Nuclei show **chromatin condensation** & ultimately, Karyorrhexis .
- ❖ The cells are **rapidly shrink** , form cytoplasmic **buds** & fragment into **apoptotic bodies** composed of membrane-bound vesicles of cytosol & organelles.
- ❖ Because these apoptotic bodies are quickly extruded (projected through cell wall) & **phagocytosed** with out eliciting an inflammatory response ...

F 80 : Apoptosis of a liver cell in viral hepatitis. The cell is reduced in size & contains brightly eosinophilic cytoplasm & a condensed nucleus.



Celula Blanca Normal

Celula Blanca Apoptotica

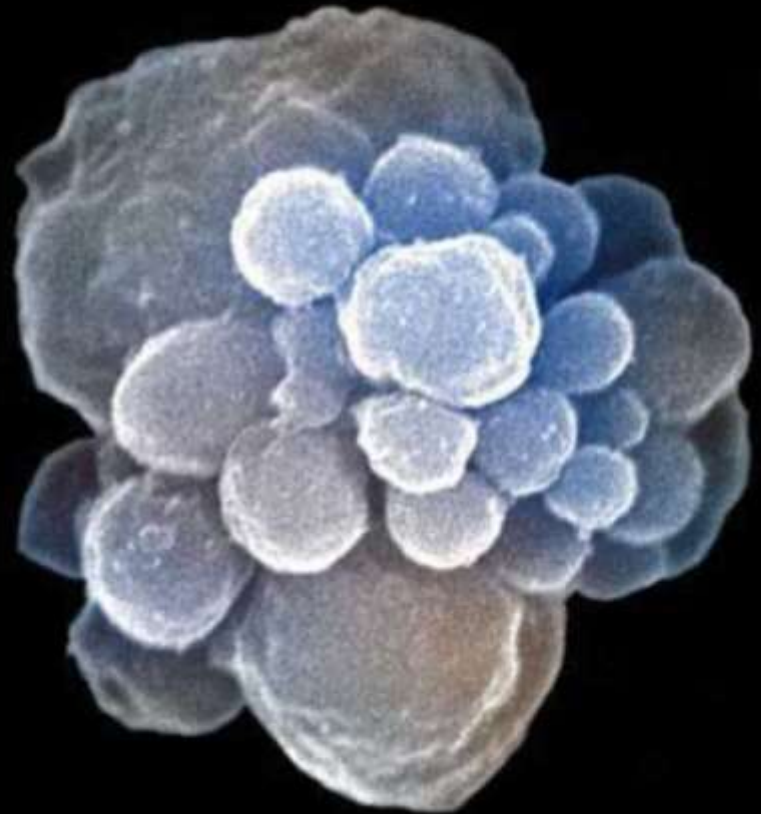
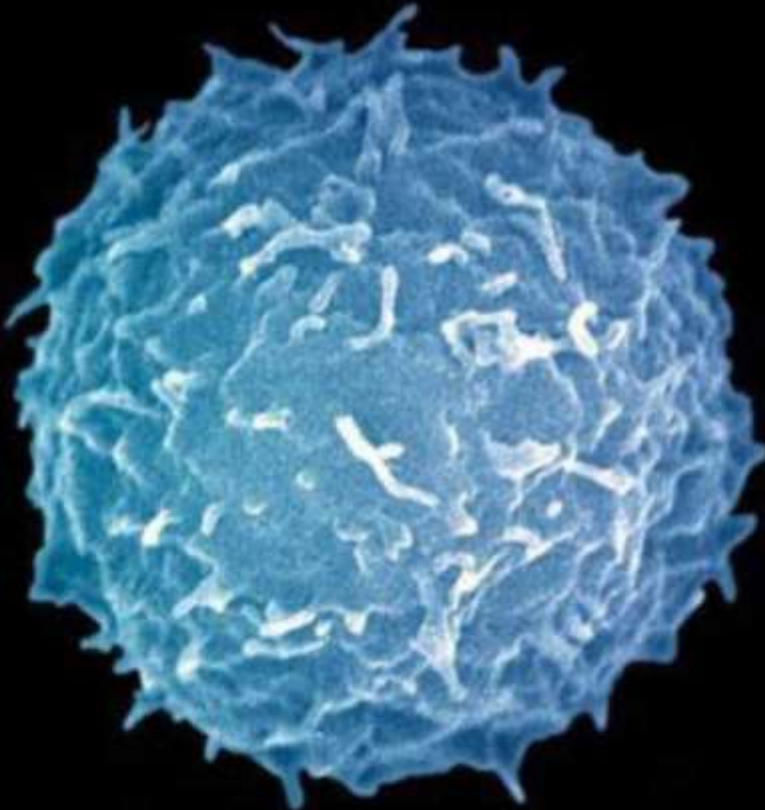


Figure 81 - Electron Microscopic (EM) appearance of normal(left) & apoptotic white Blood Cell (right).

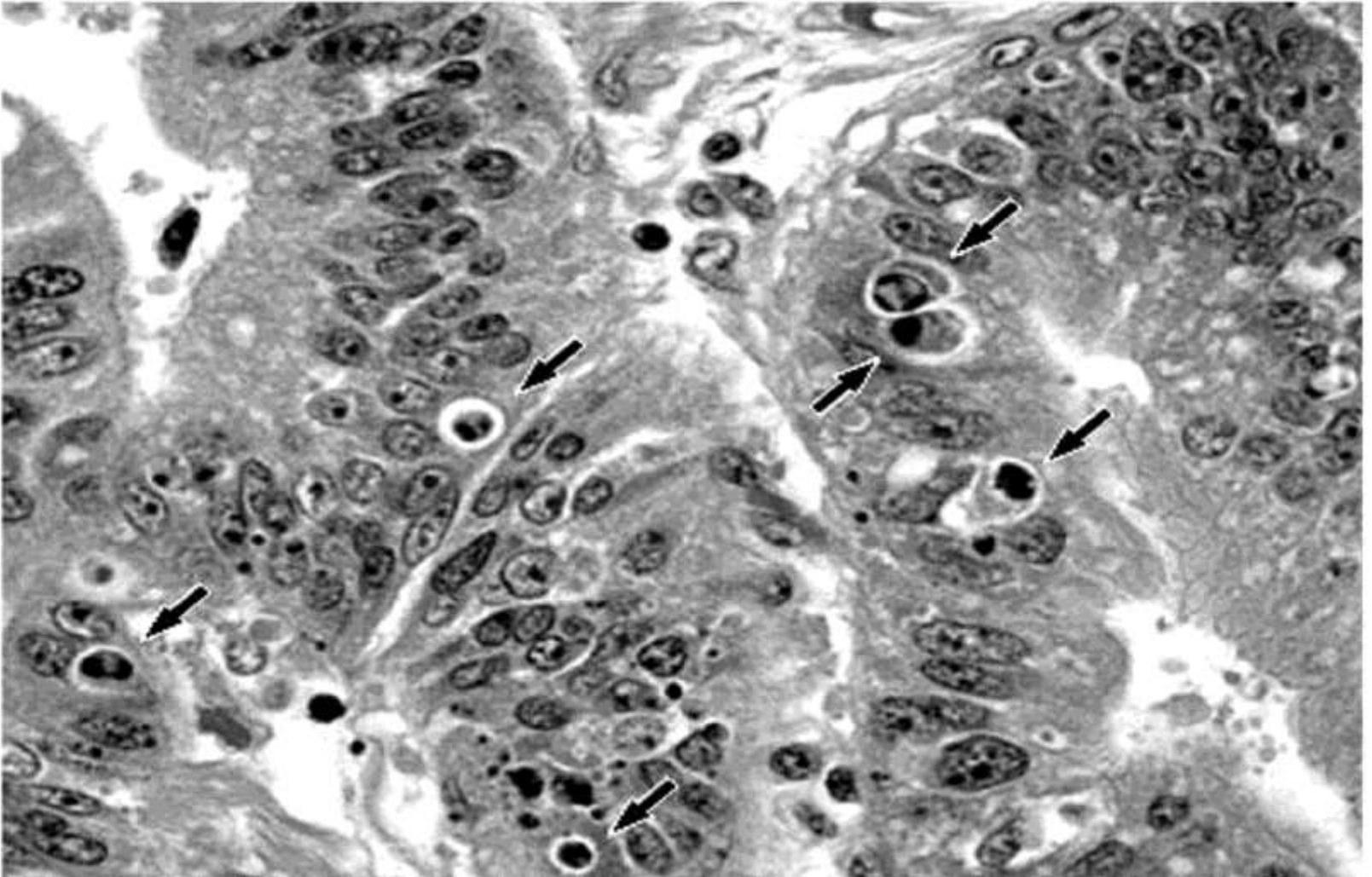


Fig. 82 : Microscopic view of apoptosis (arrows)

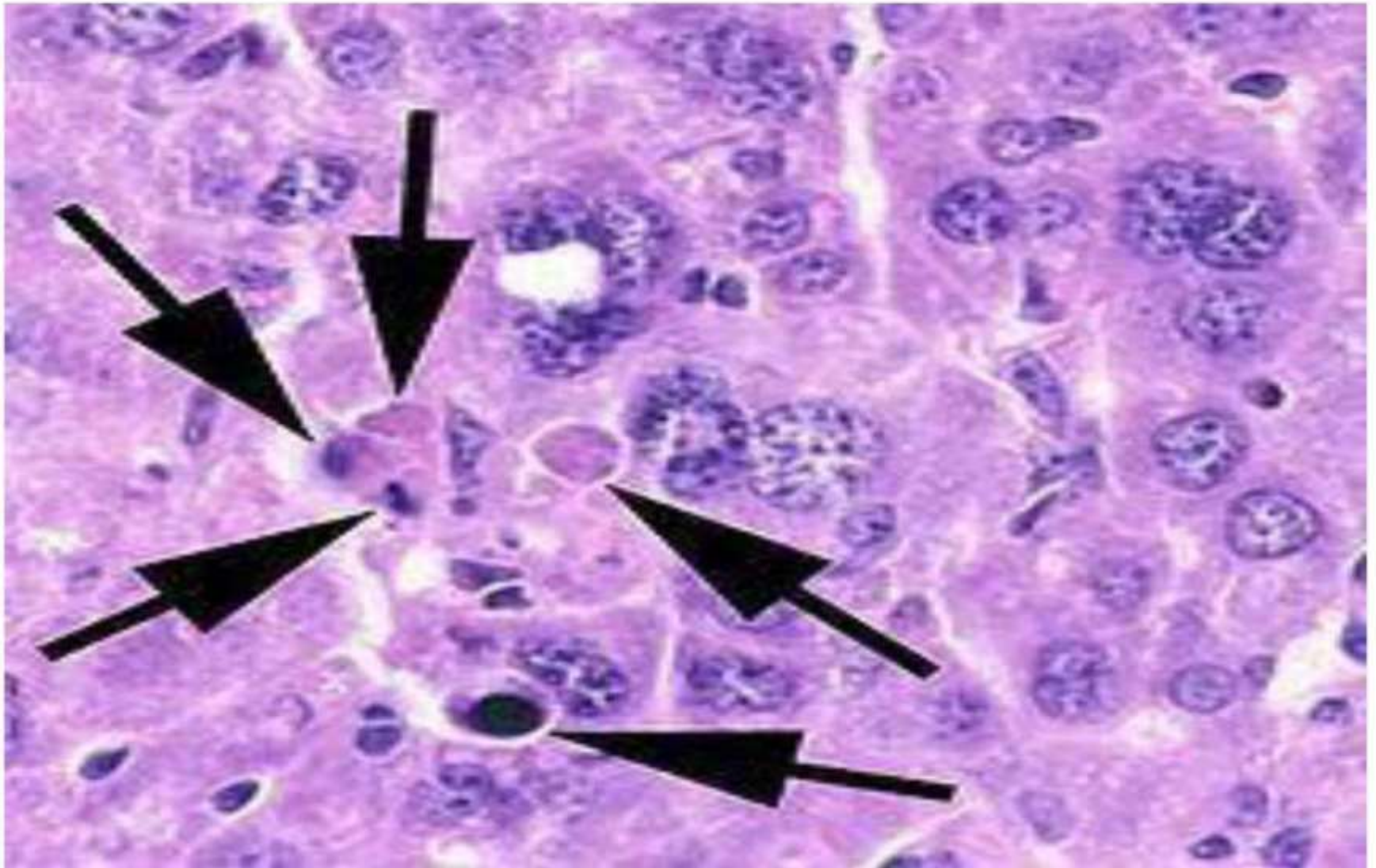


Figure 83 : Apoptotic cells (Arrows) as reddish spots of dead cells in liver tissue.

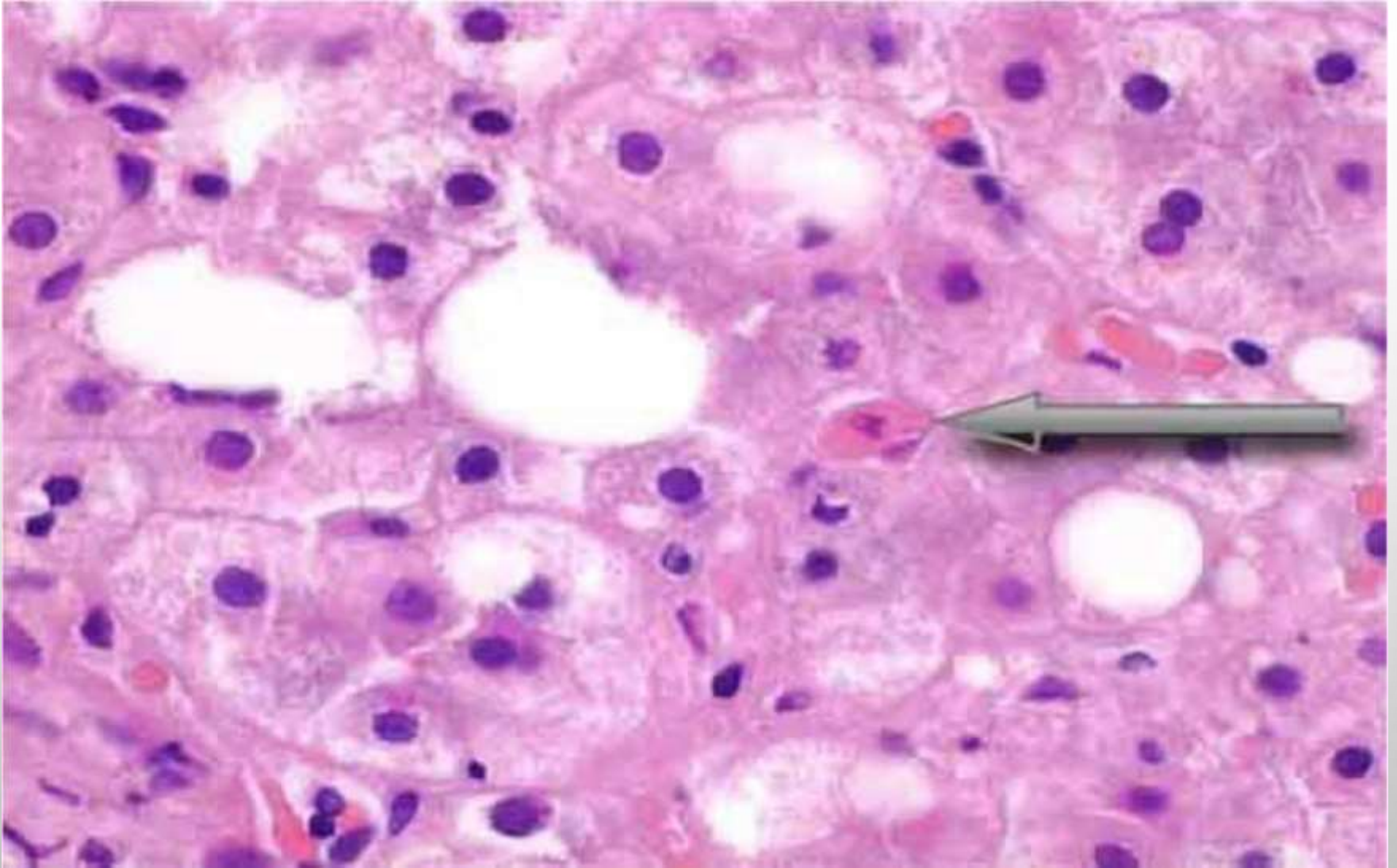
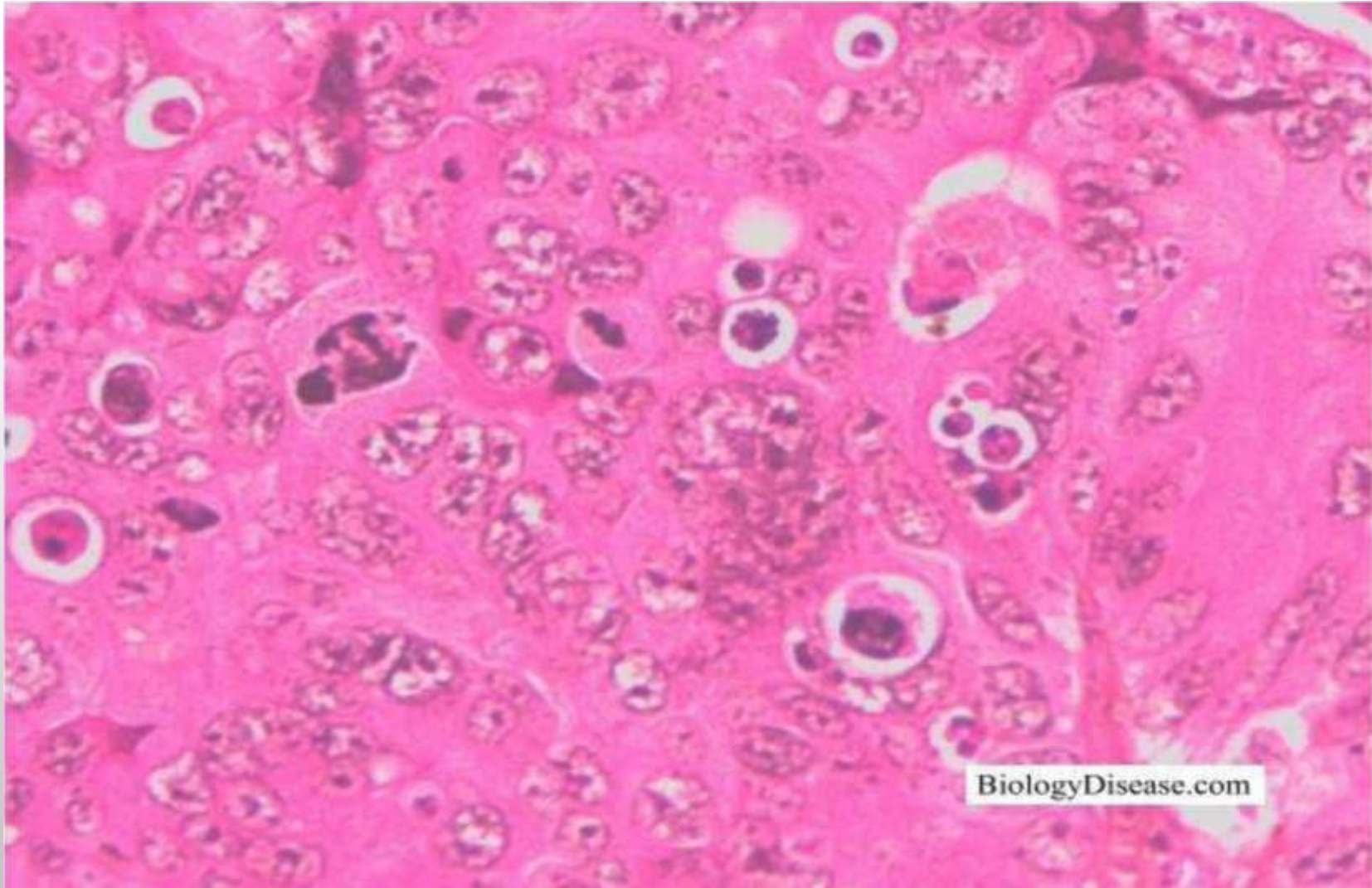


