Volgograd state medical university

Department of Pathological anatomy

Lecture 1 (часть 2)

Cell death.

Necrosis. Apoptosis.

Types of Cell Death

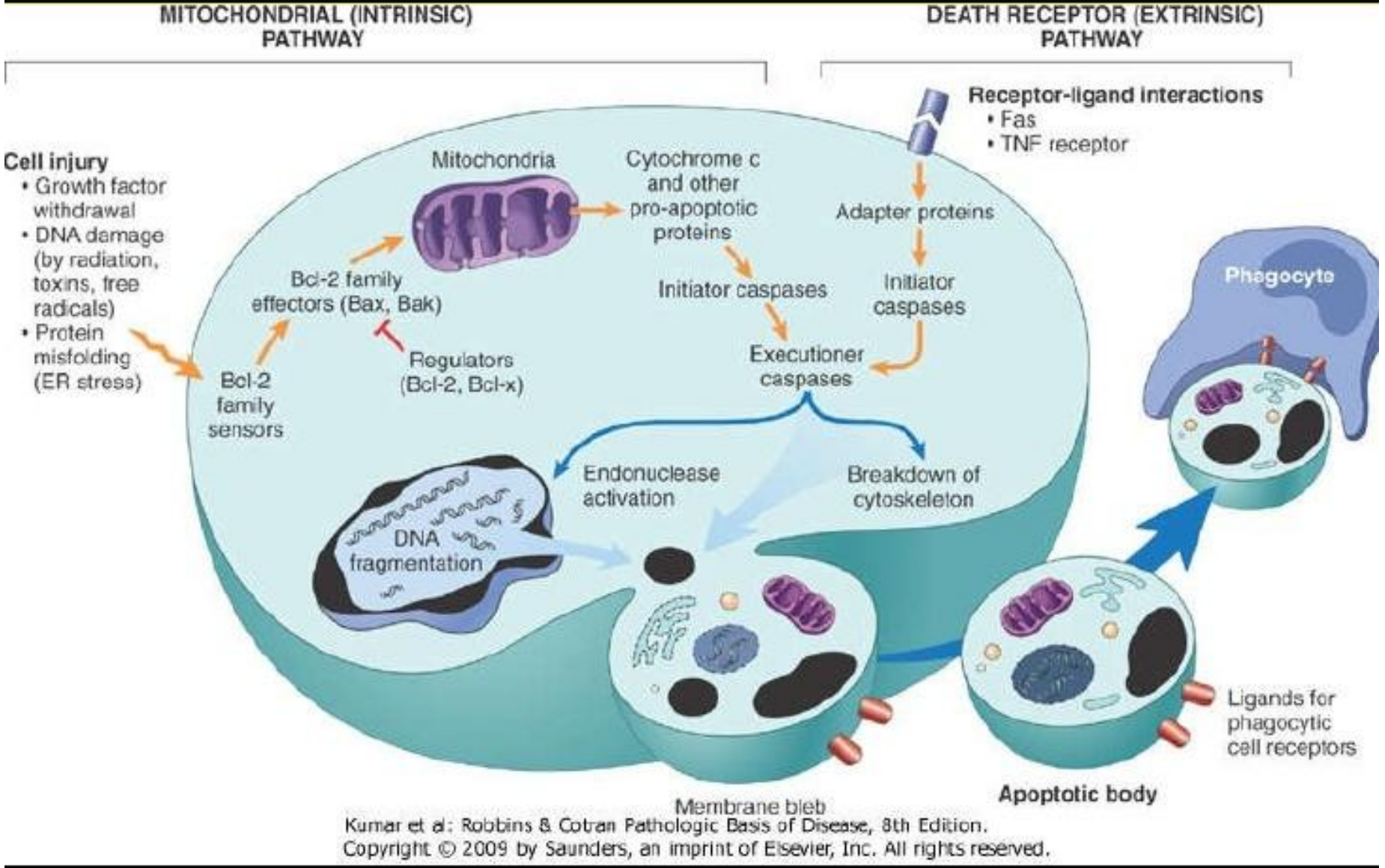
- Apoptosis
  - Usually a regulated, controlled process
  - Plays a role in embryogenesis
- Necrosis
  - Always pathologic – the result of irreversible injury
  - Numerous causes

Apoptosis

- Involved in many processes, some physiologic, some pathologic
  - Programmed cell death during embryogenesis
  - Hormone-dependent involution of organs in the adult (e.g., thymus)
  - Cell deletion in proliferating cell populations
  - Cell death in tumors
  - Cell injury in some viral diseases (e.g., hepatitis)

Apoptosis – Morphologic Features

- Cell shrinkage with increased cytoplasmic density
- Chromatin condensation
- Formation of cytoplasmic blebs and apoptotic bodies
- Phagocytosis of apoptotic cells by adjacent healthy cells



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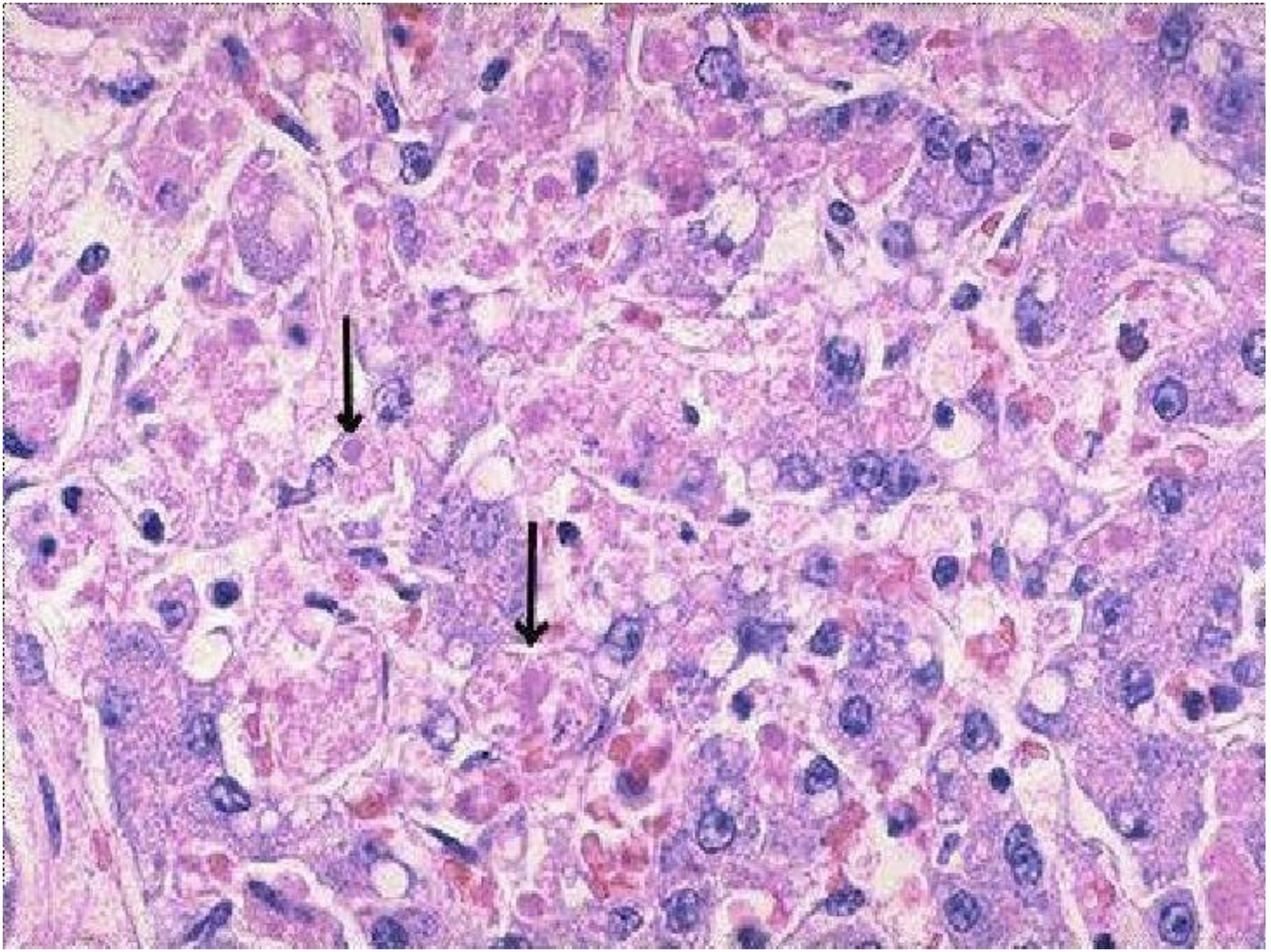


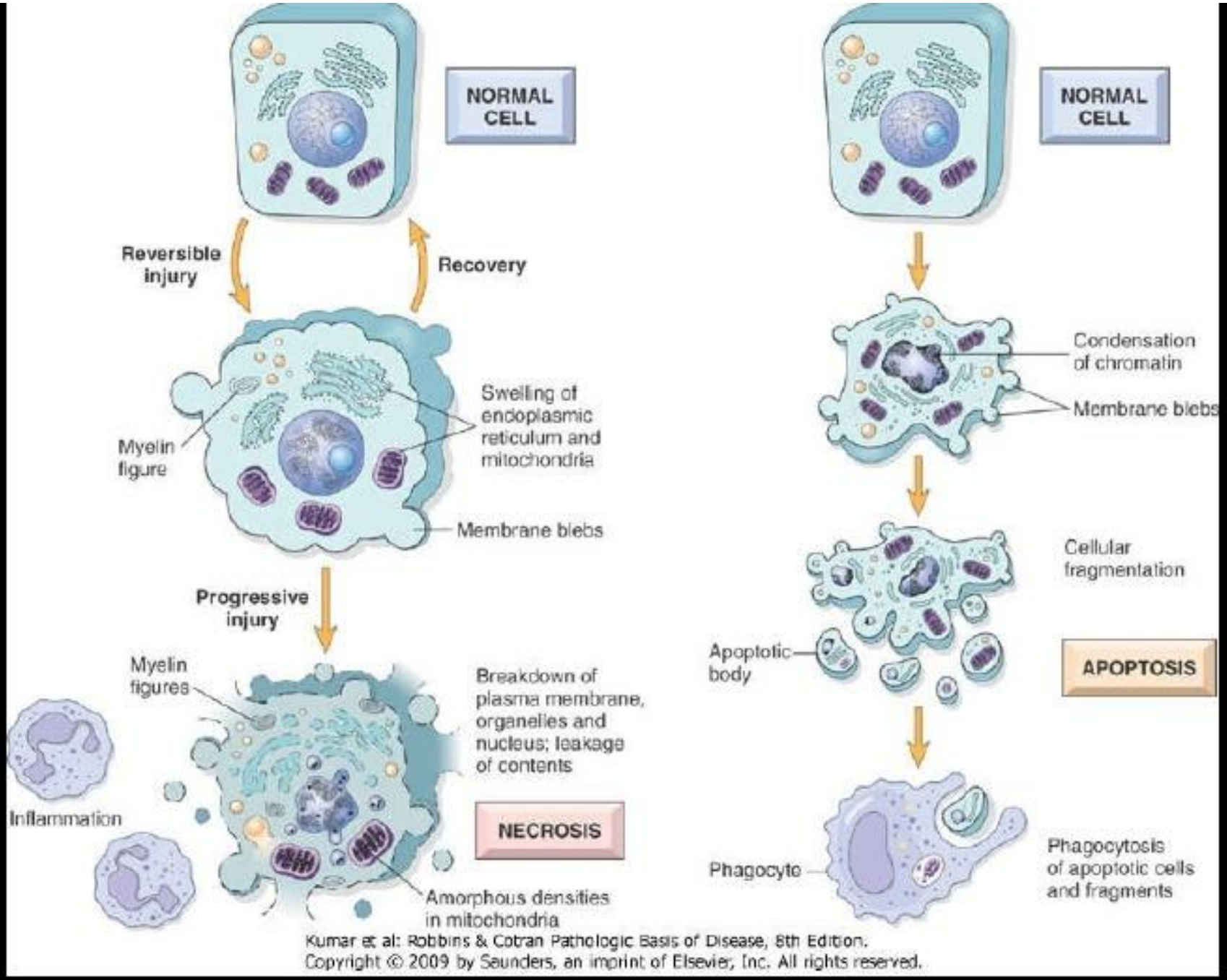
Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome size fragments
Plasma Membrane	Disrupted	Intact; altered structure, especially orientation of lipids
Cellular Contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent Inflammation	Frequent	No
Physiologic pathologic role	Invariably pathologic (culmination of irreversible cell injury)	Often physiologic, means of or eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA damage