Figure 84 : Liver biopsy : Apoptotic cells (arrow) in viral hepatitis.



Apoptotic cells in a malignant tumor appearing as reddish dots of apoptotic cells surrounded by empty spaces.

Role of P53 gene in apoptosis :

Normally, P53 gene first arrests cell cycle at G1, to allow time for repair of the damage, if the repair is successful P53 allow the cell cycle progression from G1 to S phase .

But, if the damage is irreparable, the P53 triggers apoptosis, by activating Bax & Bak, i.e.,

P53 order damaged cell : To Stop ^ Repair Or Die!

when P53 is mutant or absent (as in 70% of human cancers), it is incapable of inducing apoptosis, so that cells with damaged DNA are allowed to survive.

In such cells, the DNA damaged may result in mutations or translocations that lead to neoplastic transformation

INTRACELLULAR ACCUMULATIONS

- **Under some circumstances, cells may accumulate abnormal amounts of various substances.**
 - * Substance may be harmless or may cause injury
 - * Substance may locate either within organelles (typically lysosomes), in the cytoplasm, or in the nucleus .
 - * Substance may be endogenous synthesized by the affected cells, or may be exogenous , produced elsewhere.

General pathways by which cells can accumulate abnormal intracellular material are illustrated in

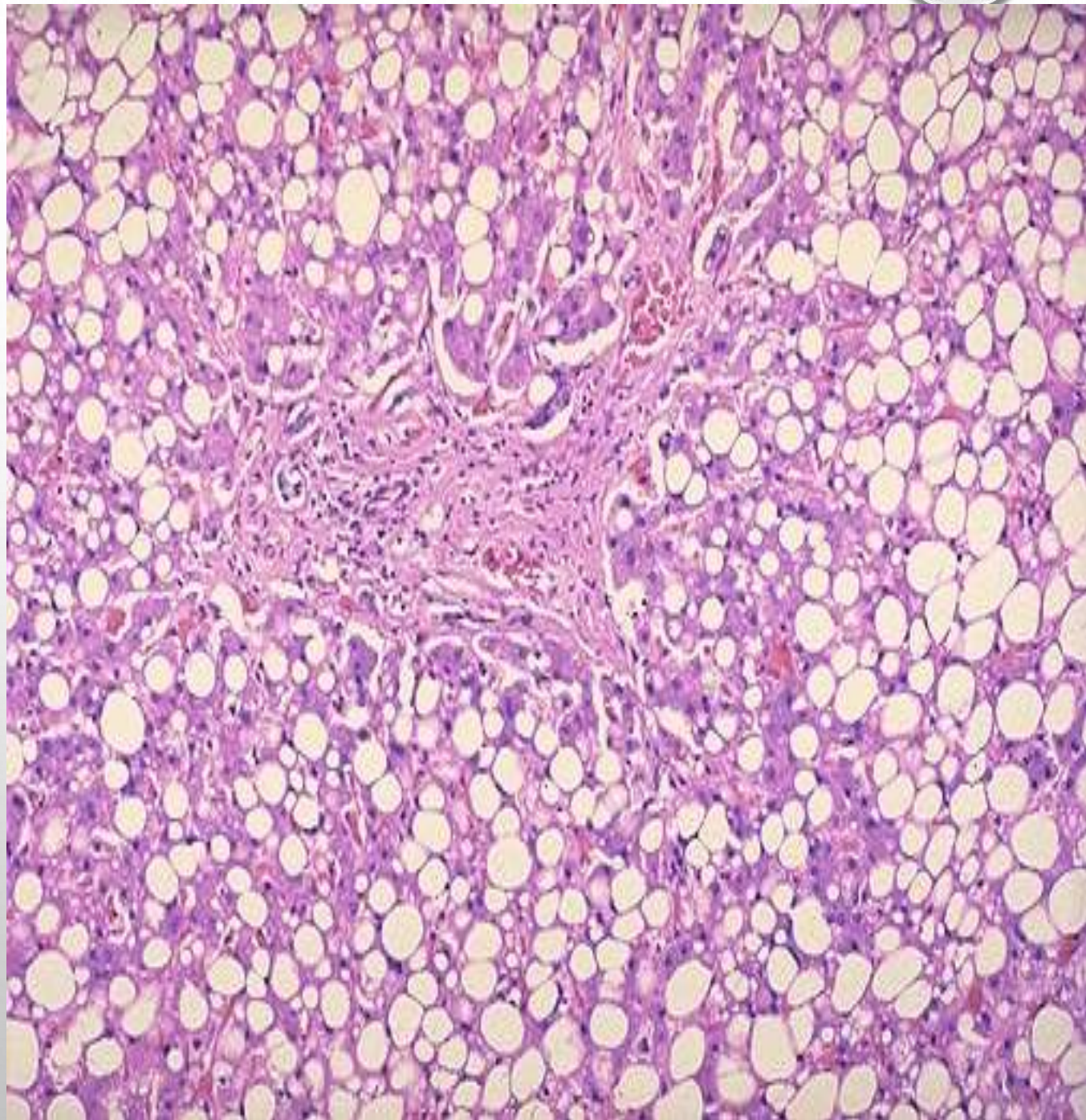
- 1- Abnormal metabolism : as in fatty change in the liver.
- 2- Protein mutation causing alteration in protein folding & transport, with defective protein accumulation in the cytoplasm . (e.g. alpha 1- antitrypsin deficiency).
- 3- Lack of enzyme responsible for breaking down certain compounds causing substrates to accumulate in lysosomes, as in e.g. lysosomal storage diseases
- 4- Ingestion of indigestible materials e.g. accumulations of carbon or silica particles.

Fatty change = Steatosis

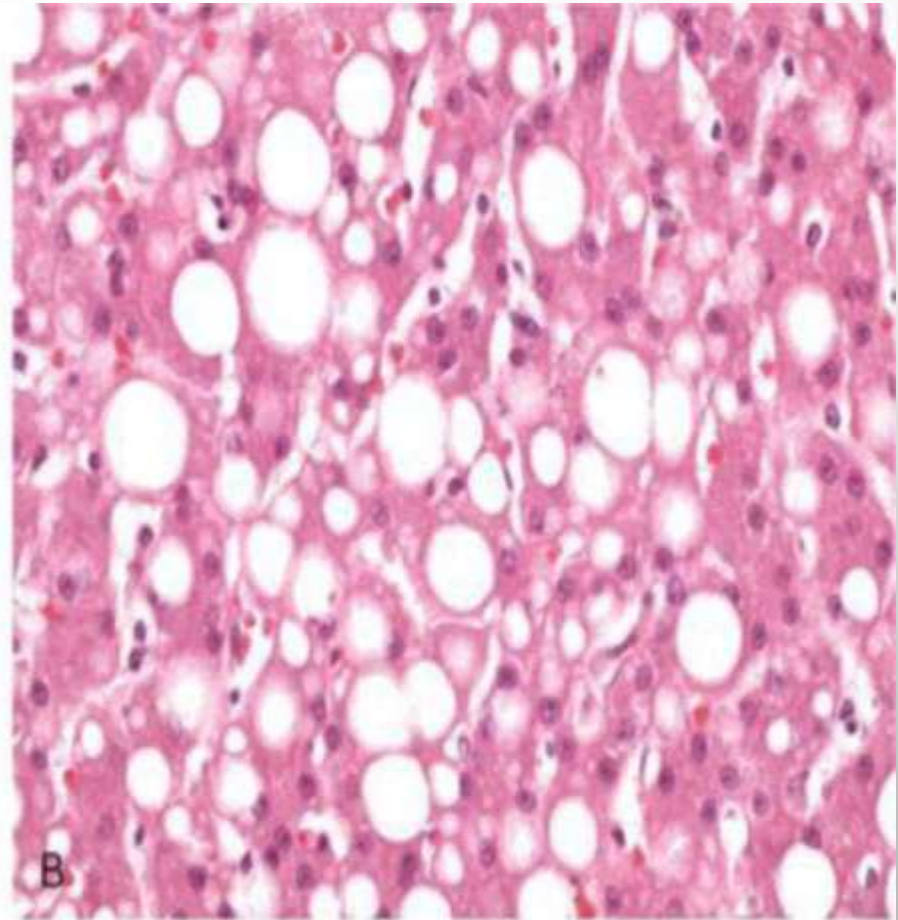
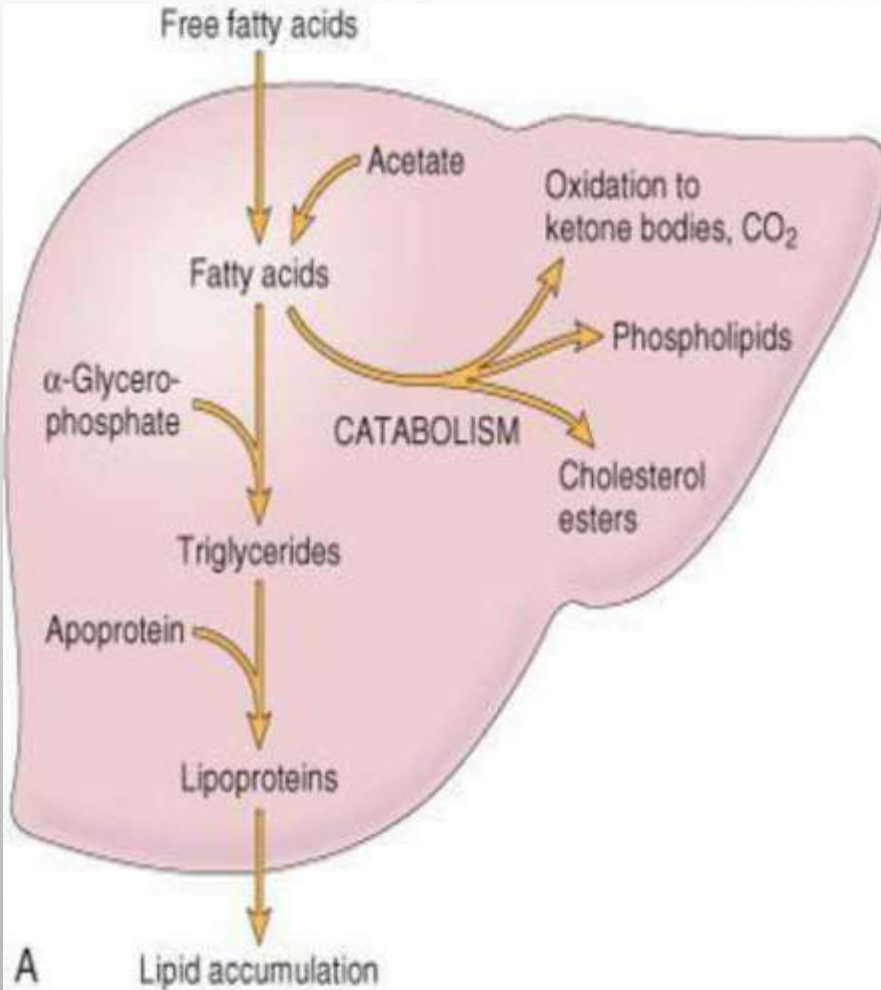
Normally, free fatty acids from adipose tissue or ingested food are transported into hepatocytes where they are...

- a. Esterified to triglycerides
 - b. Converted into cholesterol or phospholipids or
 - c. Oxidized to ketone bodies
- ❖ Transport of the triglycerides from the hepatocytes requires complexing with Apo-proteins to form lipoproteins, which then traverse the circulation.
 - ❖ Excess accumulation of triglycerides may result from defects at any step from fatty acid entry to lipoprotein exit .
 - ❖ Steatosis is an abnormal accumulation of triglycerides within parenchymal cells.
 - ❖ Steatosis mostly seen in the liver , the major organ involved in fat metabolism , it may also occur in heart , skeletal muscle, kidney & other organs.

Intracellular accumulations of a variety of materials can occur in response to cellular injury. Here is steatosis, or **fatty metamorphosis (fatty change) of the liver in which deranged lipoprotein metabolism from injury leads to accumulation of lipid in the cytoplasm of hepatocytes.** Note the large, clear lipid droplets that fill the cytoplasm of many hepatocytes.



Fatty Liver. A, The mechanisms leading to accumulation of triglycerides in fatty liver. Defects in any of the steps of uptake, catabolism, or secretion can lead to Steatosis. B, Microscopically, the fat vacuole in the cytoplasm displace & squeeze the nucleus to the periphery of the cell.



The liver injury with chronic alcoholism leads to fibrosis and regeneration of the hepatocytes in nodules. This firm, nodular appearance of the liver as seen here is called cirrhosis.



- **Alcohol abuse** is the most common cause of fatty change in the liver in industrialized nations .
- **Protein malnutrition ,obesity, diabetes mellitus, anemia, anoxia,& toxins** are other causes of fatty change .
- **CCL4 & protein malnutrition** decrease the synthesis of Apo proteins.
- **Anoxia** inhibits fatty acid oxidation .
- **Starvation** increase fatty acid mobilization from peripheral stores.
- Effects of fatty change depend on the cause & the severity of accumulation.
 - When mild, it may have no effect on the cellular function. More sever fatty change may transiently impair cellular function, but unless some vital intracellular process is irreversibly impaired (e.g., in CCL4 poisoning) , fatty change is reversible.
 - In a severe form, fatty change may precede cell death .

- ❑ GROSSLY : in the liver , mild fatty change may not affect the gross appearance. With accumulation, the liver *becomes* yellow, *enlarges*, in extreme cases, it may weigh 3 to 6 Kg (normal weight 1.5 Kg) & appears *bright yellow, soft & greasy*.
- ❑ Microscopically : early fatty change is seen as small fat vacuoles in the cytoplasm around the nucleus. Later, the vacuoles coalesce (unite together) to create cleared large space that displace the nucleus to the cell periphery .
- ❑ In the heart , prolonged moderate hypoxia (as in severe anemia) result in focal intracellular fat deposits, with a characteristic (‘tiger-like, tabby-cat’ pattern) , while more severe hypoxia or toxic myocarditis (e.g., diphtheria infection) result in diffuse fatty change .