Irreversible Injury -- Morphology

- Light microscopic changes
  - Increased cytoplasmic eosinophilia (loss of RNA, which is more basophilic)
  - Cytoplasmic vacuolization
  - Nuclear chromatin clumping
- Ultrastructural changes
  - Breaks in cellular and organellar membranes
  - Larger amorphous densities in mitochondria
  - Nuclear changes

Irreversible Injury – Nuclear Changes

- Pyknosis
  - Nuclear shrinkage and increased basophilia
- Karyorrhexis
  - Fragmentation of the pyknotic nucleus
- Karyolysis
  - Fading of basophilia of chromatin



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Morphology

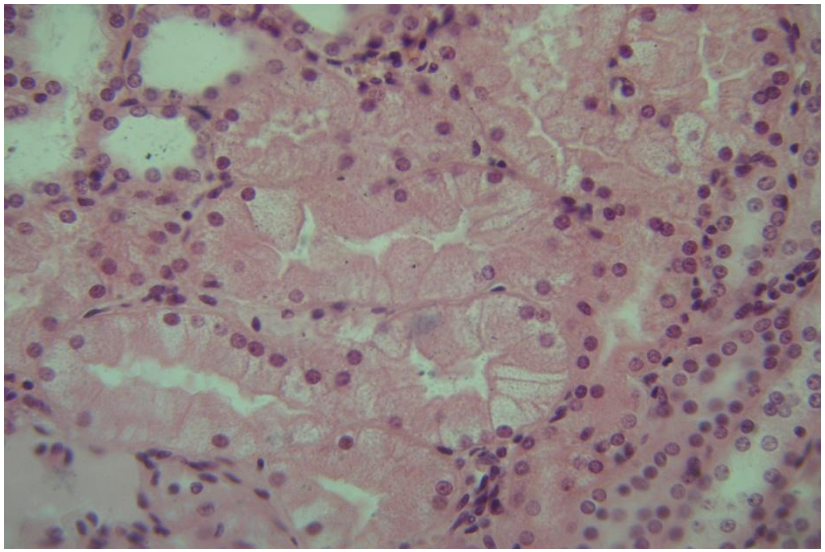
- Necrotic cells show increased eosinophilia in hematoxylin and eosin (H & E) stains, attributable in part to the loss of cytoplasmic RNA (which binds the blue dye, hematoxylin) and in part to denatured cytoplasmic proteins (which bind the red dye, eosin).

- The necrotic cell may have a more glassy homogeneous appearance than do normal cells, mainly as a result of the loss of glycogen particles
- When enzymes have digested the cytoplasmic organelles, the cytoplasm becomes vacuolated and appears moth-eaten. Dead cells may be replaced by large, whorled phospholipid masses called myelin figures that are derived from damaged cell membranes

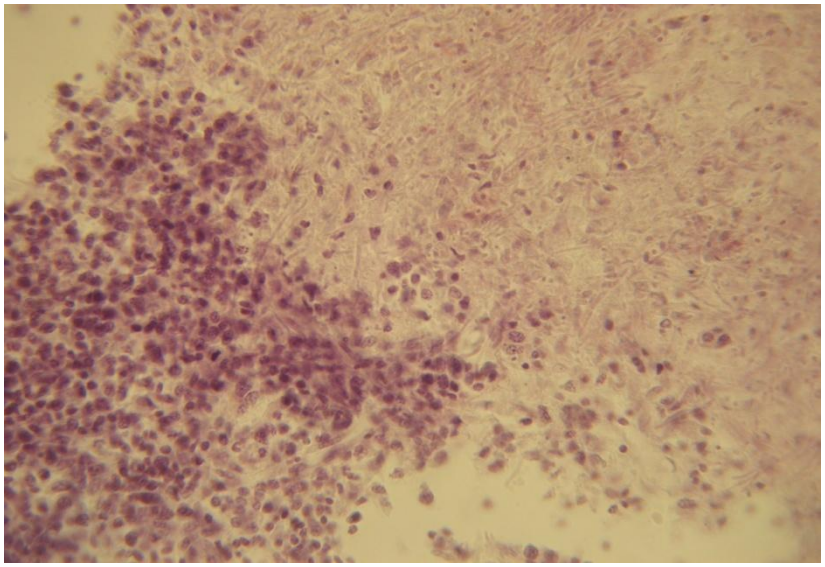
- These phospholipid precipitates are then either phagocytosed by other cells or further degraded into fatty acids; calcification of such fatty acid residues results in the generation of calcium soaps. Thus, the dead cells may ultimately become calcified.

- By electron microscopy, necrotic cells are characterized by discontinuities in plasma and organelle membranes, marked dilation of mitochondria with the appearance of large amorphous densities, intracytoplasmic myelin figures, amorphous debris, and aggregates of fluffy material probably representing denatured protein

Morphology of necrosis



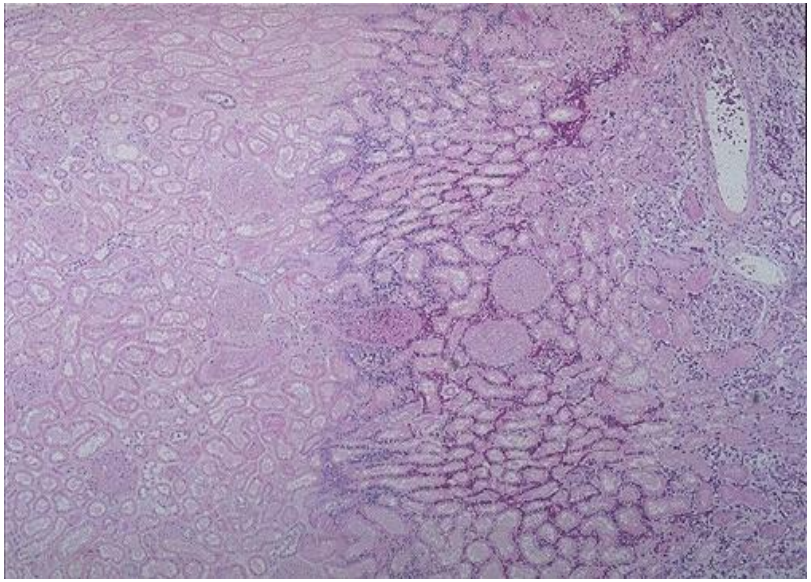
- *Kariolysis of epithelium of renal tubules*



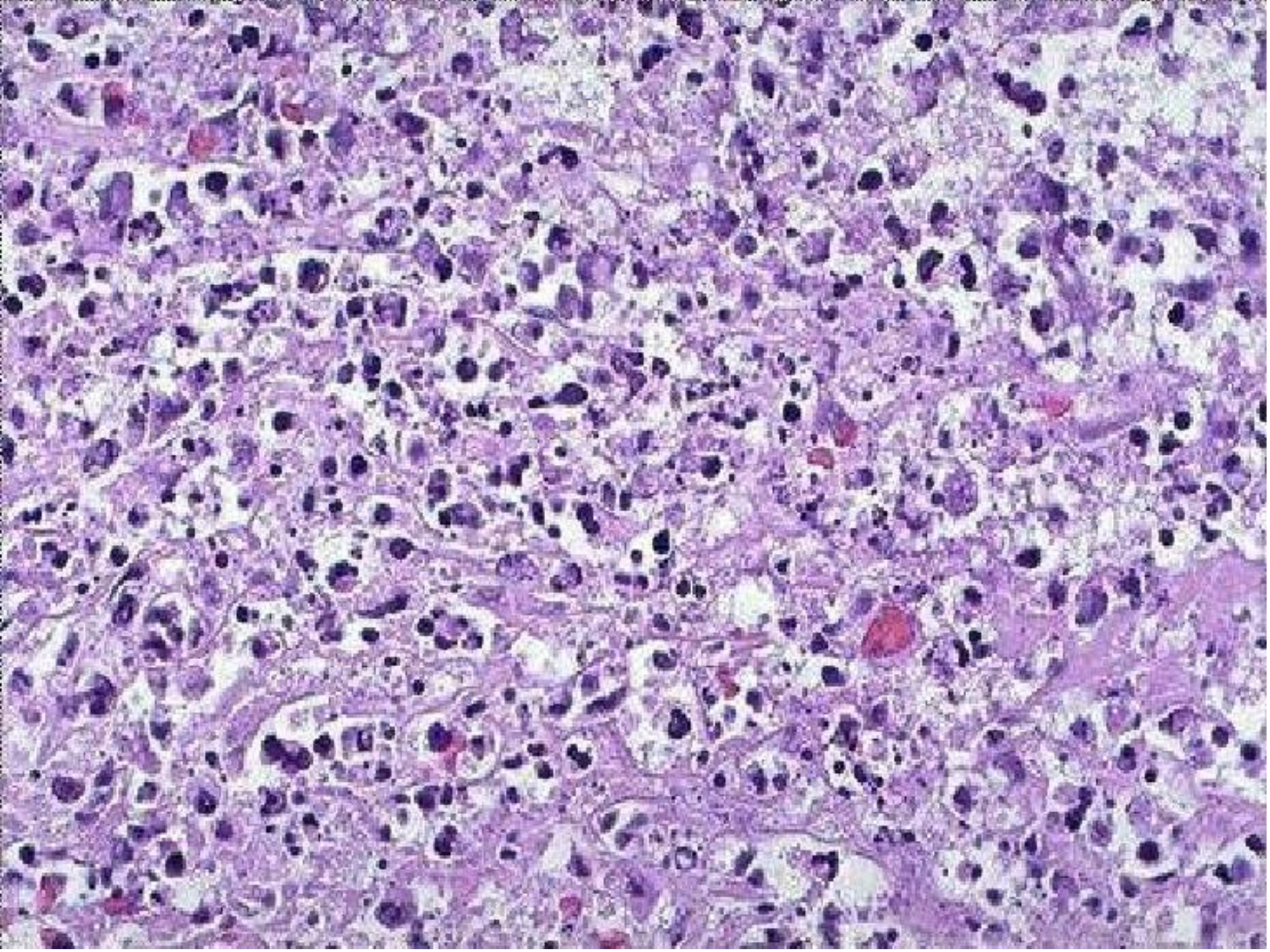
- *Necrosis of lymphoid tissue of tonsils in scarlet fever*

Morphology of necrosis

- Zone of necrosis – reactive inflammation (demarcation inflammation) – unchanged tissue
- ***Demarcation inflammation*** is characterized by red line with yellow border, adjacent directly to the necrotic tissue;



- Accumulations of leukocytes.