Fatty change: liver. The patient was chronic alcoholic. The presence of large quantities of neutral fat within the liver cells result in a uniform yellow appearance of the liver section.



5.4 Fatty change: liver



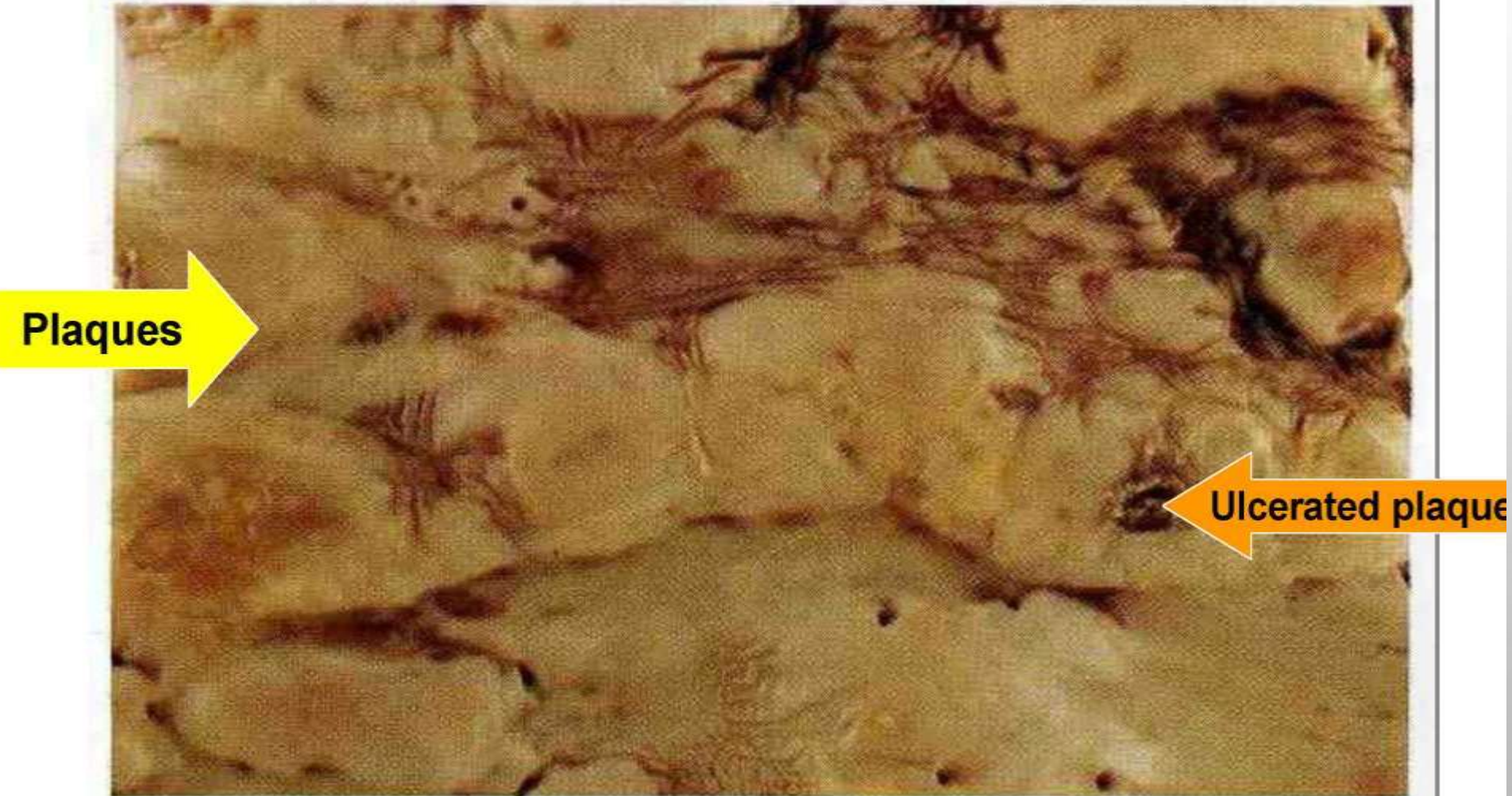
F 90 : Fatty change:
Heart . The muscles of the
LV shows patchy mottling
as yellow streaks & lines
(due to focal fatty change)
alternating with
unaffected muscle to
produce : A 'tiger-like ,
tabby-cat' pattern
characteristic of heart
fatty change in anemia.

Fatty change: heart

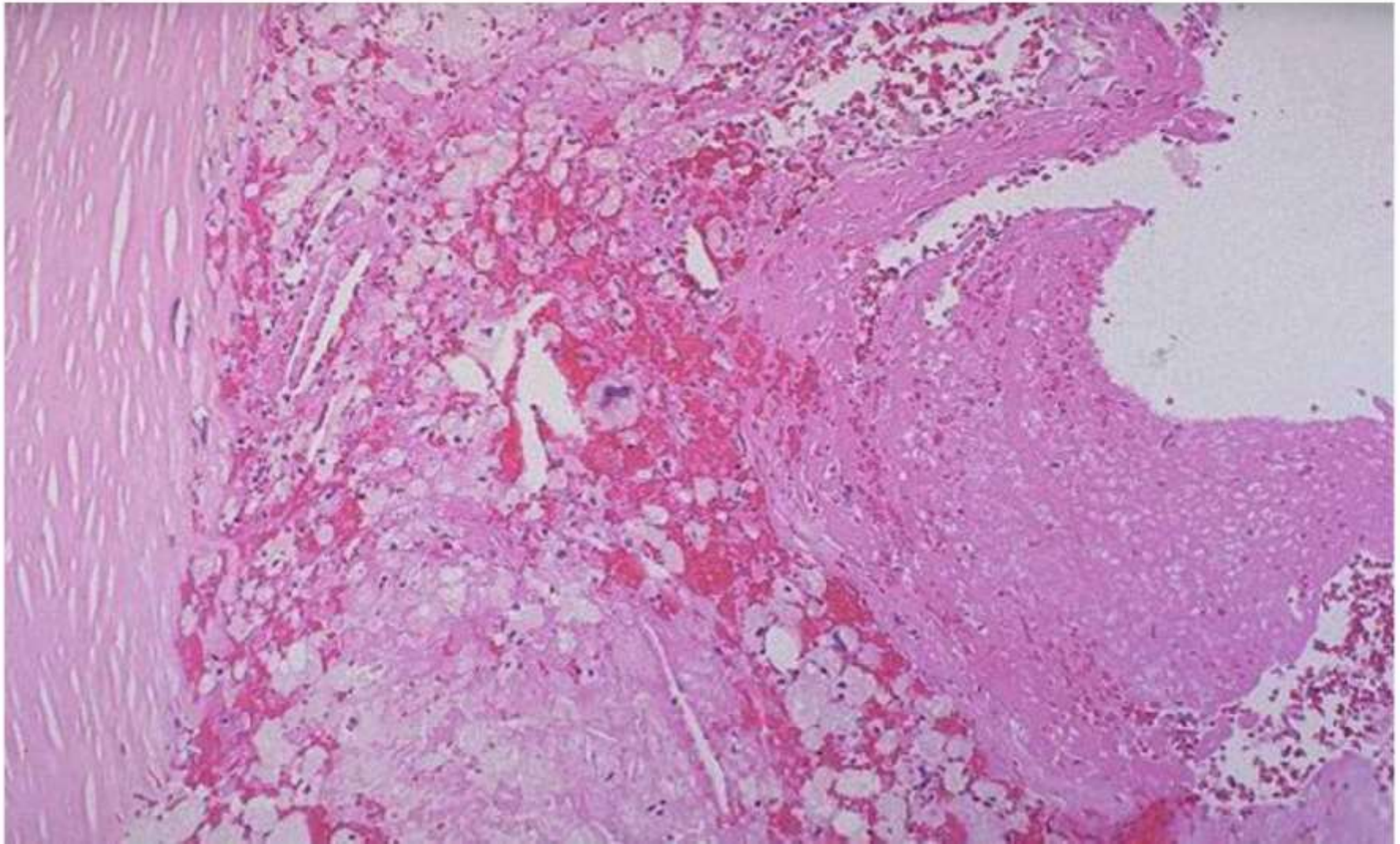
Cholesterol & Cholesteryl Esters

- ❑ Normally, cellular cholesterol metabolism is tightly regulated to ensure normal cell membrane synthesis without significant intracellular accumulation.
- ❑ Phagocytic macrophages in contact with the lipid debris of necrotic cells may become stuffed with lipid, imparting a foam appearance to their cytoplasm, called foam cells .
- ❑ **In atherosclerosis** , smooth muscle cells & macrophages are filled with lipid vacuoles composed of cholesterol & cholesterol esters; these give atherosclerotic plaques their characteristic yellow color .
- ❑ In hereditary & acquired hyperlipidemic syndromes , macrophages accumulates intracellular cholesterol in the skin
- ❑ or in tendons, forming masses called Xanthomas .

Abundant fat within large atheromatous plaques, as confirmed by the yellow color of the lesions shown. The plaque on the right has ulcerated.



Atherosclerosis: aorta



Atherosclerosis in aorta: microscopic view shows deposition of cholesterol & cholesterol esters appear as white vacuoles within macrophages & smooth muscle cells in the wall of blood vessel.



Figure 93 : Xanthelasma : Yellowish skin nodules ,
consisting of cholesterol filled subcutaneous lesions .



Figure 94 : Xanthoma of hands.

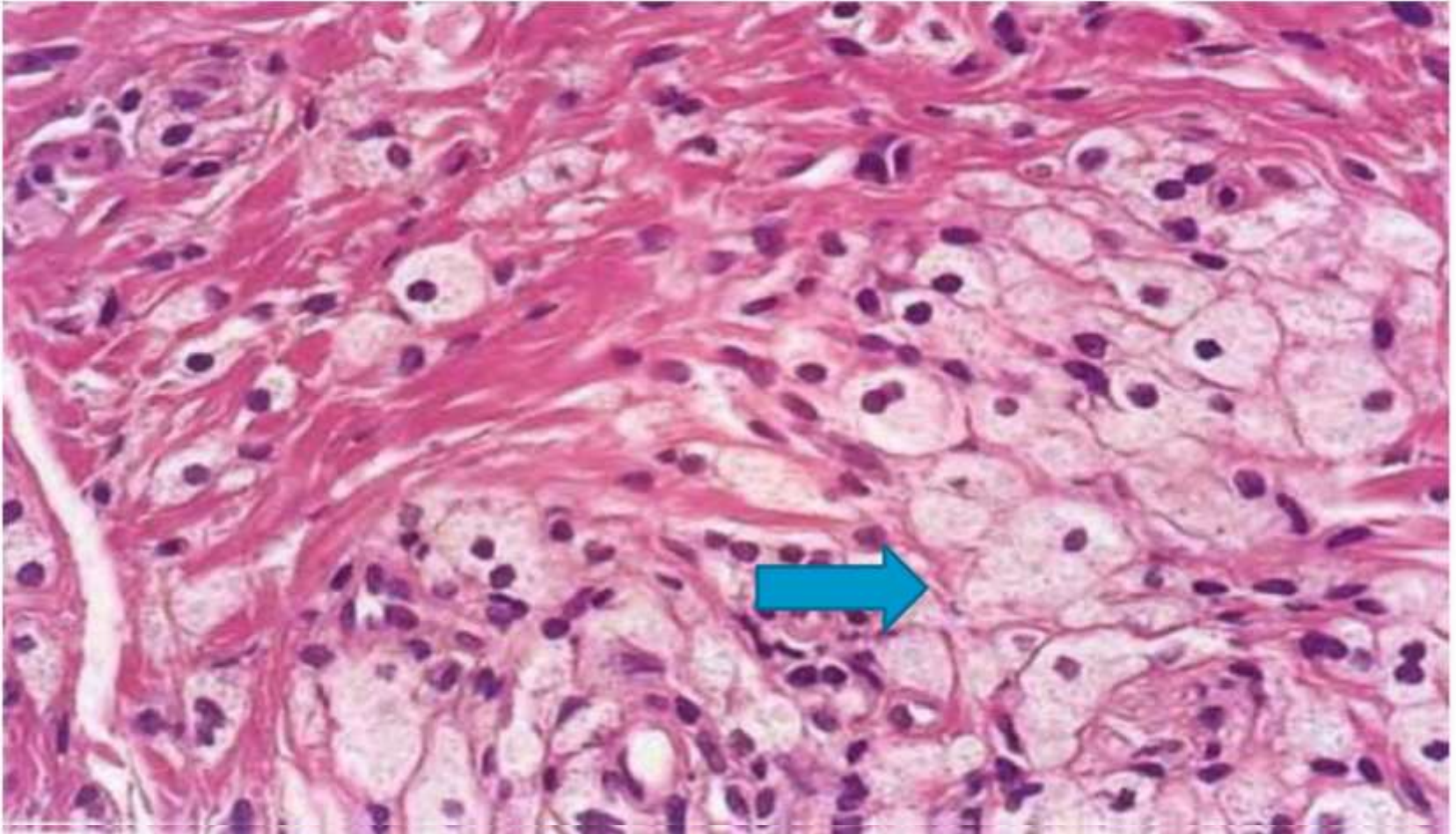


Figure 95 : Skin biopsy in xanthoma : showing foamy lipid-laden macrophages filled with cholesterol (arrow) .

Proteins

(1) In disorders with heavy protein leakage across the glomerular filter proteinuria, e.g., in **nephrotic syndrome**, there is marked increased pinocytotic reabsorption of the protein, resulting in the appearance of pink, hyaline cytoplasmic droplets in the renal tubular epithelium .

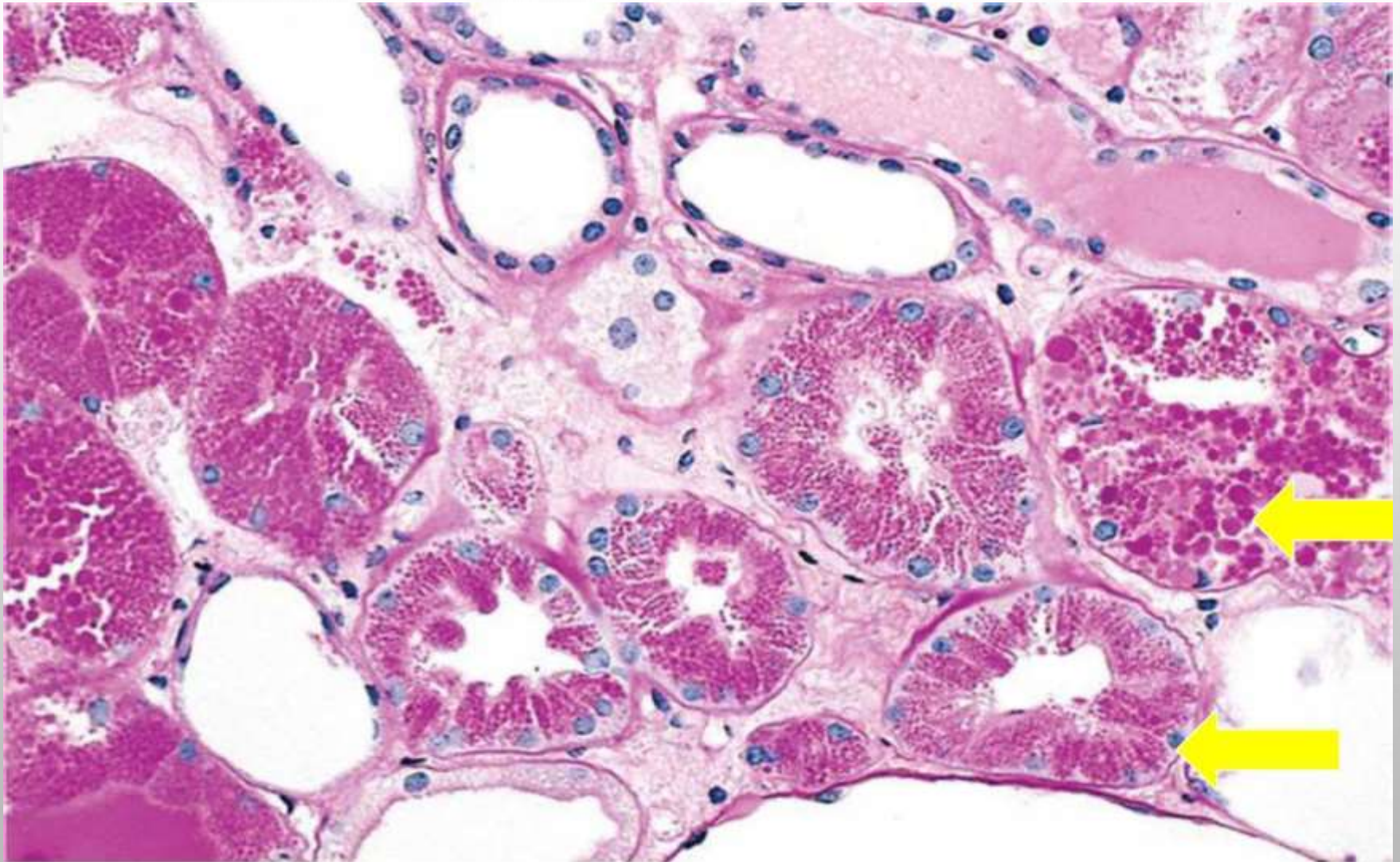
The process is reversible ; if the proteinuria ends, the protein droplets disappear.

(2) Marked accumulations of synthesized immunoglobulines in the RER of some plasma cells, resulting Russell bodies.

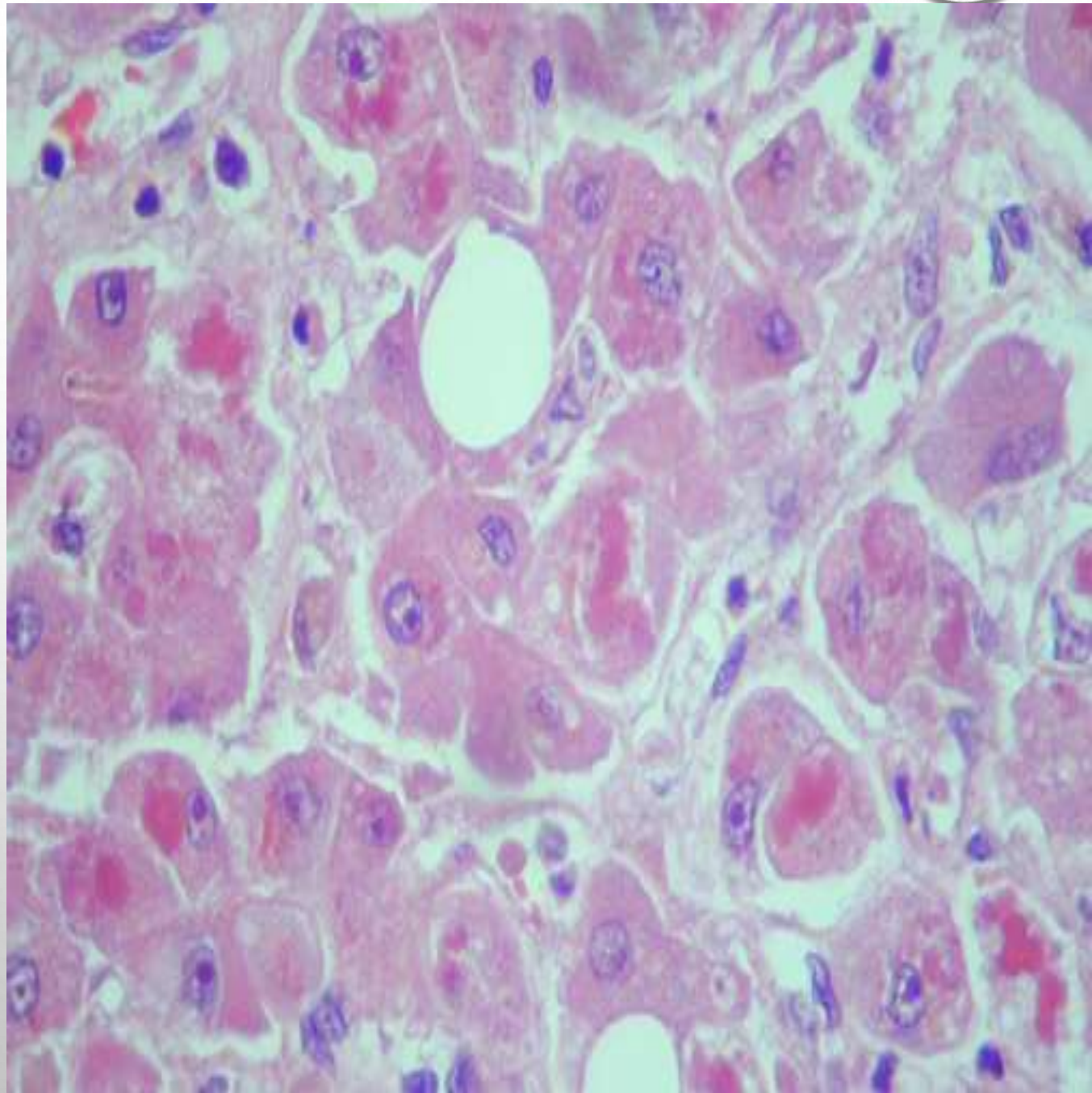
(3) Eosinophilic intracytoplasmic, protein inclusions in the liver cells that are highly characteristic of alcoholic liver disease are called “**alcoholic hyaline**” or **Mallory bodies**

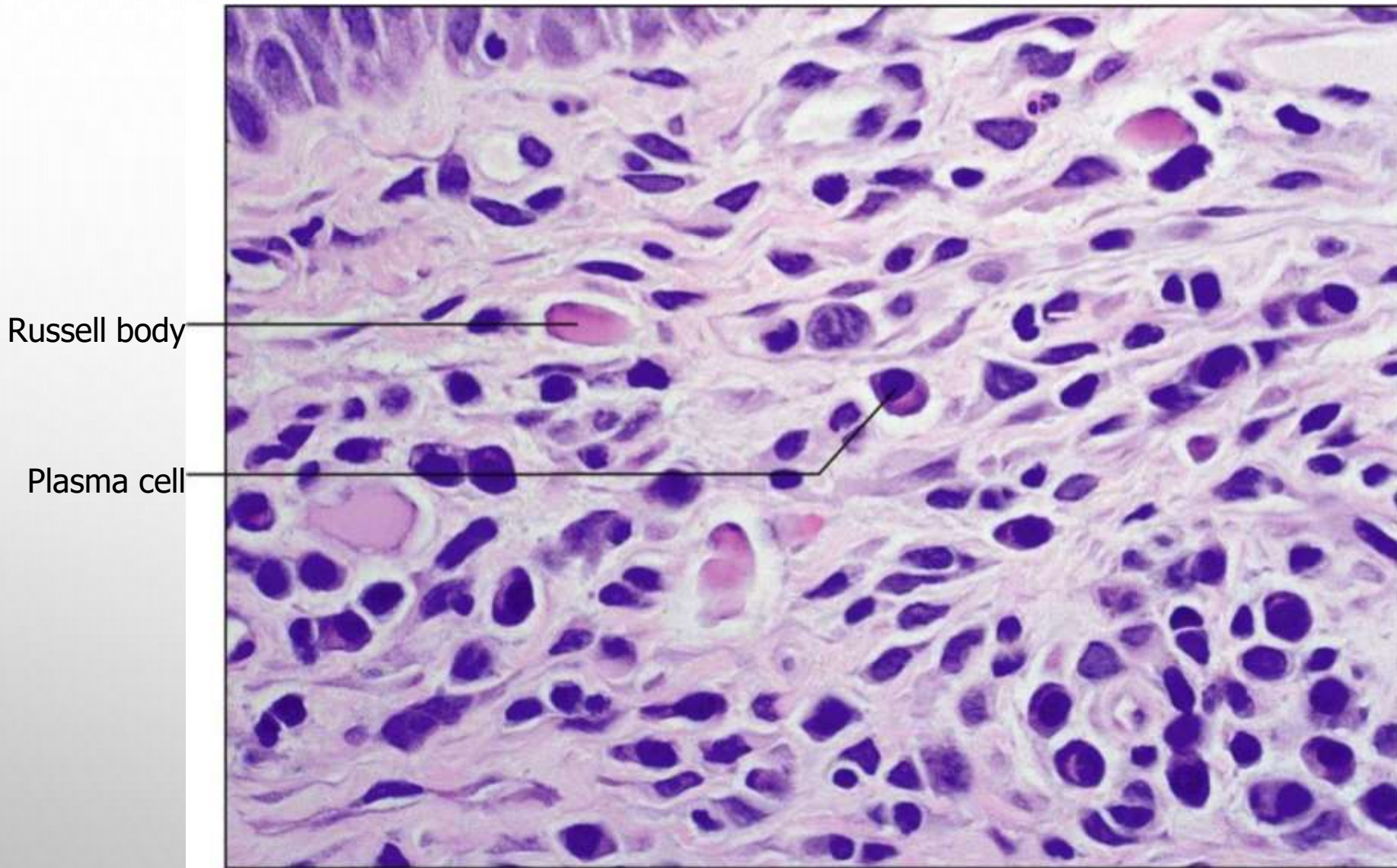
These inclusions composed predominantly of aggregated intermediate filaments that resist degradation.

F 96 : Protein reabsorption droplets in the renal tubular epithelium. In nephrotic syndrome .

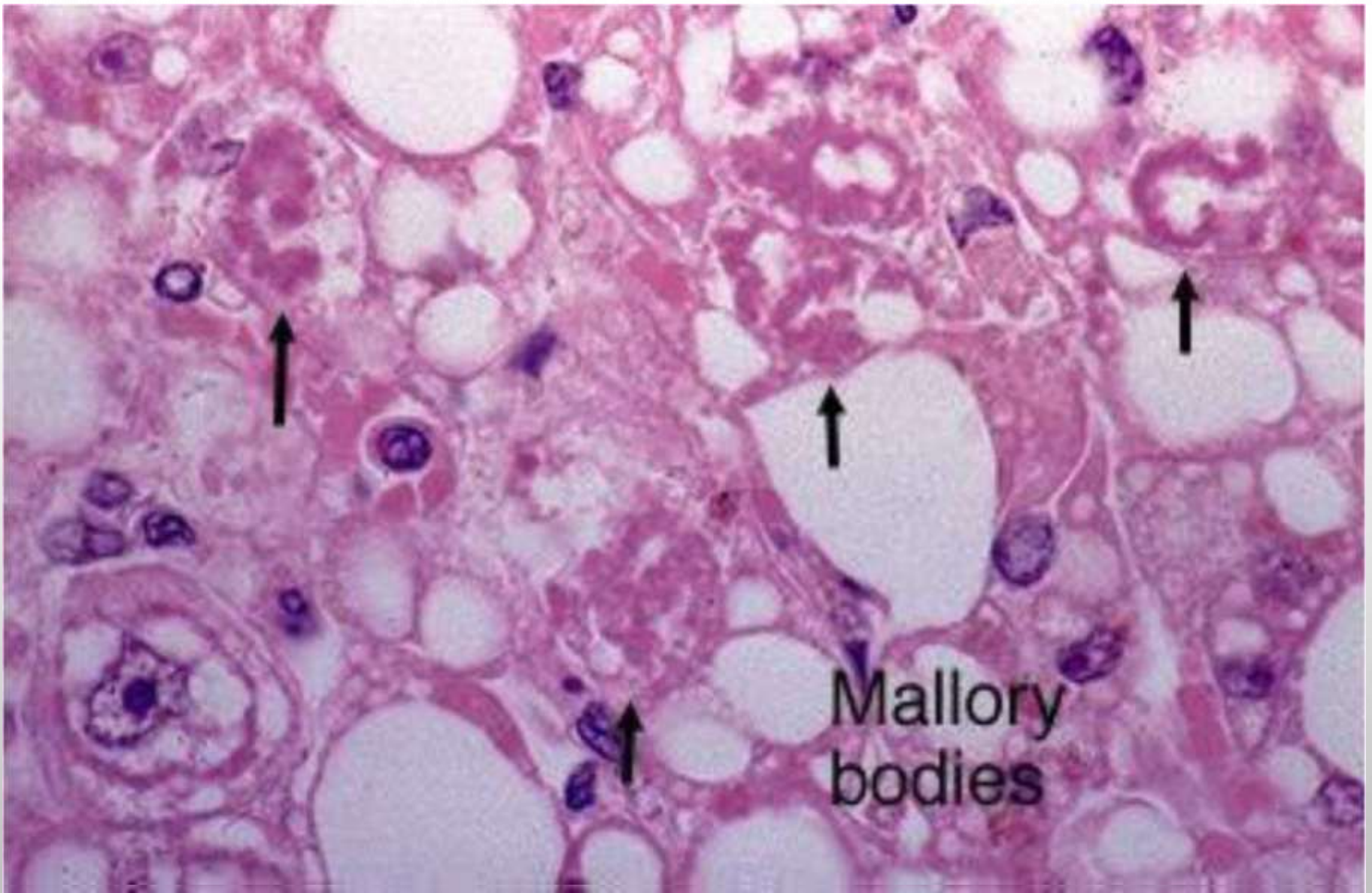


Here are Mallory bodies (the red globular material) composed of cytoskeletal filaments in liver cells chronically damaged from alcoholism. These are a type of "intermediate" filament)





Russel bodies (red colored dots, consisting of immunoglobulins) in plasma cells



Mallory hyaline in alcoholic liver disease.

Glycogen

❑ Excessive intracellular deposits of glycogen are associated with abnormalities in the metabolism of either glucose or glycogen.

(1) In poorly controlled diabetes mellitus : **glycogen accumulates in renal tubular epithelium, cardiac myocytes, & beta cells of the islets of Langerhans.**

(2) In a group of related **genetic disorders** collectively referred to as glycogen storage diseases, or glycogenosis, glycogen accumulates within cells.

In these diseases, enzymatic defects in the synthesis or breakdown of glycogen result **in massive accumulation of glycogen with secondary Injury & cell death.**