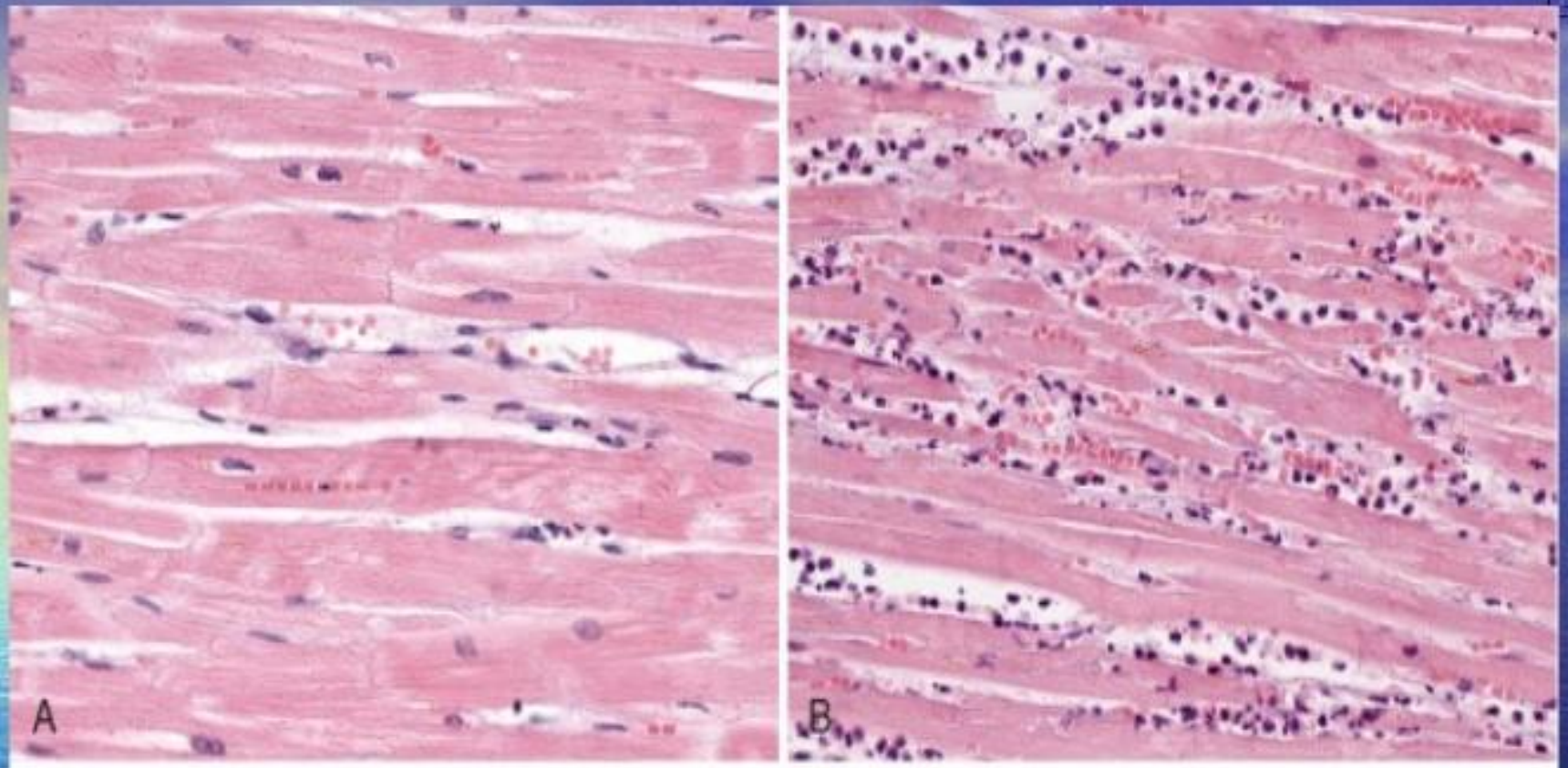


Figure 1-18 Ischemic necrosis of the myocardium. *A*, Normal myocardium. *B*, Myocardium with coagulation necrosis (upper two thirds of figure), showing strongly eosinophilic anucleate myocardial fibers. Leukocytes in the interstitium are an early reaction to necrotic muscle. Compare with *A* and with normal fibers in the lower part of the figure.

Classification of necrosis according to the cause:

- - traumatic necrosis;
- - toxic necrosis;
- - trophoneurotic necrosis;
- - allergic necrosis;
- - vascular or ischemic necrosis.

Classification of necrosis due to mechanisms of development

- **Direct necrosis** (traumatic, toxic)
- **Indirect necrosis** (trophoneurotic, allergic, vascular)

What are the types of necrosis?

- Coagulation Necrosis
- Liquefactive or Colliquative Necrosis
- Fat Necrosis
- Caseous Necrosis
- Gangrenous Necrosis
- Fibrinoid Necrosis

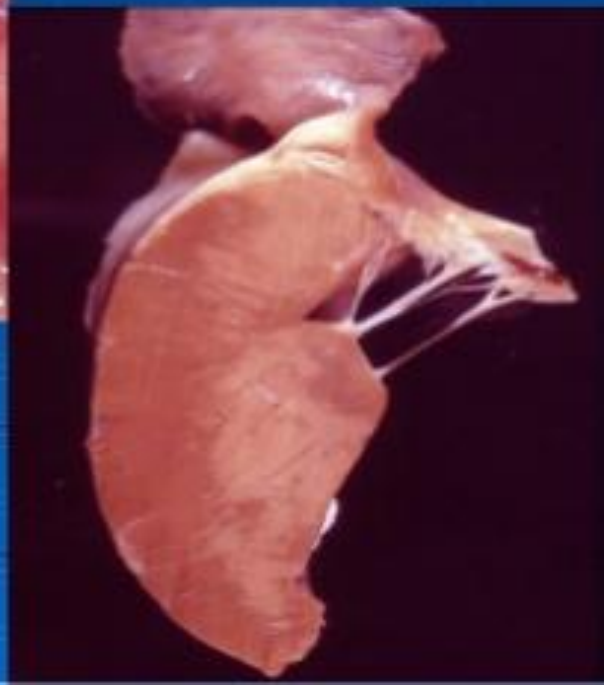
Coagulative Necrosis

- Cell's basic outline is preserved
- Homogeneous, glassy eosinophilic appearance due to loss of cytoplasmic RNA (basophilic) and glycogen (granular)
- Nucleus may show pyknosis, karyolysis or karyorrhexis

Coagulation Necrosis

Gross Appearance

- architecture resembles normal tissue, but colorant texture are different.
- lighter in color (pale) -due to coagulation of cytoplasmic proteins and decreased blood flow (eg infarcts).
- usually firm.
- tissue may be swollen or shrunken.
- may see a local vascular / inflammatory reaction to necrotic tissue.



Coagulation Necrosis

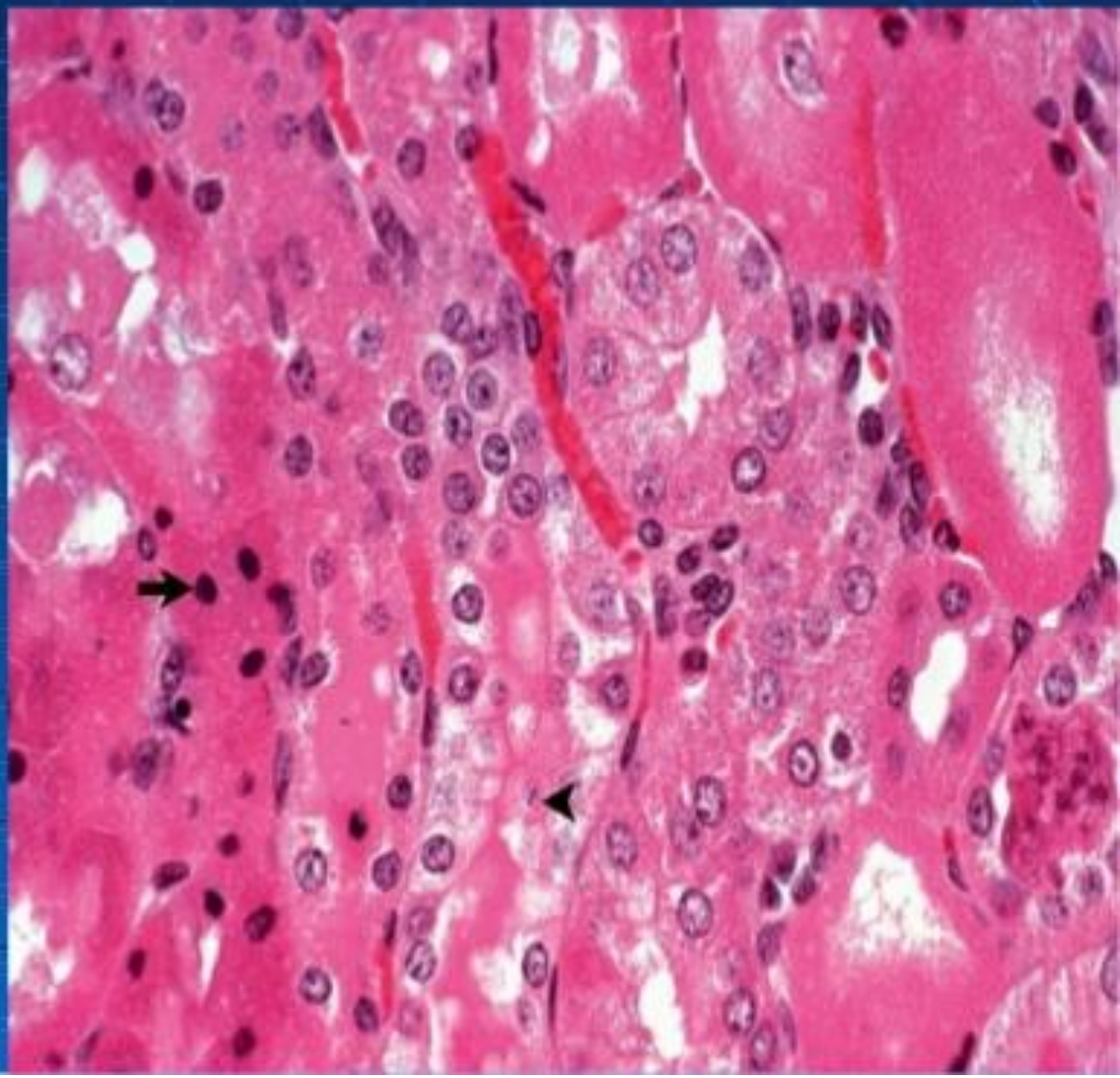
Microscopic Appearance

- original cell shape & tissue architecture is Preserve die dead cells resemble an eosinophilic "shadow" of the original cells.
- cytoplasm: increased eosinophilia (H&E stain)usually hyalinized (homogeneous glassy appearance) may be mineralized.