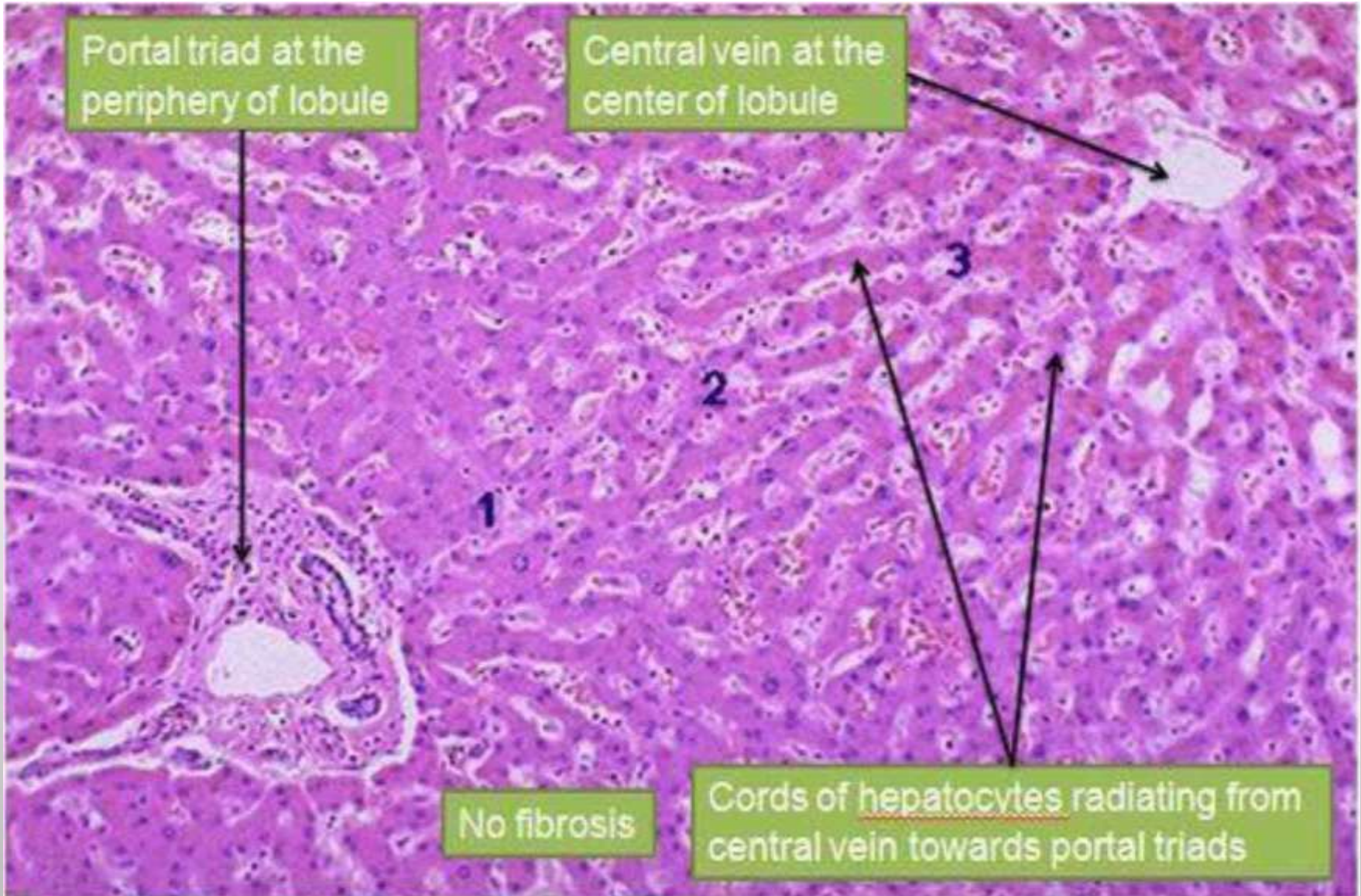
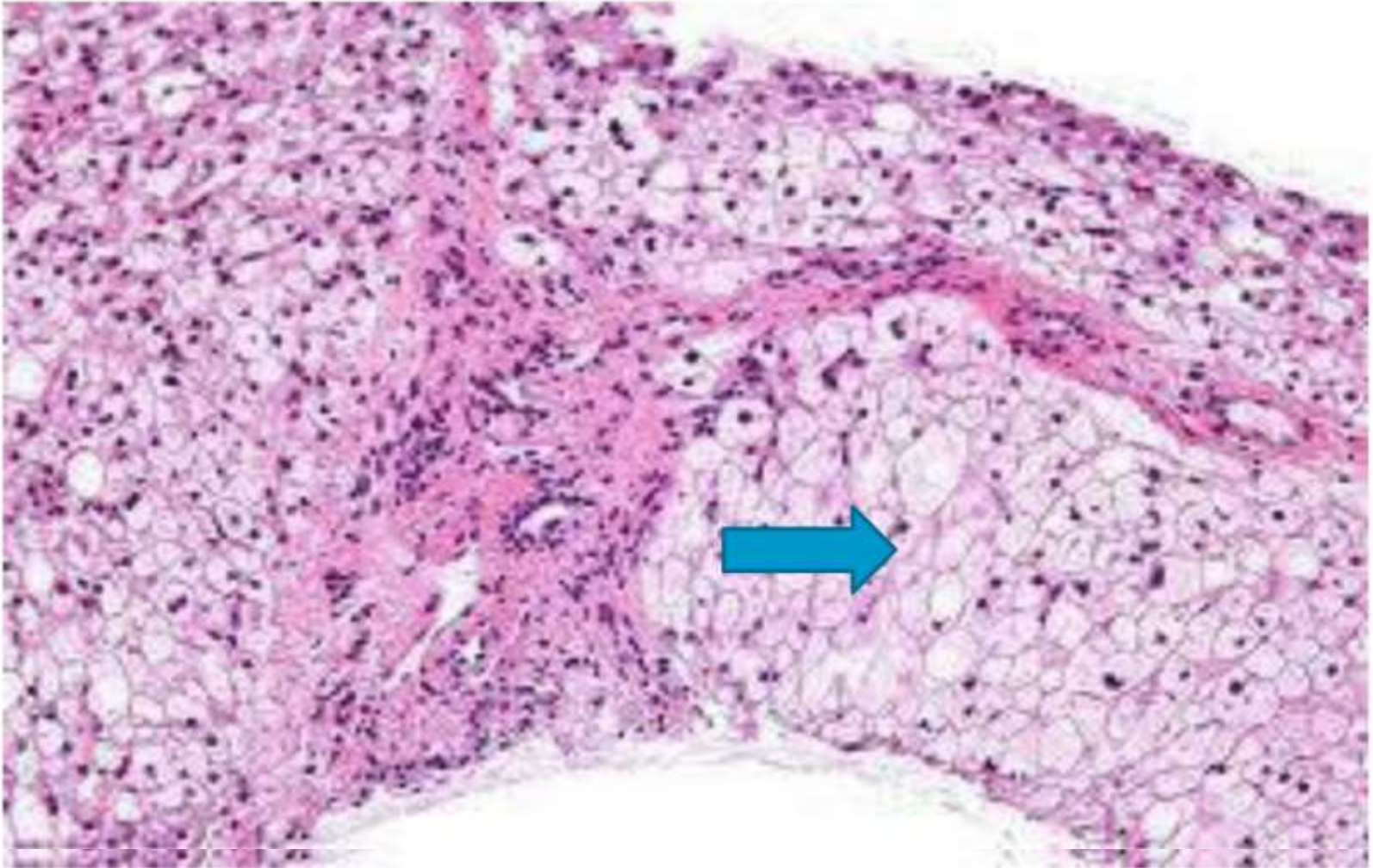
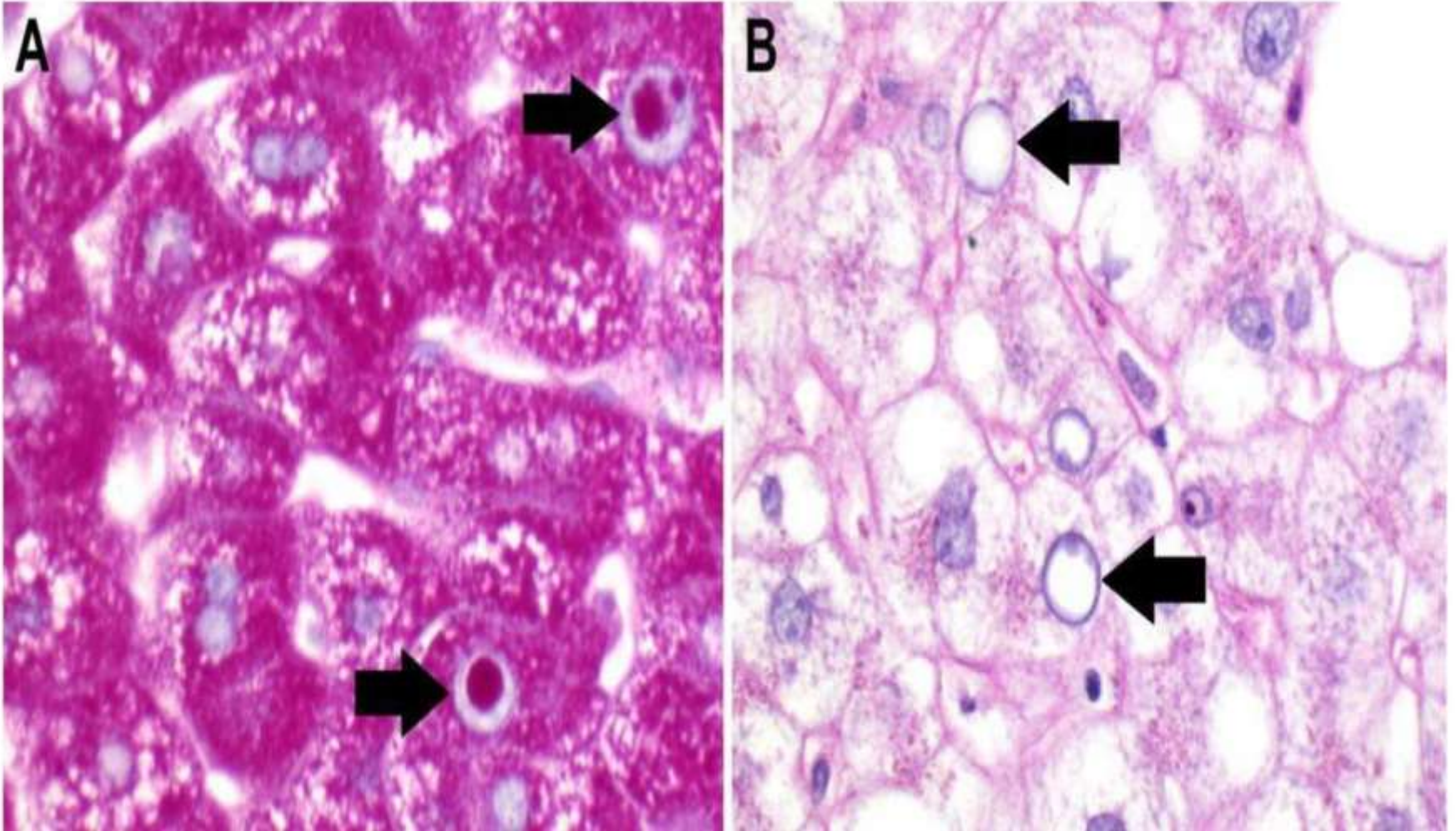


Figure 100 : Normal liver



Microscopic view of liver biopsy from case of Glycogen storage disease , showing vacuoles of glycogen in hepatocytes .



Liver tissue : Glycogen droplets left :(red colored by PAS stain) .Right : white vacuole in the cytoplasm by H&E stain.

Pigments

Are colored substances can be either :

Endogenous pigments i.e. synthesized within the body itself, or exogenous pigments coming from outside the body

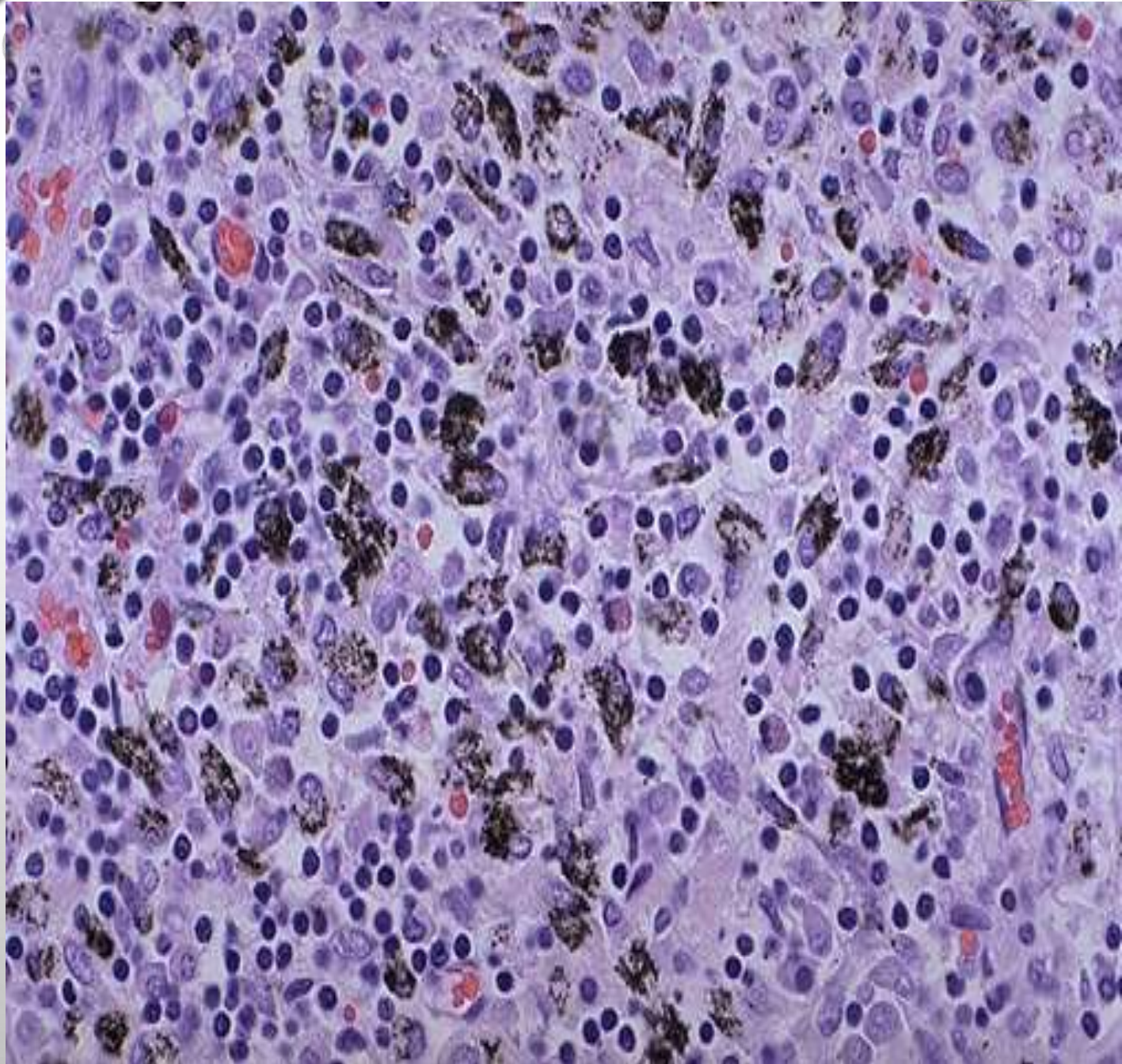
I- Exogenous pigments :

The most common exogenous pigment is **carbon** (e.g. coal dust), a universal air pollutant. When inhaled, carbon is phagocytosed by alveolar macrophages & transported through lymphatic channels to the regional lymph nodes (LN).

Aggregates of the carbon pigment grossly blacken the draining LN & the pulmonary parenchyma (anthracosis).

Heavy accumulations may induce a fibroblastic reaction that can result in a serious lung disease called coal dust worker **pneumoconiosis** .

Here is **anthracotic pigment in macrophages** in a hilar lymph node. **Anthracosis is nothing more than accumulation of carbon pigment from breathing dirty air.** Smokers have the most pronounced anthracosis. The anthracotic pigment looks bad, but it causes no major organ dysfunction.



II- Endogenous pigments

include lipofuscin , melanin & derivatives of hemoglobin .

(1) Lipofuscin : or “wear-&- tear pigment” seen due to aging , is an insoluble, brownish-yellow granular intracellular material that accumulates in a variety of tissues (particularly the heart , liver, & brain) . It causes a brownish color of the tissue e.g. the brown atrophy of the heart .

(2) Melanin : is an endogenous, brown-black pigment.

It is synthesized exclusively by melanocytes , specific cells characteristically found in the epidermis of skin & acts as an endogenous screen against harmful ultraviolet radiation.

(3) Hemosiderin : Is a hemoglobin-derived granular pigment that is golden-yellow to brown & accumulates in tissues when there is a local or systemic excess of iron. Local excess of iron, & consequently of hemosiderin, result from hemorrhage, e.g., in the skin, where it called bruise.

The iron ions of hemoglobin are accumulated as golden-yellow hemosiderin .

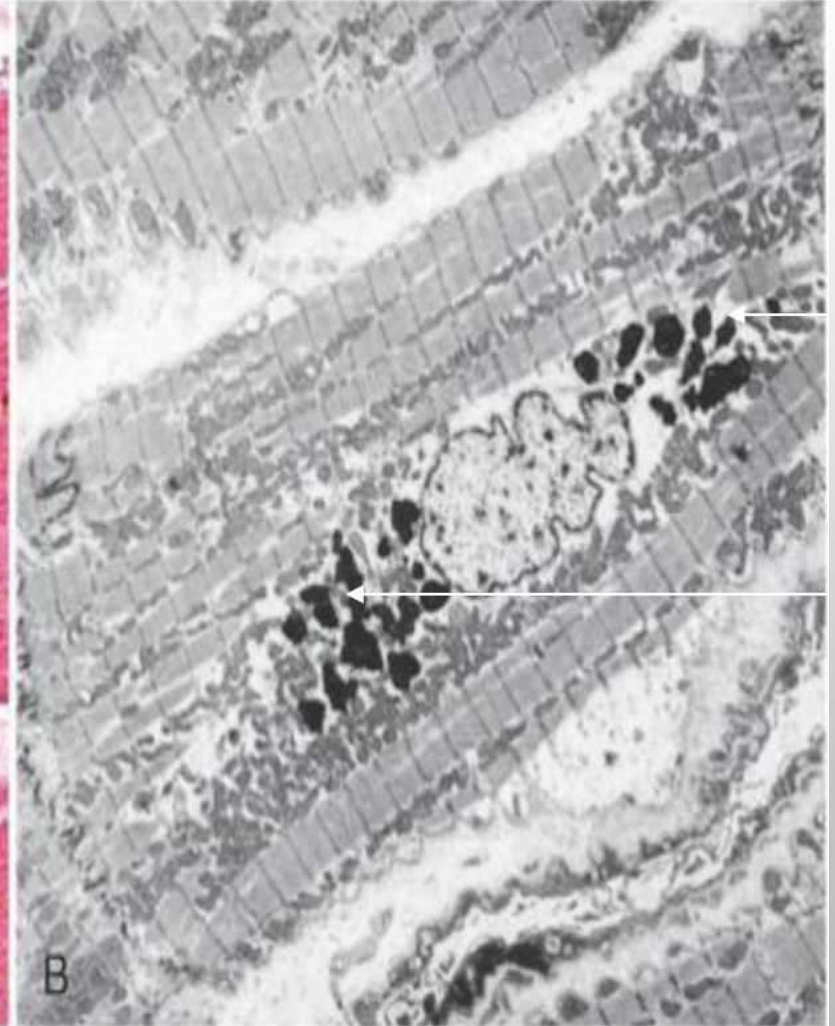
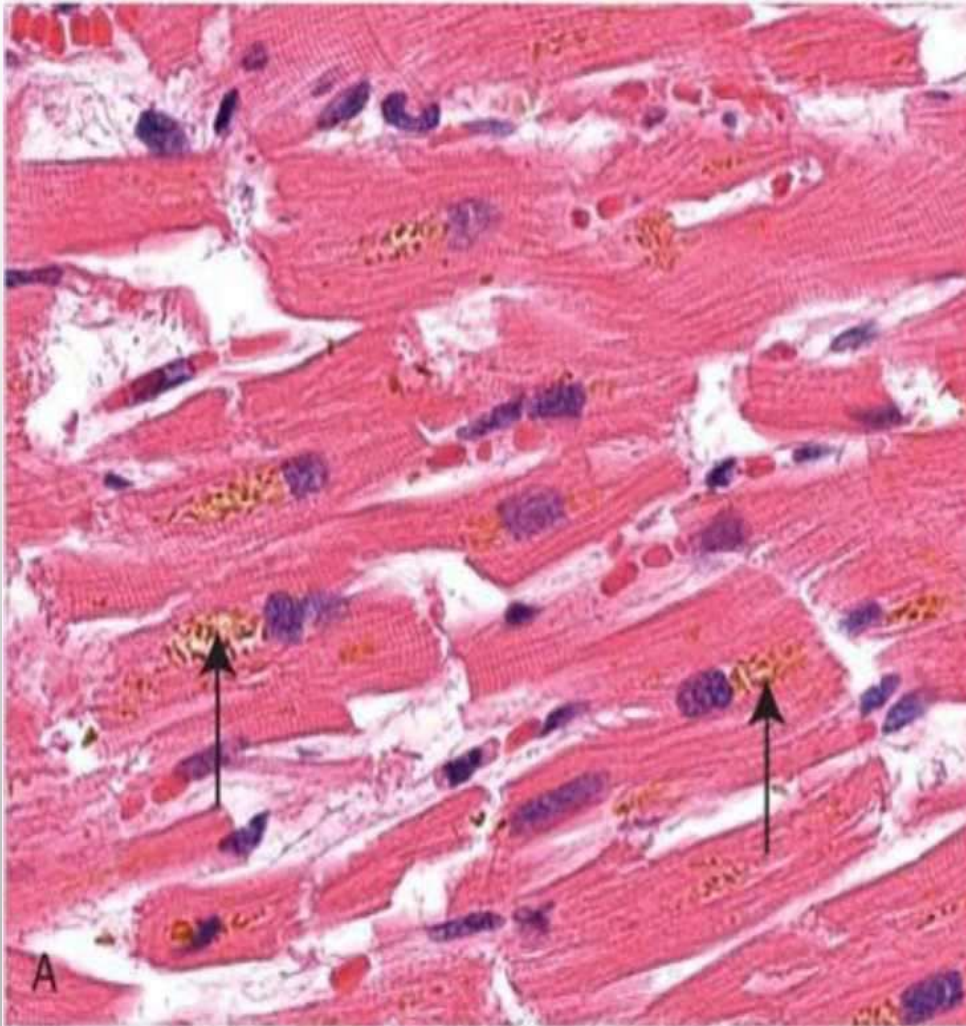