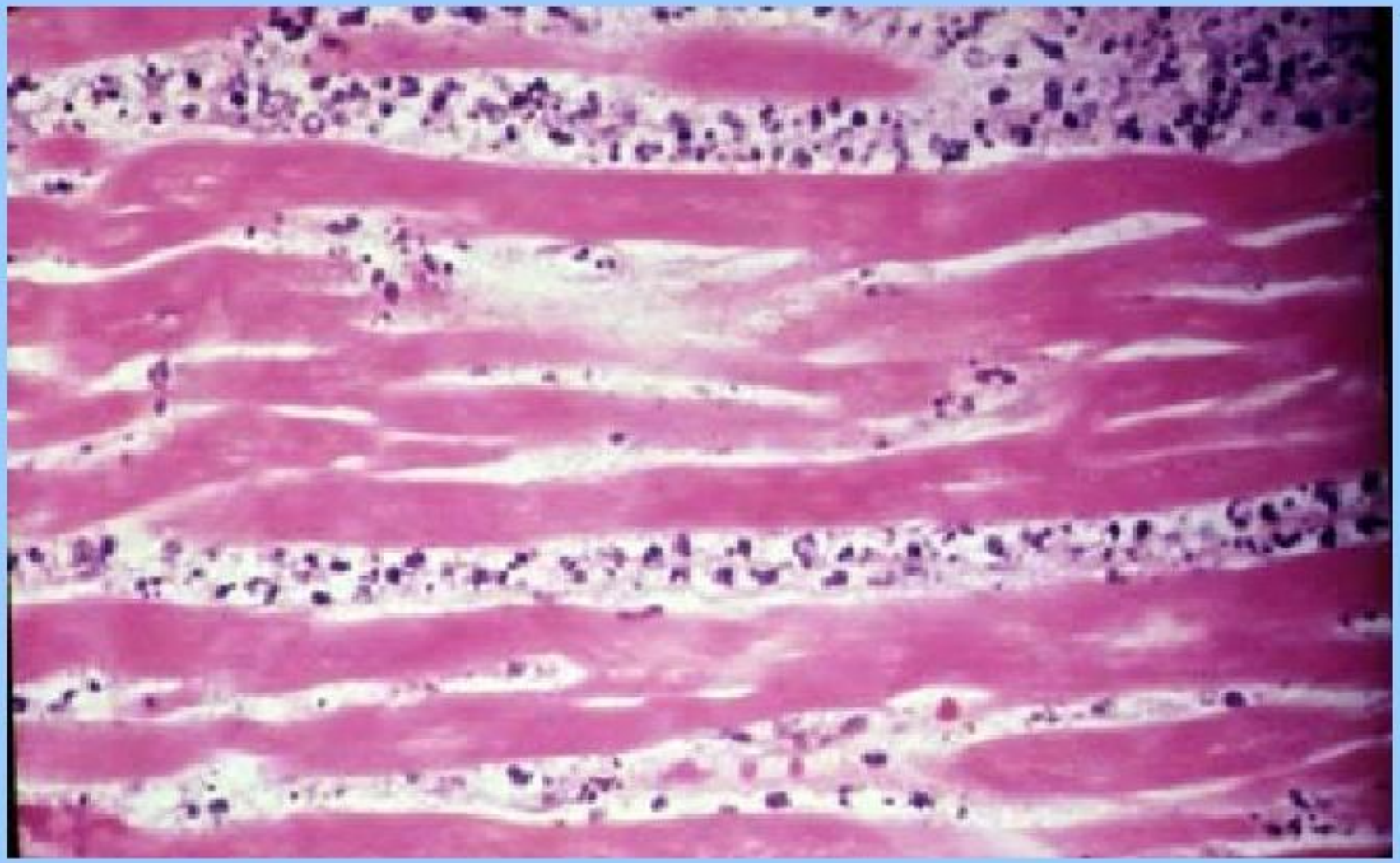
a) Coagulation Necrosis

- nucleus:

1. karyolysi
2. pyknosis
3. karyorrhexis

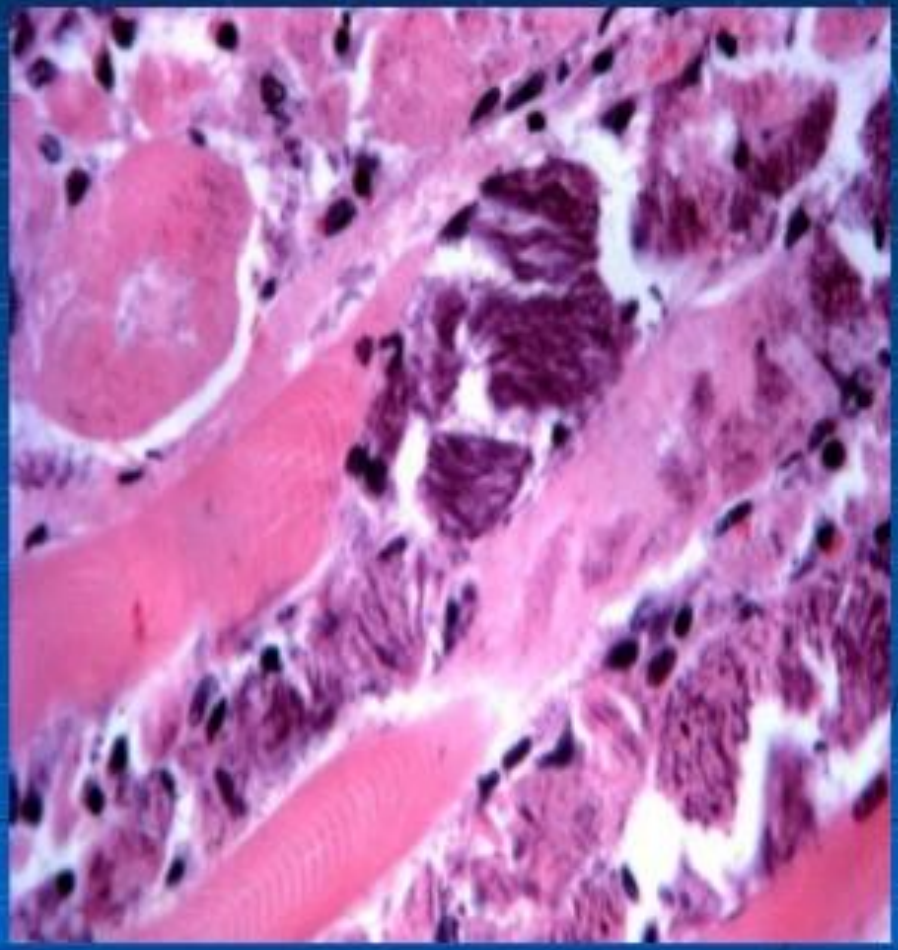


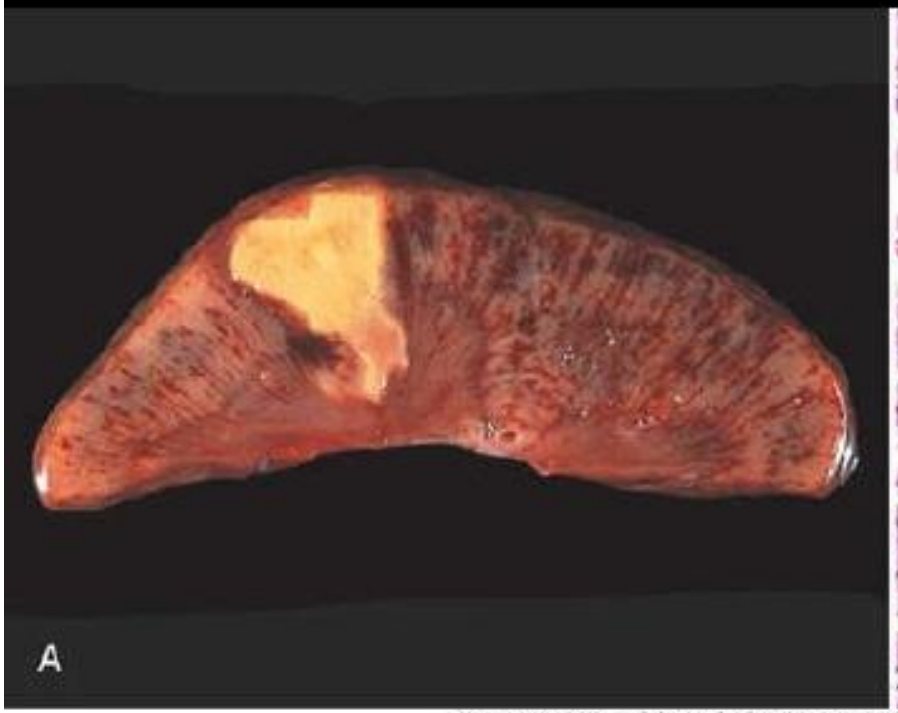
COAGULATION NECROSIS-HEART

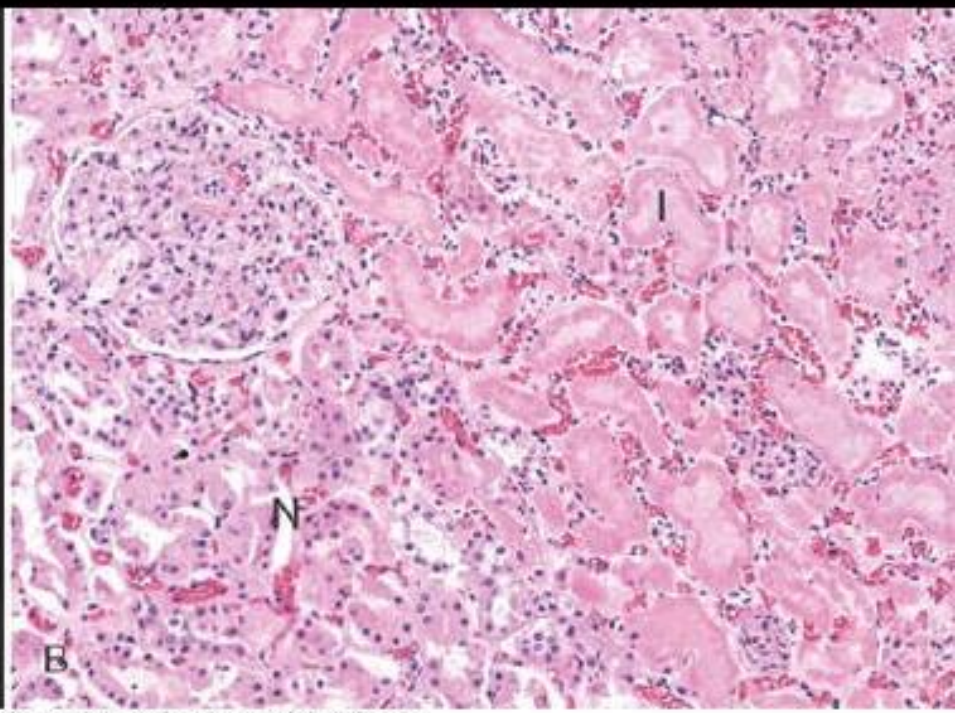


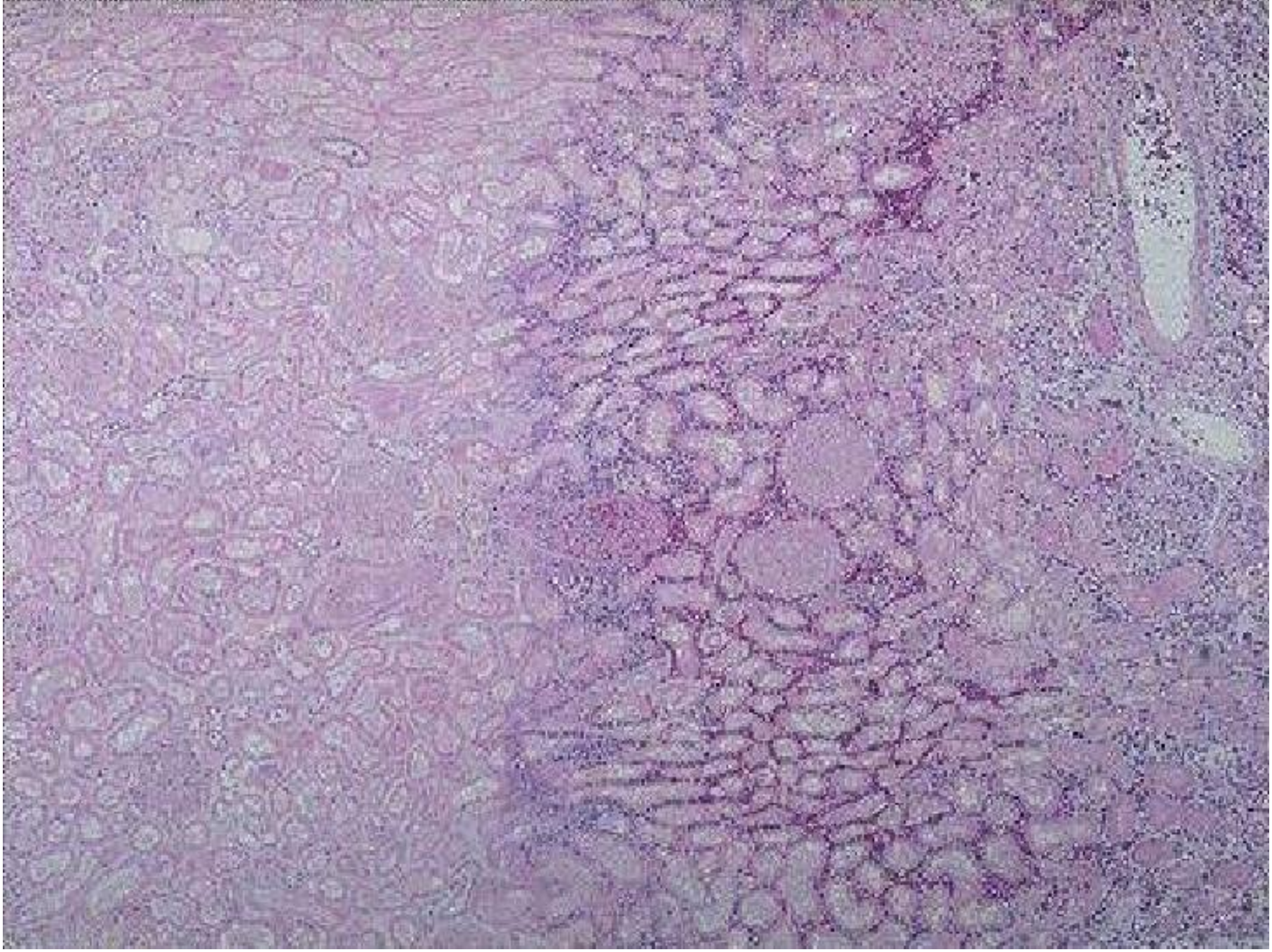
Skeletal muscle

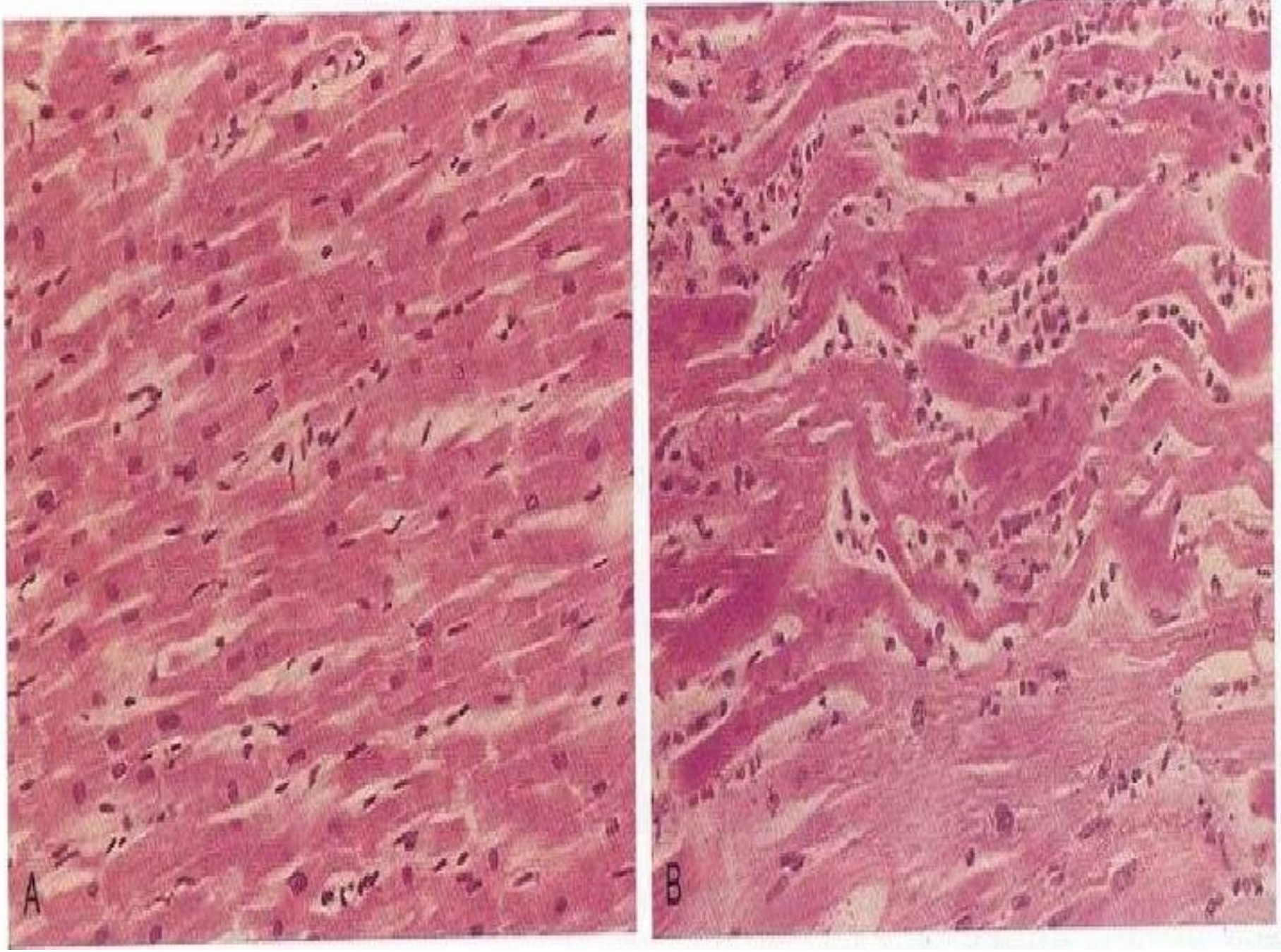
note coagulation necrosis of myofibers characterized by fragmentation and hyalinization; also note extensive mineralization (blue-purple staining)