Brown atrophy: heart. The heart is small & atrophic, its brown color is due to accumulation of lipofuscin pigment within the myocardial muscle .

: Lipofuscin granules in a cardiac myocyte.

A, Light microscopy (deposits indicated by arrows).

B, Electron microscopy.





Skin : Malignant melanoma. Brownish color of skin lesion due to melanin pigment deposition .

❑ **Hemosiderosis** : is a condition characterized by systemic overload of iron, with hemosiderin deposited: **first in the mononuclear phagocytes of the liver**, bone marrow, spleen, & lymph nodes, but, later, in the **parenchymal cells of the body, principally in the liver, heart, & pancreas.**

Hemosiderosis occurs in the setting of:

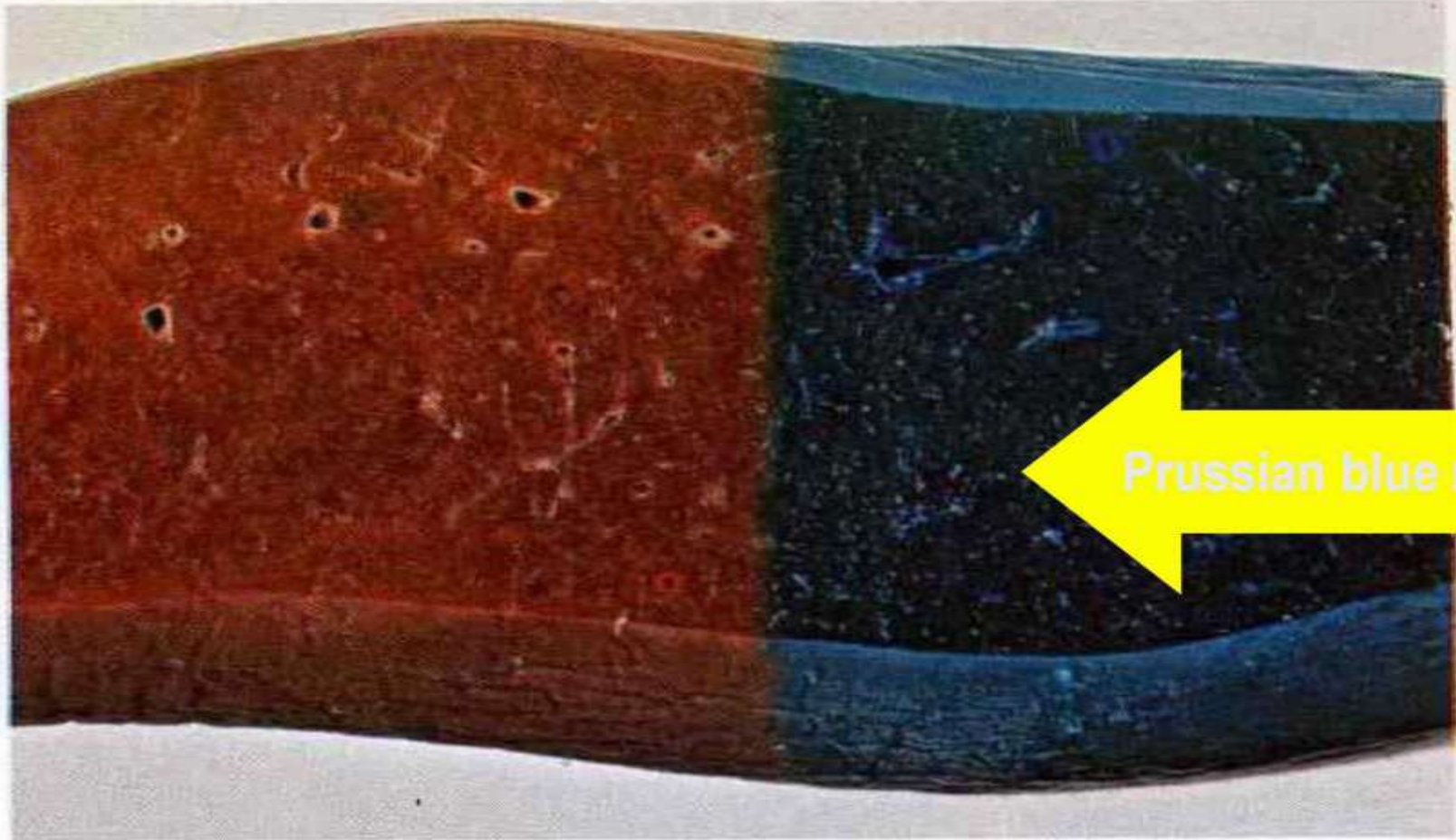
(1) **Increased absorption of dietary iron**, a disease called hereditary hemochromatosis, one of the most common inborn errors of metabolism, in which excessive absorption of iron from the intestine lead extensive accumulations of iron in tissue, causing liver cirrhosis, heart failure & diabetes mellitus .

(2) **Hemolytic anemias**

(3) **Frequent Blood transfusions**, in which the transfused red cells constitute an exogenous load of iron.

(3) **Localized hemosiderosis** : occurs at sites **of trauma** , commonly seen in hands feet, trunk or face as dark red patches due to local hemorrhage its color gradually changes into brownish , bluish , yellowish then disappears .

Hemosiderosis in the Spleen .The section of the splenic tissue on the right has been immersed in Perls' solution & the deep blue color (Prussian blue reaction) confirms the presence of iron-containing pigment.

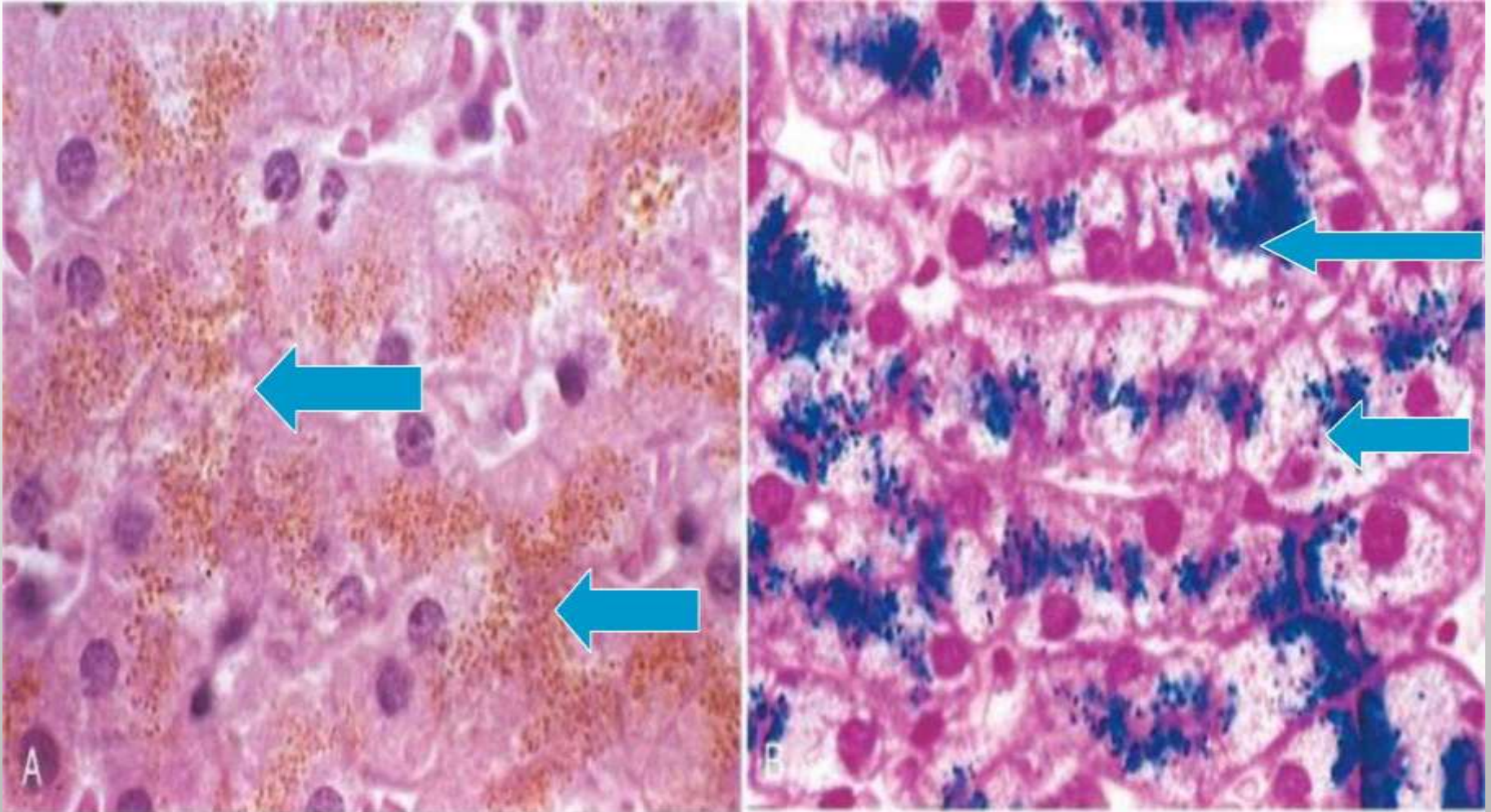


2.8 Haemosiderosis: spleen

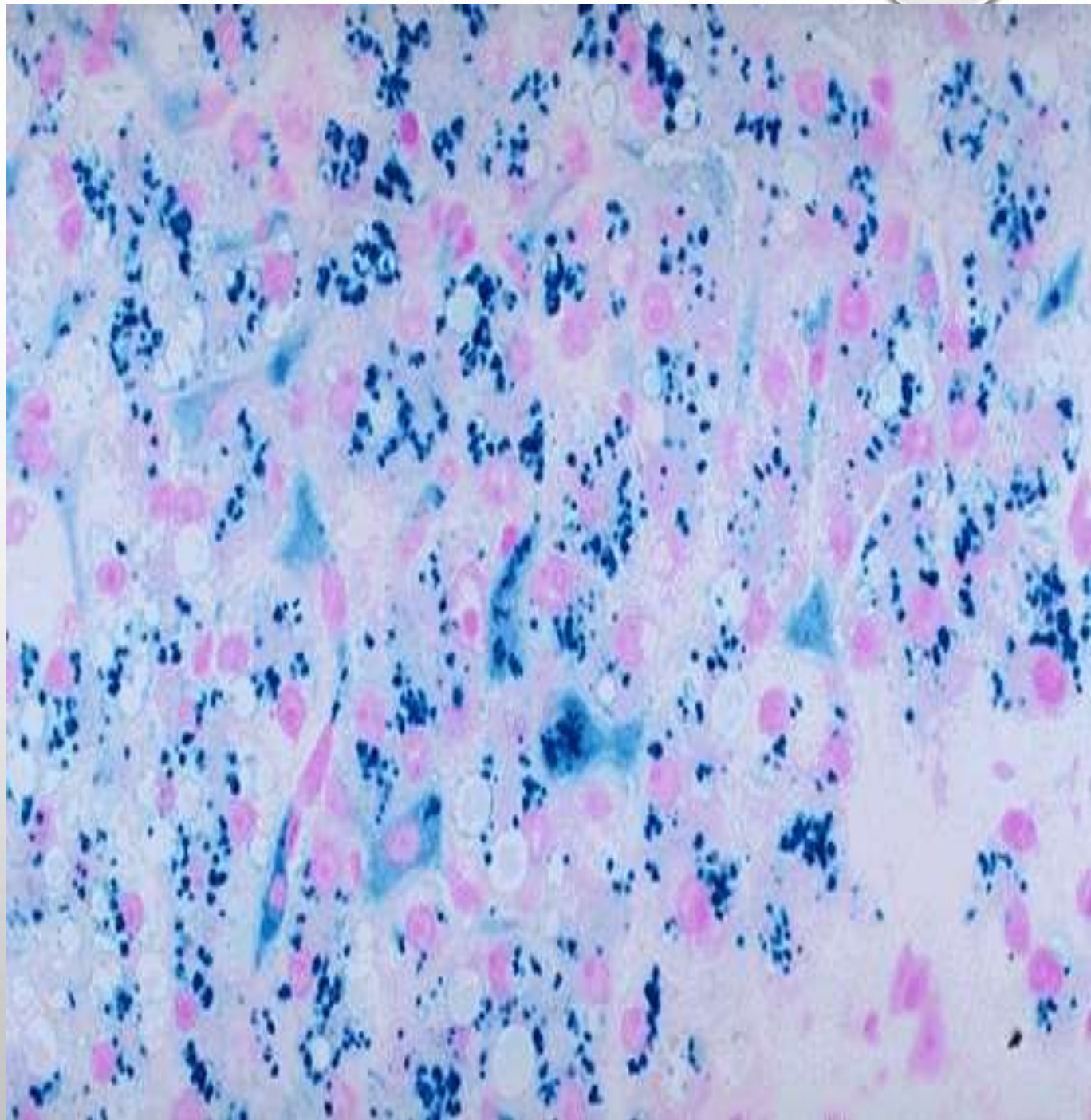
Hemosiderin granules in liver cells.

A, H&E section showing golden-brown, finely granular pigment.

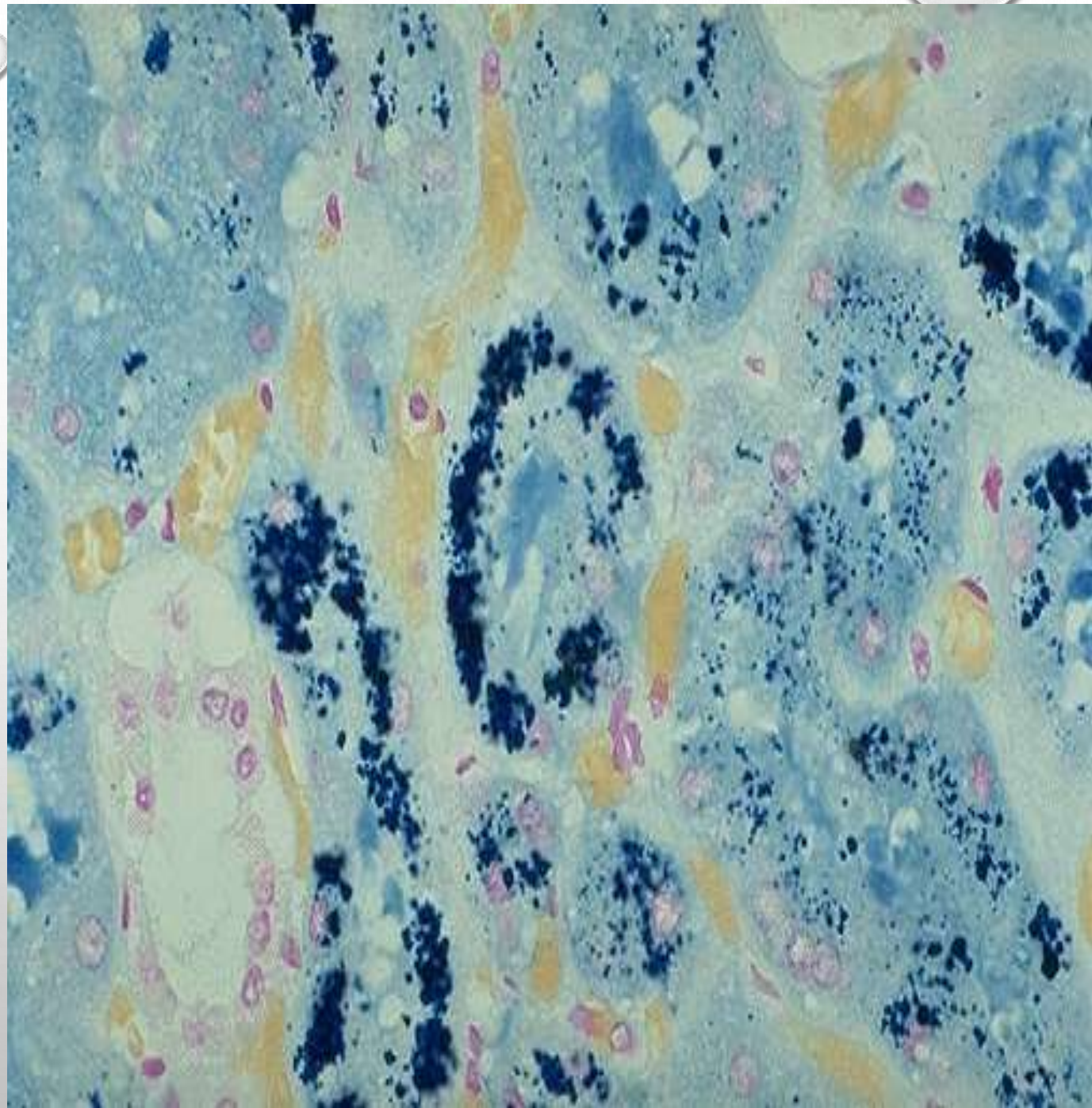
B, Positive Prussian blue reaction, iron stains blue .



A Prussian blue reaction is seen in this iron stain of the liver to demonstrate large amounts of hemosiderin that are present within the cytoplasm of the hepatocytes and Kupffer cells. Ordinarily, only a small amount of hemosiderin would be present in the fixed macrophage-like cells in liver, the Kupffer cells, as part of iron recycling.



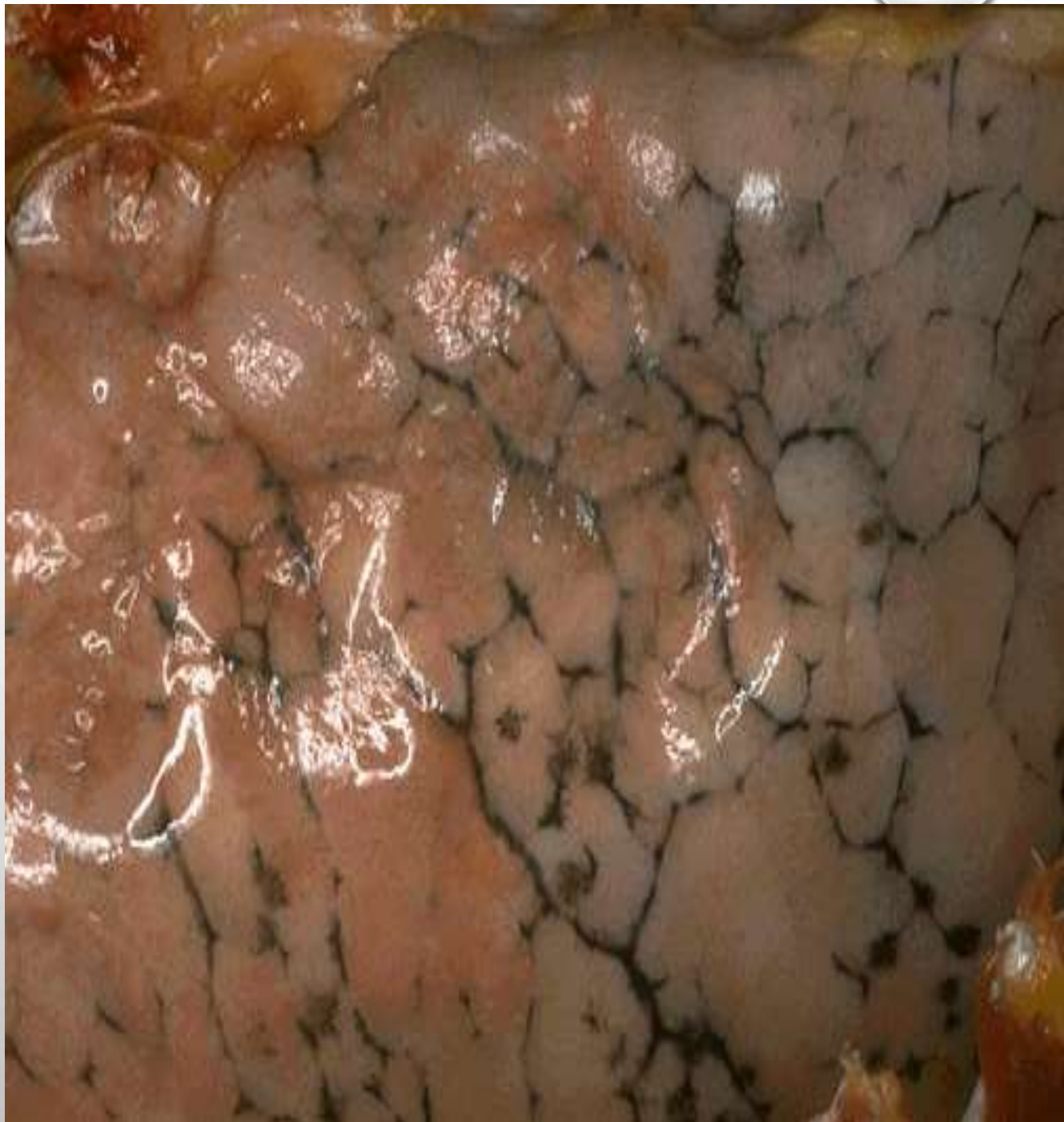
These renal tubules contain large amounts of hemosiderin, as demonstrated by the **Prussian blue** iron stain. This patient had chronic hematuria.



The black streaks seen between lobules of lung beneath the pleural surface are due to accumulation of **anthracotic pigment**.

This anthracosis of the lung is not harmful and comes from the carbonaceous material breathed in from dirty air typical of industrialized regions of the planet.

Persons who smoke would have even more of this pigment.



Pathologic Calcification

Is the abnormal deposition of calcium salts in tissue. Can be :

I-Dystrophic calcification . II-Metastatic calcification.

I- Dystrophic calcification:

- In this form calcium salts are **deposited in necrotic tissue** with **normal calcium level in blood** .

- **Dystrophic calcification can be seen in :**

- (1) **TB caseous necrosis** .

- (2) Calcification in atheromas of advanced **atherosclerosis** , is • extremely common

- (3) **Calcific aortic valve** in the elderly.

- (4) **Carcinoma of the breast**.

Grossly:

- the calcium salts are seen as fine, white granules or clumps, often felt as gritty deposits, or stony hard white nodules .

II- Metastatic calcification :

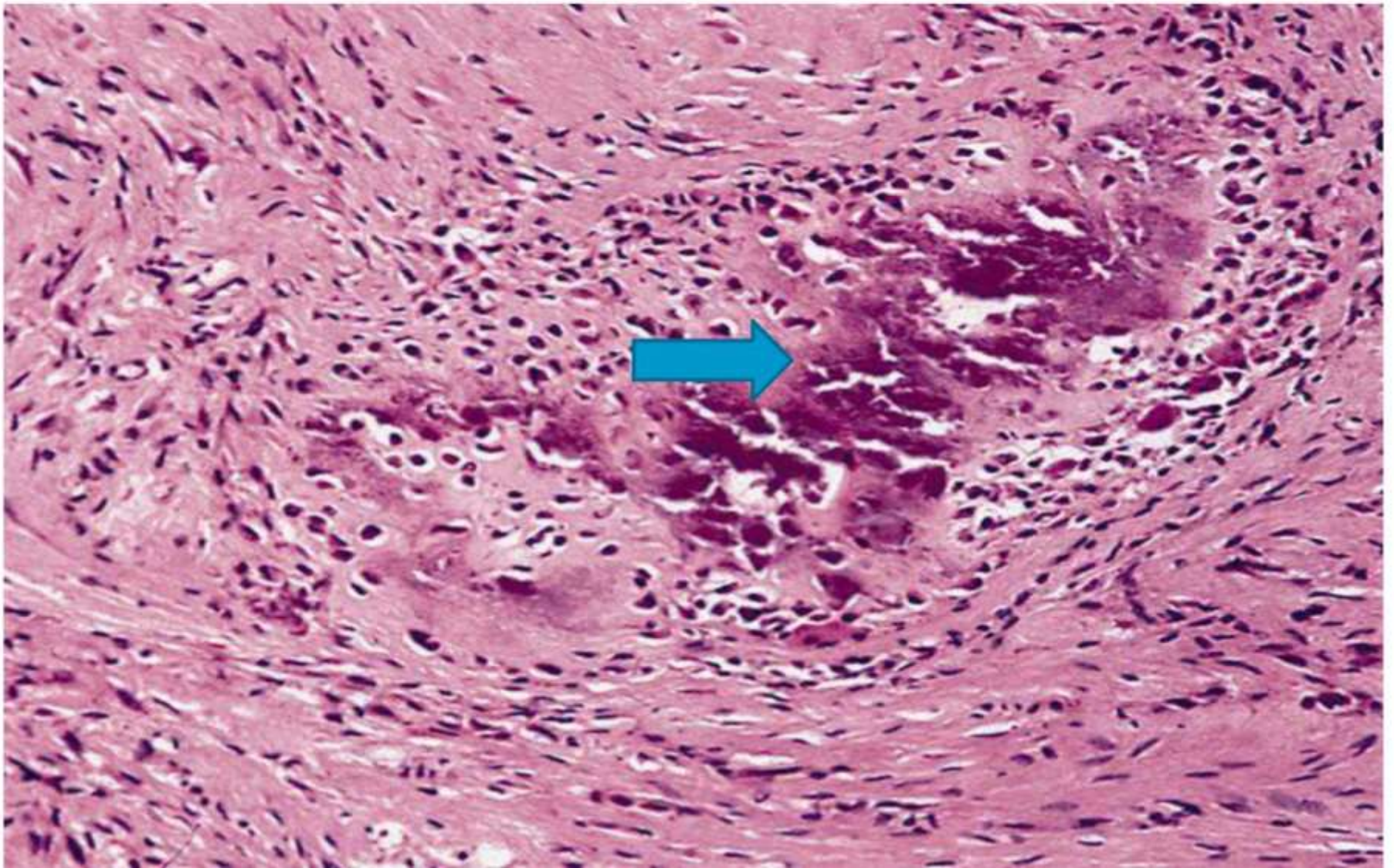
It is characterized by deposition of **calcium salts in normal tissues** due to **increased calcium level** in blood :

Causes of hypercalcemia are :

- 1- Increased secretion of parathyroid hormone
- 2- Destruction of bone : due to immobilization, or bone involvement by tumors as in multiple myeloma, leukemia, or diffuse skeletal metastases.
- (3) Vitamin D-related disorders .
- (4) Renal failure in which phosphate retention leads to secondary hyperthyroidism .

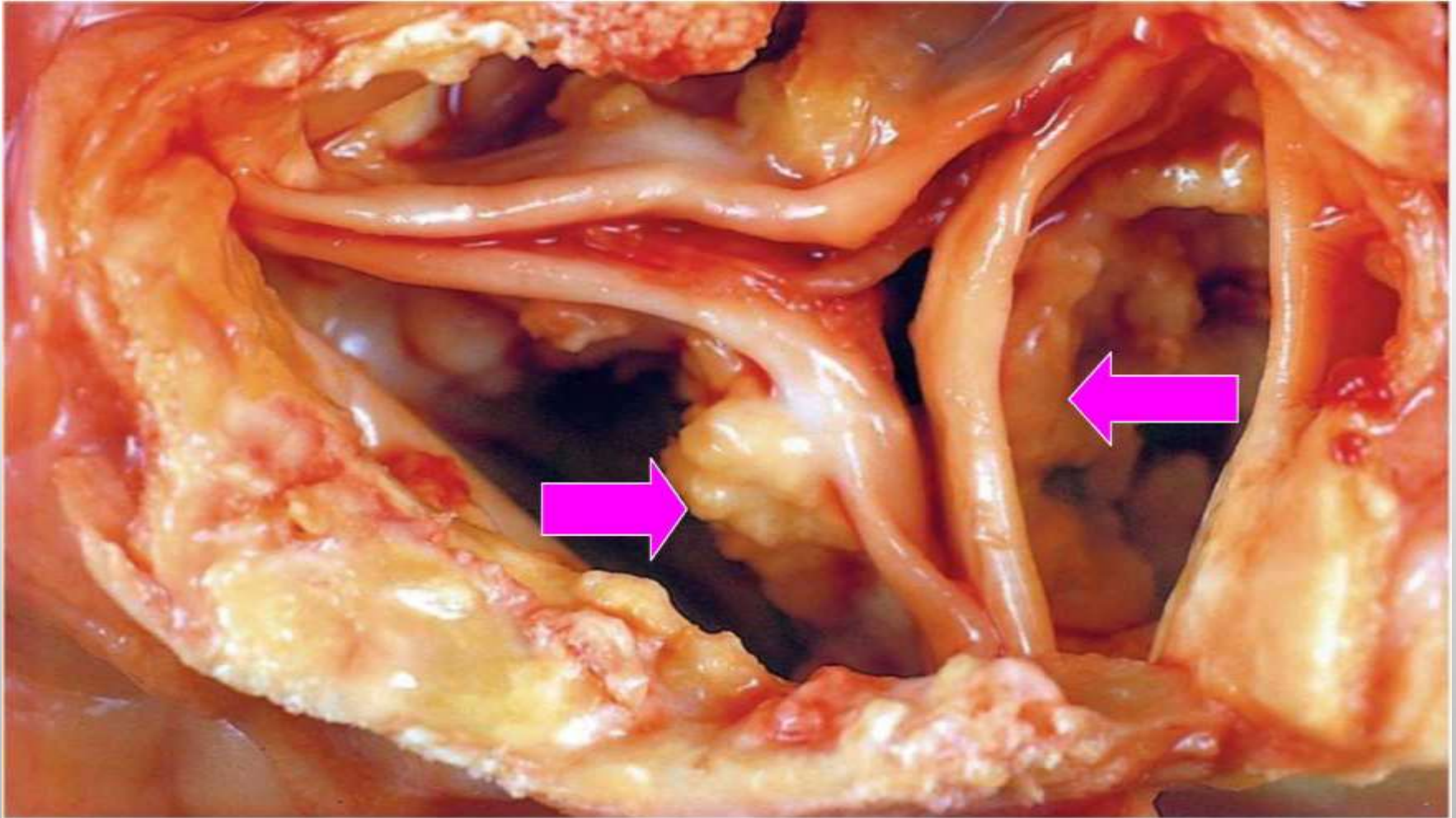
Metastatic calcification resemble dystrophic calcification.