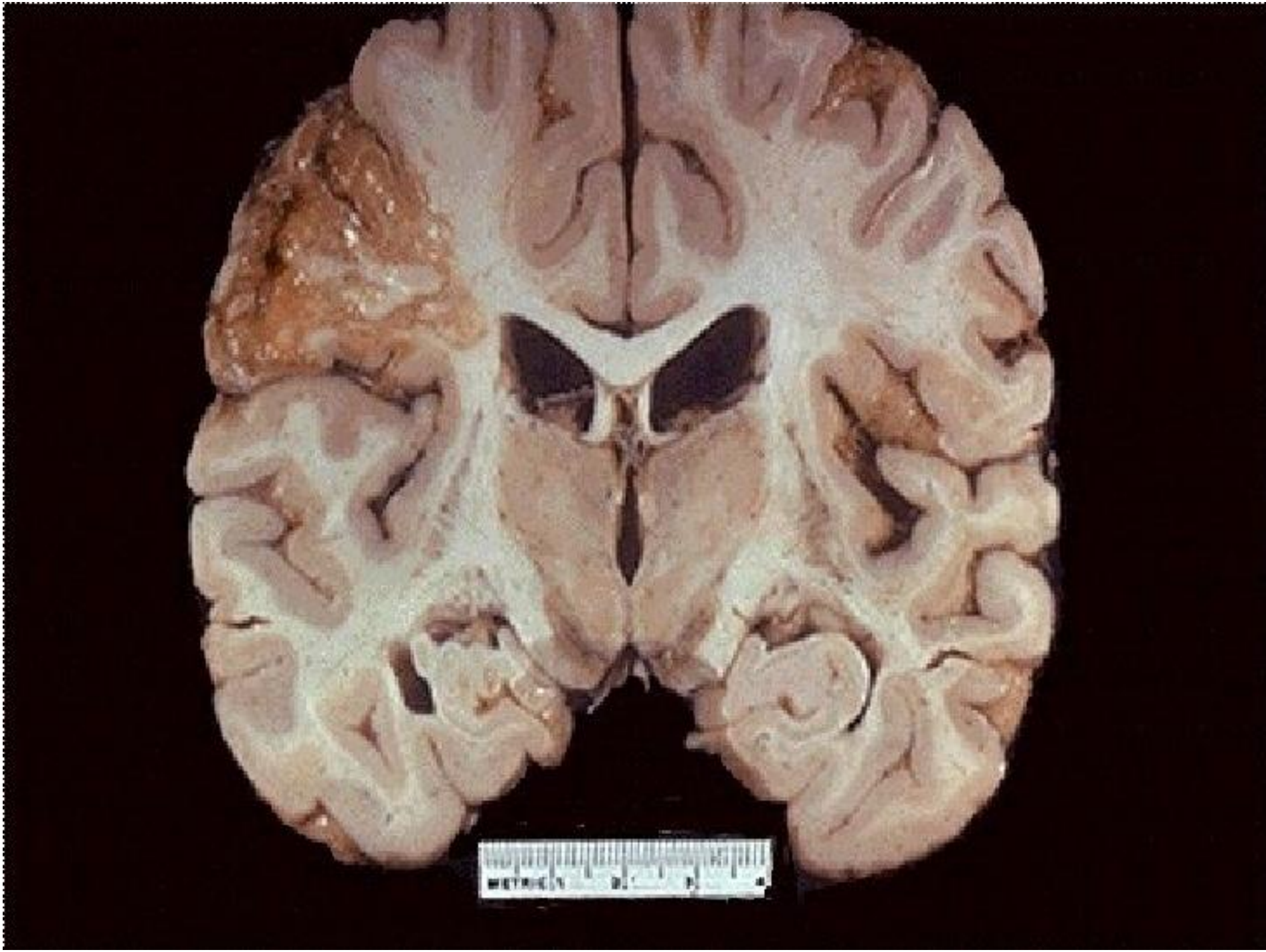

A
B

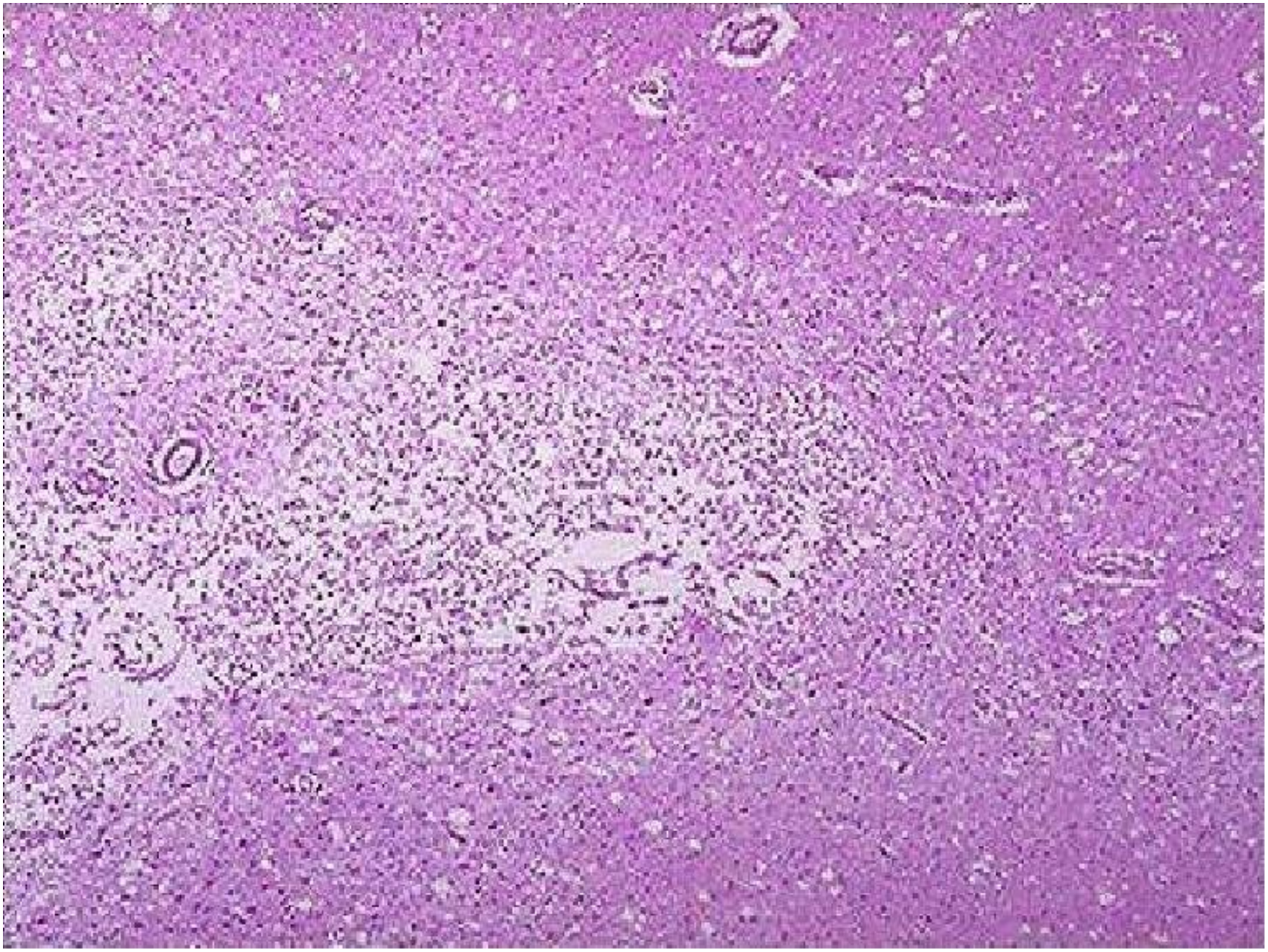
Liquefactive Necrosis

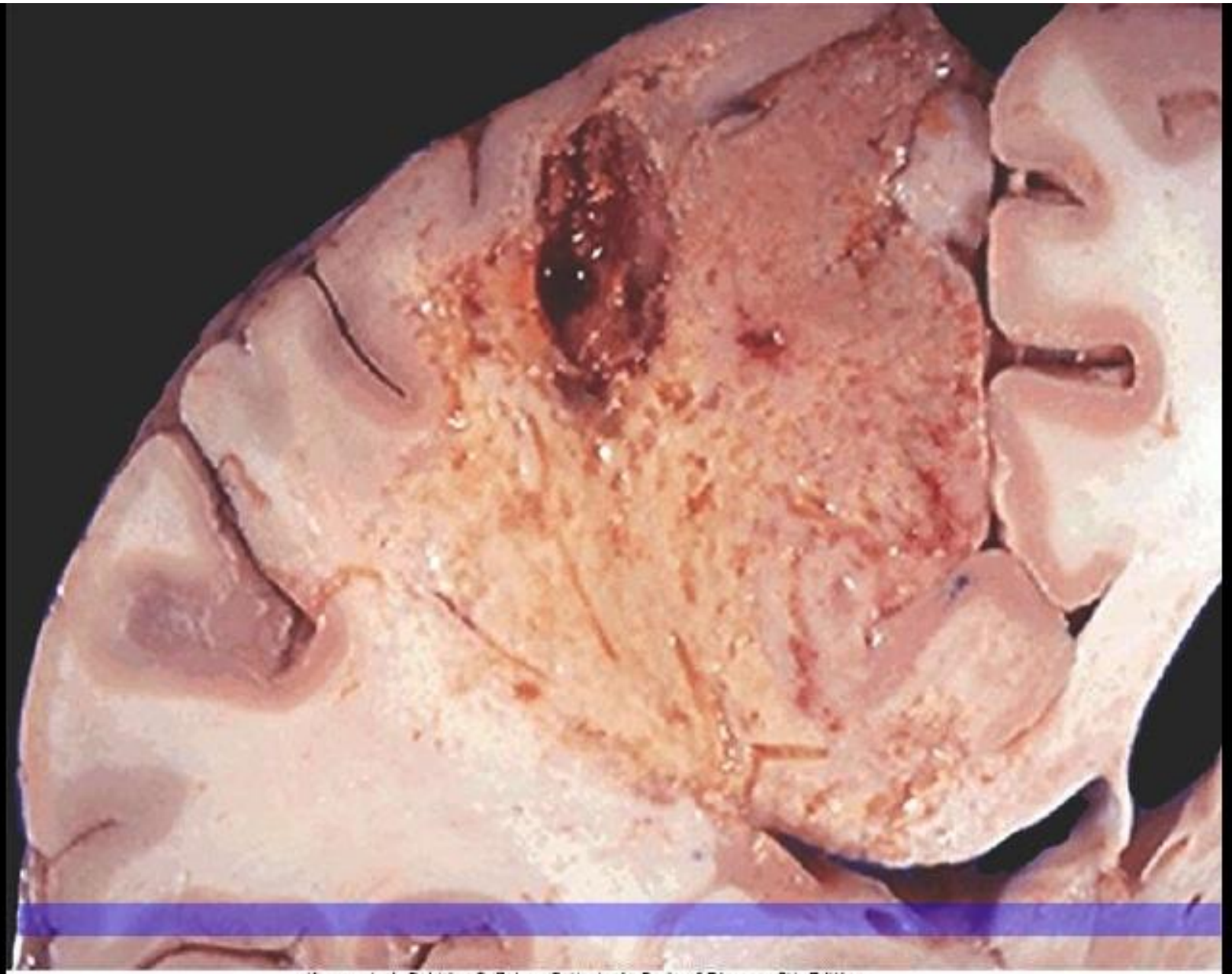
- Usually due to enzymatic dissolution of necrotic cells (usually due to release of proteolytic enzymes from neutrophils)
- Most often seen in CNS and in abscesses

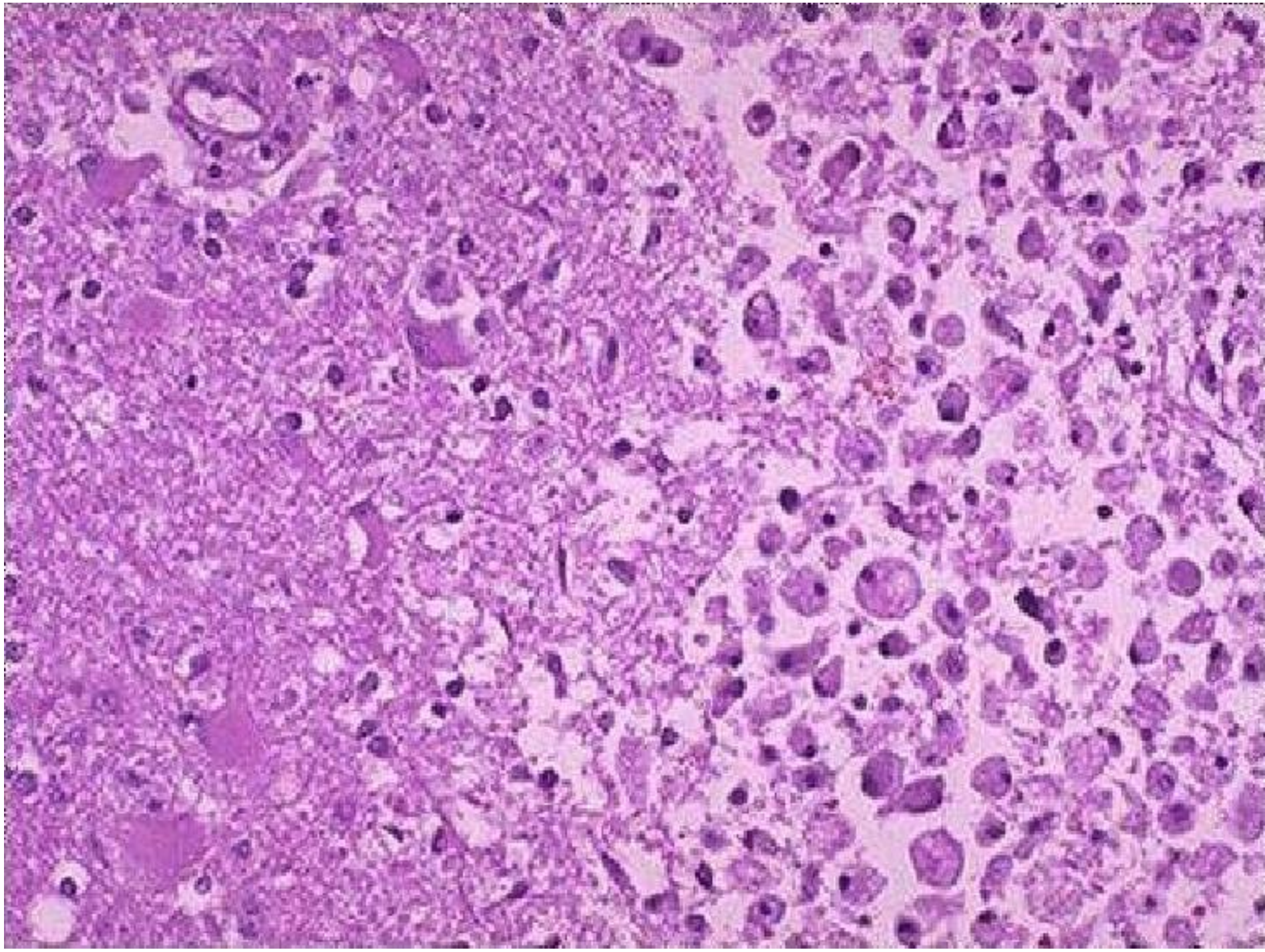
Liquefactive Necrosis

- when enzymatic digestion of necrotic cells predominates.
- esp bacterial infections; neutrophils contain potent hydrolases.
- in hypoxic damage (and other types of damage) of the CNS.
- affected tissue is liquefied to a soft, viscous, fluid mass.
- in acute inflammation, the liquid is often mostly dead WBC's (pus).
- may see degenerate neutrophils and/or amorphous necrotic material.







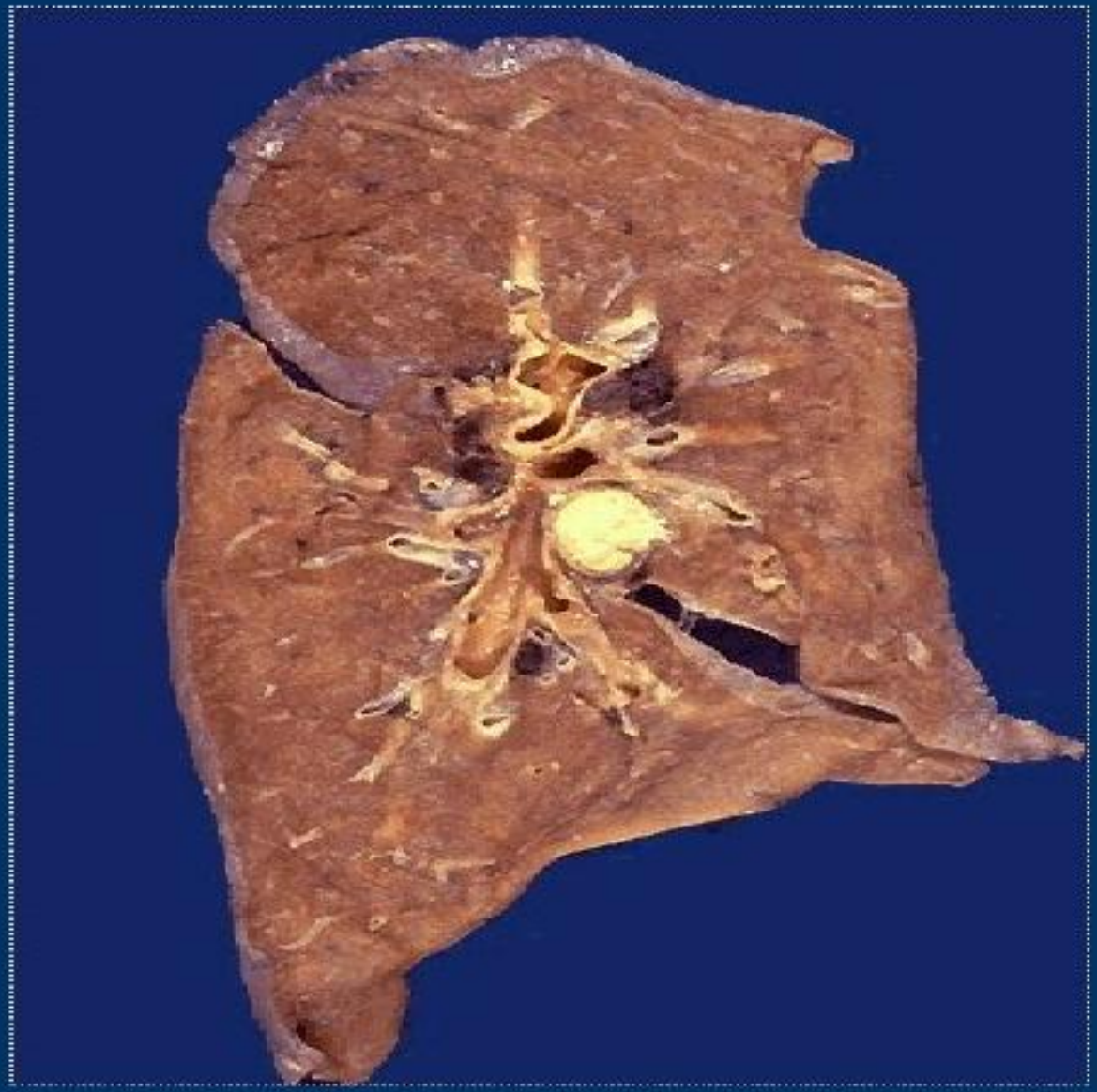


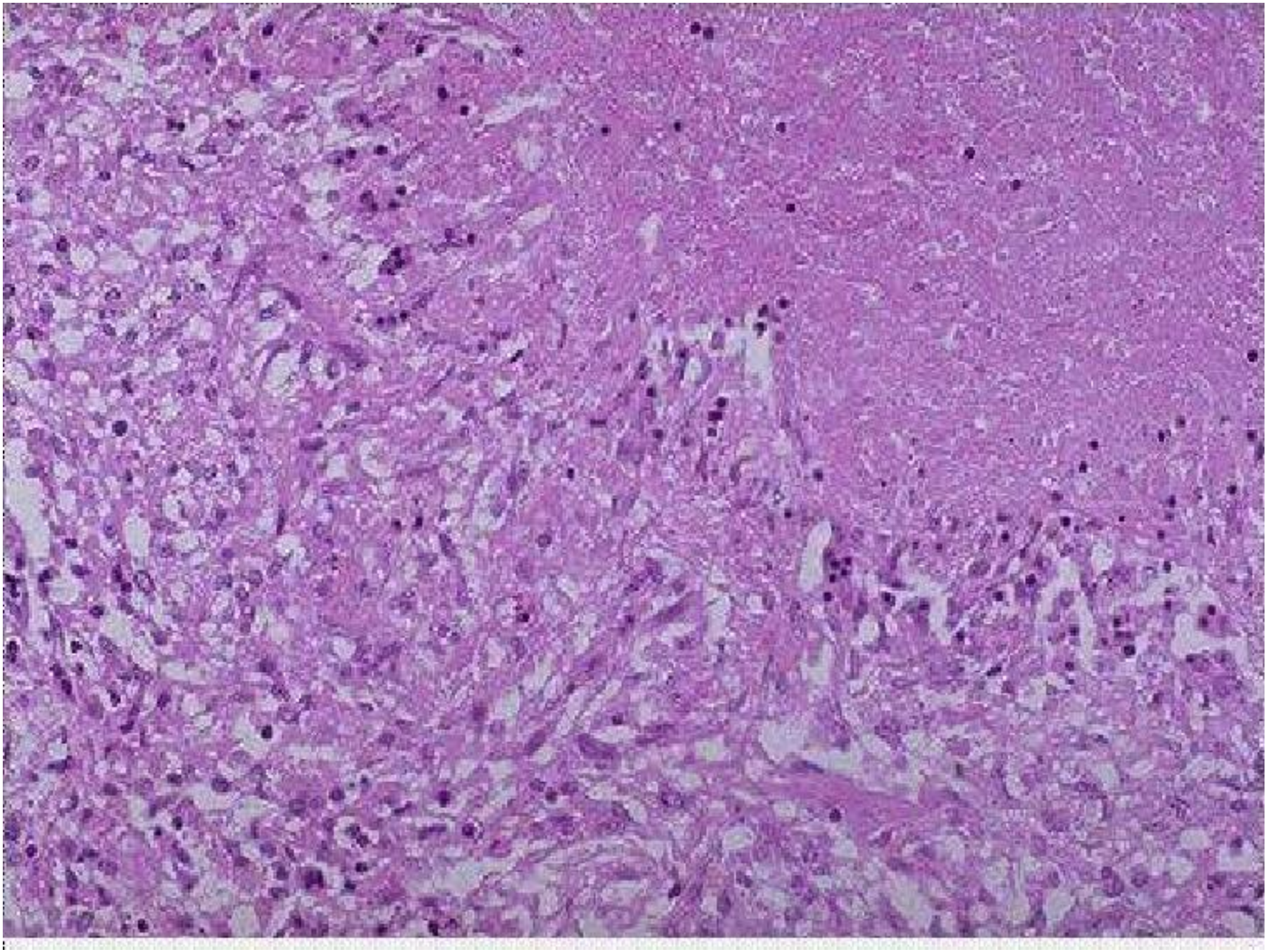
Caseous Necrosis

- Gross: Resembles cheese
- Micro: Amorphous, granular eosinophilc material surrounded by a rim of inflammatory cells
  - No visible cell outlines – tissue architecture is obliterated
- Usually seen in infections (esp. mycobacterial and fungal infections)

Caseous Necrosis

- •typical seen with specific bacterial diseases, eg TB, caseous lymphadenitis.
- Gross appearance• grey-white, dry and friable to pasty (caseous = cheese like).
- Microscopic appearance• dead cells persist as amorphous, coarsely granular, eosinophilic debris.• don't retain cellular outline but don't undergo complete dissolution either.