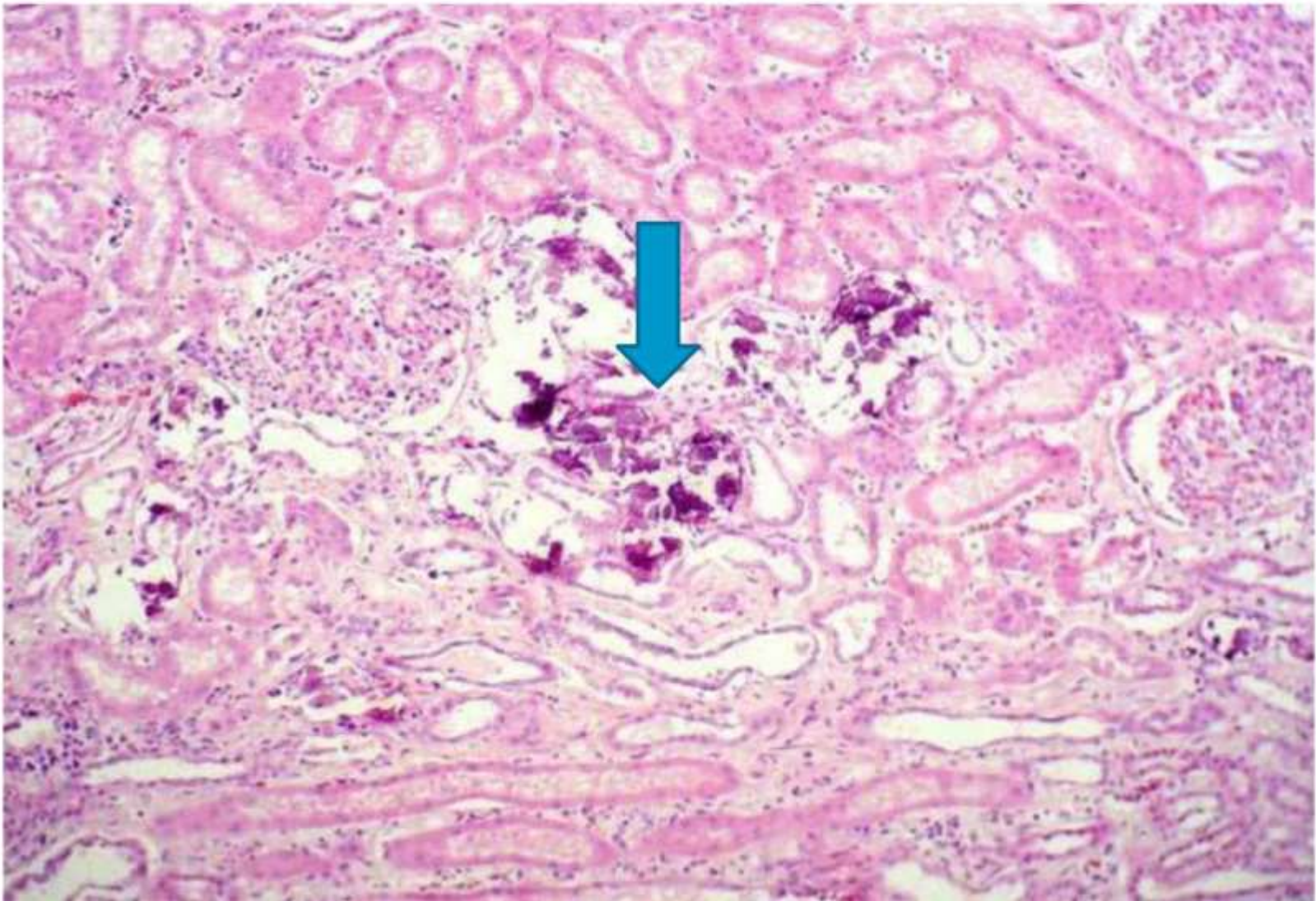
It can occur widely throughout the body but principally affects the interstitial tissues of the blood vessels , , kidneys, lungs & gastric mucosa .



Microscopic view , showing dystrophic calcification in soft tissue granuloma looks purple color

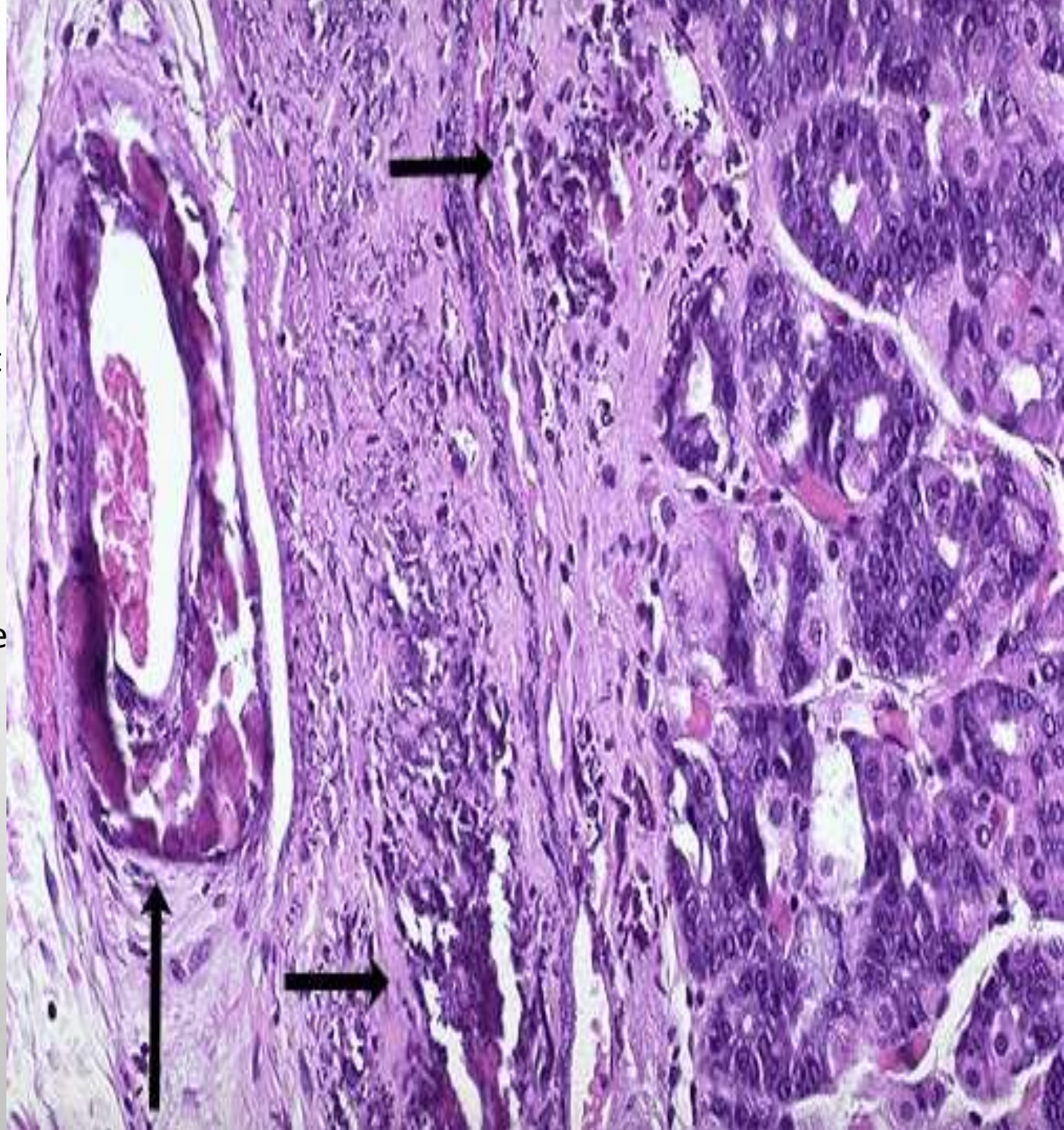
Calcification of the aortic valve in elderly people. Seen as large, irregular white masses of dystrophic calcification (arrows) .





Microscopic view of kidney showing metastatic calcification called nephrocalcinosis .Dark purple calcium salts seen in renal tubules (arrow) .

This is **dystrophic calcification** in the wall of the stomach. At the far left is an artery with calcification in its wall. There are also irregular bluish-purple deposits of calcium in the submucosa. Calcium is more likely to be deposited in tissues that are damaged.



Aging

- ❑ Aging is one of **the strongest independent risk factor** for chronic diseases like cancer , Alzheimer disease & ischemic heart diseases .
- ❑ Aging of any individual is simply due to their **cellular aging** •
- ❑ Aging is regulated by limited number of genes and signaling pathways that are evolutionary conserved from yeasts to mammals .
- ❑ Cellular aging is the result of a progressive decline in the life span and functional capacity of cells •
- ❑ Aging is caused by changes in **genetic information, chromosome structure, and protein homeostasis**
- ❑ Cellular senescence, defined as irreversible cell cycle arrest, is another important characteristic of aging cells.

Several mechanisms are responsible for cellular aging, including :

DNA damage :

- ❑ A variety of metabolic insults that accumulate overtime may result in damage to nuclear & mitochondrial DNA . Although most DNA damage is repaired by DNA repair enzymes ,some persists and accumulates as cells age .

Decreased cellular replication :

- ❑ All normal cells have a limited capacity for replication .and after a fixed number of divisions cells become arrested in a terminally non dividing state , known as replicative senescence .
- ❑ Aging is associated with **progressive replicative senescence** .
- ❑ **replicative senescence can be triggered by a DNA damage response due to the shortening of telomeres. Cells can also be induced to senesce by DNA damage in response to elevated reactive oxygen species (ROS), activation of oncogenes, and cell-cell fusion.**

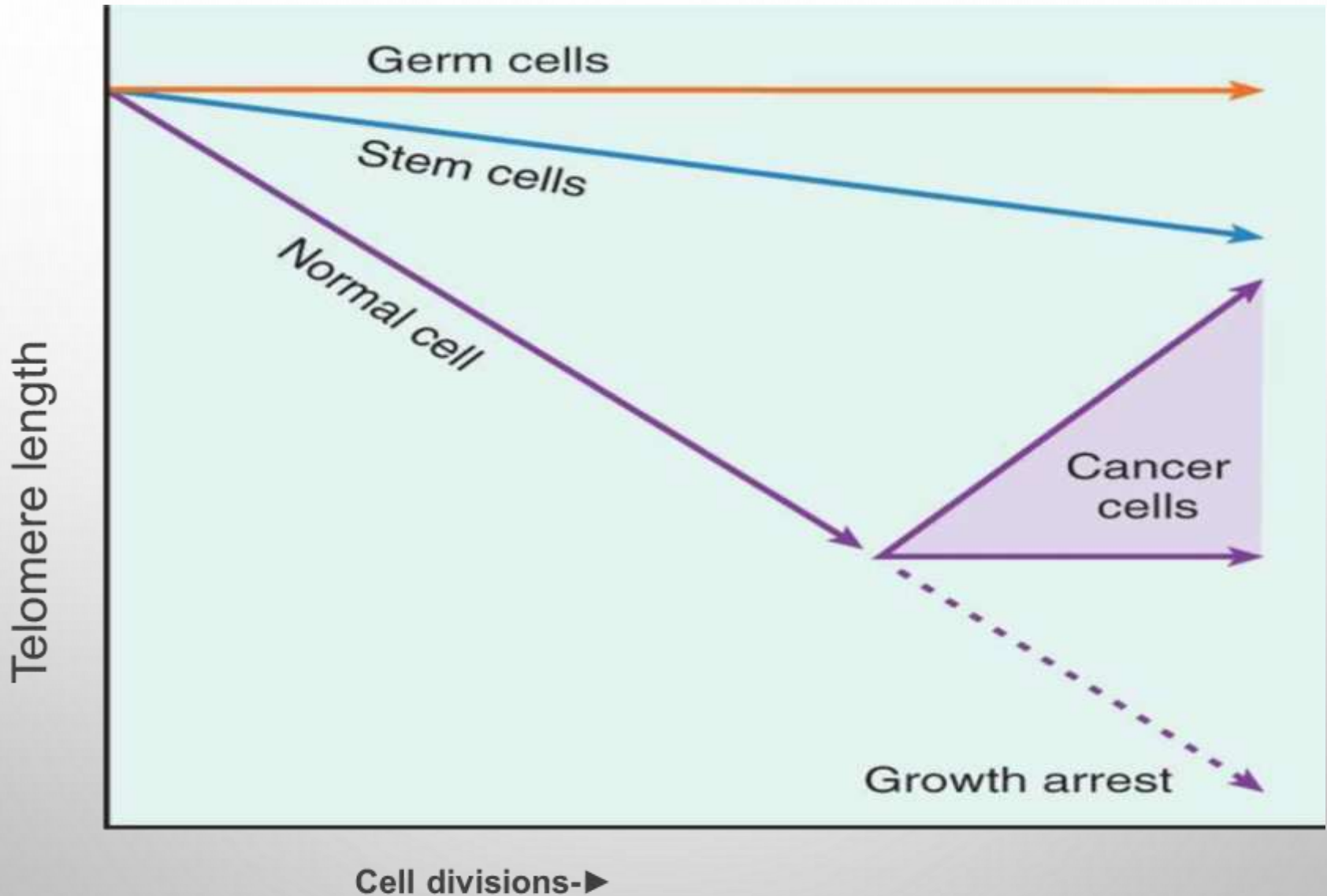
- CELLS FROM PATIENTS WITH **WERNER SYNDROME** , A RARE DISEASE CHARACTERIZED BY **PREMATURE AGING** , HAVE A MARKEDLY REDUCED IN VITRO CELLULAR LIFE SPAN (TISSUE CULTURE) , AS COMPARED TO CELLS TAKEN FROM HEALTHY CHILDREN WHICH HAVE THE CAPACITY TO UNDERGO MORE ROUNDS OF REPLICATIONS & LONGER LIFE SPAN ALSO SEEN IN PROGERIA SYNDROME .



Progeria syndrome , photograph of a child showing premature aging.

- ❑ In human cells , the mechanism of replicative senescence involves progressive shortening of telomeres , which ultimately results in cell cycle arrest.
- ❑ Telomeres are short repeated sequence of DNA present at the ends of linear chromosomes ,that are important for ensuring the complete replication of chromosome ends & for protecting the ends from fusion & degradation .
- ❑ When somatic cells replicate a small fraction of the telomere is not duplicated and telomeres become progressively shorter, & as a consequence for its shortening the DNA may break & its ends cannot be protected .

- ❑ Telomere length is maintained by nucleotide addition mediated by enzyme called Telomerase which is a specialized RNA-protein complex that uses its own RNA as a template for adding nucleotides to the end of chromosomes .
- ❑ Telomerase activity is present at germ cells , less in stem cells & absent in most somatic cells .
In cancer cells, telomerase is often reactivated.



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Figure 114 : The role of telomeres in replicative senescence of cells .

Defective protein Homeostasis:

Overtime the cells cannot maintain normal protein homeostasis , because of increased turnover & decreased synthesis.

Abnormal protein homeostasis can have many effects on cell survival , replication & function , as well accumulated misfolded proteins , which trigger apoptosis .

Other factors : progressive accumulation of metabolic damage ; possible roles of growth factors that promote aging in simple model organisms

SAMPLE QUESTIONS

- **<Q> CHOOSE THE CORRECT ONE OF THE FOLLOWING STATEMENTS ?**
- **A-CLOUDY SWELLING AND HYDROPIK SWELLING ARE THE SAME.**
- **B- RUSSELL'S BODIES REPRESENTING EXCESSIVE IMMUNOGLOBULIN IN PLASMA CELLS' ROUGH ENDOPLASMIC RETICULUM WHICH IS A FORM OF HYALINE CHANGES THAT SEEN IN MULTIPLE MYELOMA.**
- **C-IN NEPHROTIC SYNDROME THERE IS EXCESSIVE LEAKING OF LIPOFUSION THROUGH GLOMERULI RESULTING IN PUFFINESS OF THE FACE AND PERIORBITAL EDEMA.**
- **D-MALLORY'S HYALINE IS SEEN IN HEPATOCYTES IN CHOLESTASIS.**
- **E-METASTATIC CALCIFICATION OCCURS IN ABNORMAL NECROTIC TISSUE.**

- **<Q>WHICH OF THE FOLLOWING STATEMENTS ABOUT CELL INJURY IS TRUE?**
- A- PHOSPHOLIPID RICH AMORPHOUS DENSITIES ARE SEEN IN MITOCHONDRIA IN IRREVERSIBLE CELL INJURY.
- **B- ISCHEMIA-REPERFUSION INJURY IS MAINLY BECAUSE OF OXIDATIVE DAMAGE TO CELL.**
- C- GENERATION OF OXYGEN FREE RADICALS OCCURS IN CYTOPLASM.
- D-SUPEROXIDE OXYGEN IS THE MOST REACTIVE OF THE OXYGEN FREE RADICALS.
- E-NORMALLY NO OXYGEN FREE RADICALS PRODUCED IN THE CELLS.

• **<Q>WHICH OF THE FOLLOWING STATEMENTS REGARDING CELL INJURY IS TRUE?**

• A- IRREVERSIBLE CELL INJURY CHARACTERIZED BY ACUTE CELLULAR SWELLING.

• **B-INABILITY TO REVERSE MITOCHONDRIAL FUNCTION AFTER REMOVAL OF CAUSATIVE AGENT AND MEMBRANE DAMAGE ARE TWO DEFINING DIFFERENCES BETWEEN REVERSIBLE AND IRREVERSIBLE INJURY.**

• C-INTRACELLULAR ACCUMULATION OF SODIUM AND WATER IS A CAUSE OF CHROMATIN CLUMPING.

• D-INTRACELLULAR ACCUMULATION OF POTASSIUM CAUSES HYDROPIK SWELLING OF CELL.

• E-MYELIN FIGURES ARE FOUND ONLY IN IRREVERSIBLE INJURY.