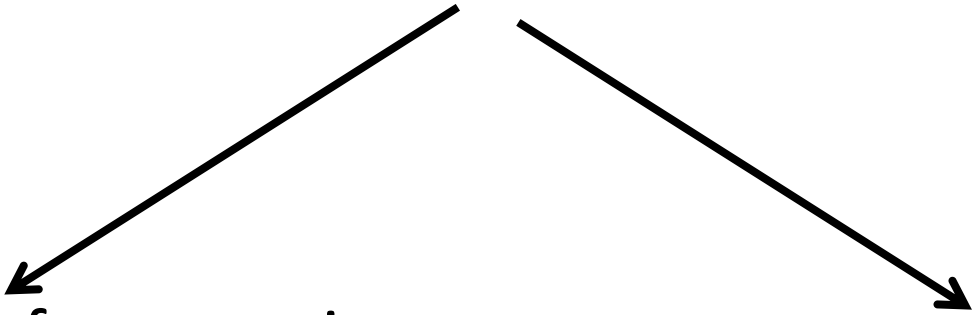


Fat Necrosis

- distinguished by its location in body fat stores.

- etiology: inflammation (eg pancreatitis), Vit E deficiency, trauma, idiopathic

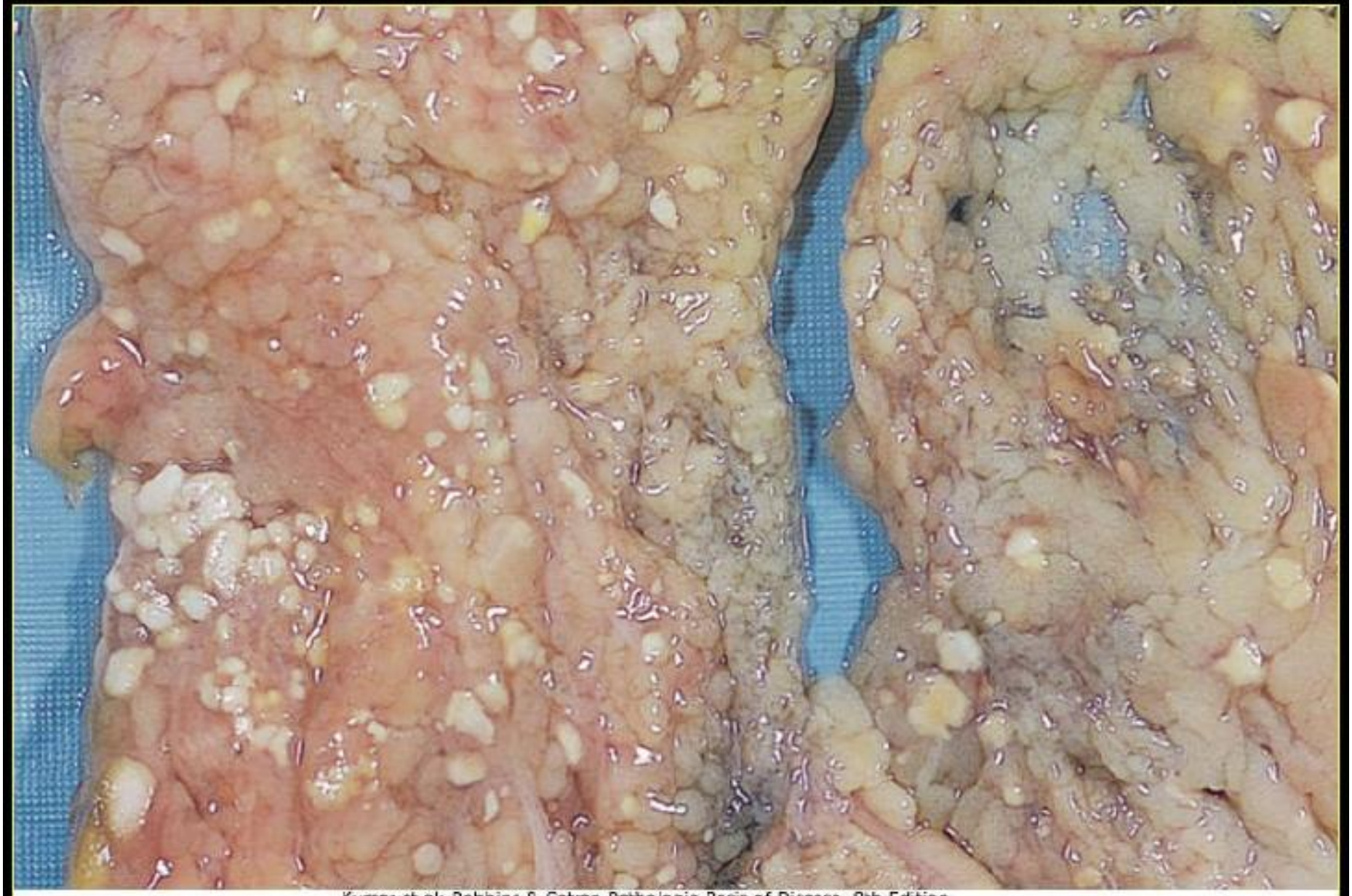
Fat necrosis



- Enzymatic fat necrosis
- Non-enzymatic fat necrosis

Enzymatic Fat Necrosis

- Results from hydrolytic action of lipases on fat
- Most often seen in and around the pancreas; can also be seen in other fatty areas of the body, usually due to trauma
- Fatty acids released via hydrolysis react with calcium to form chalky white areas → "saponification"



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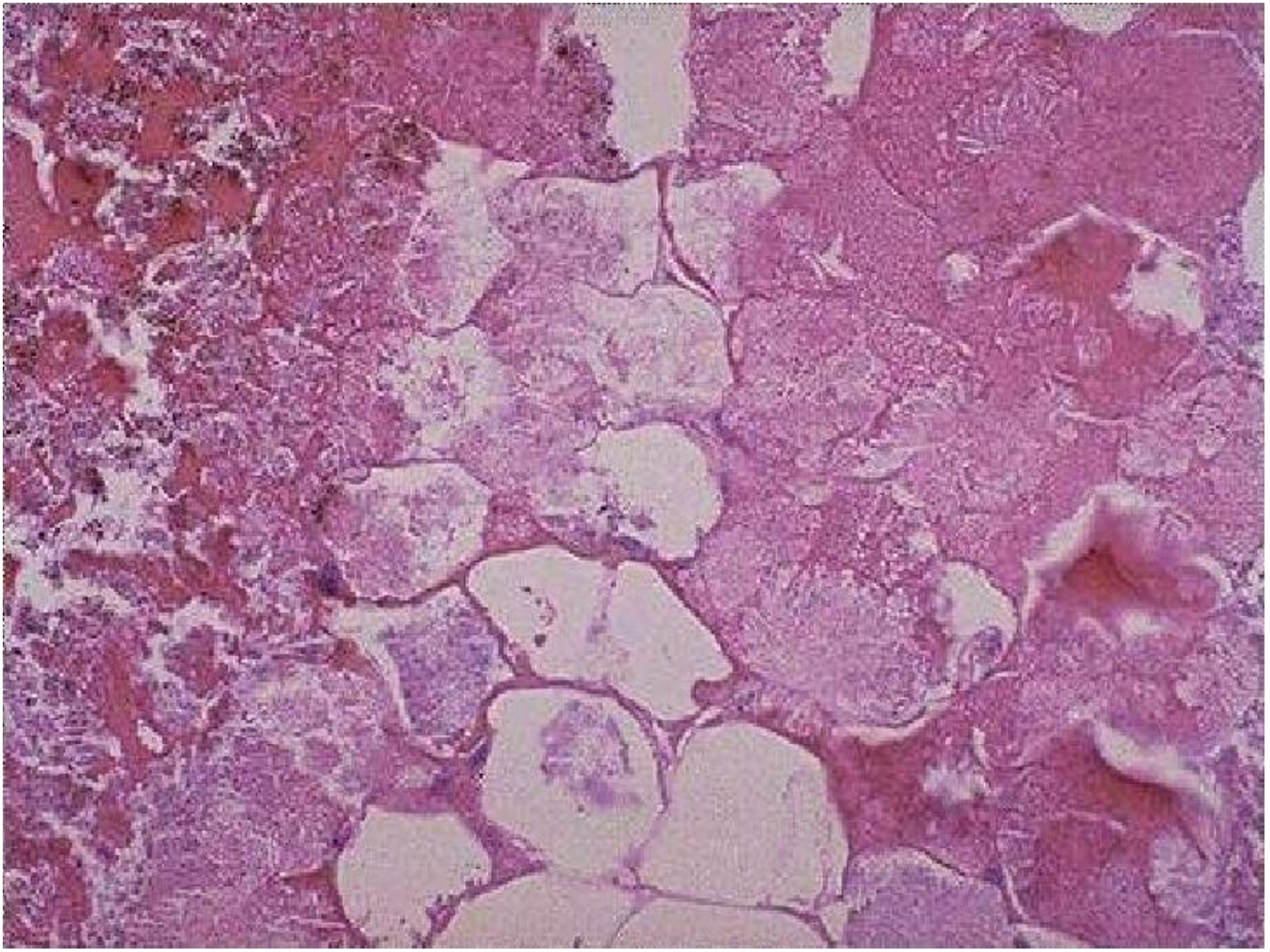

Non-enzymatic fat necrosis

- in the mammary gland, subcutaneous adipose tissue and in the abdominal cavity.

- Most patients have a history of trauma.= traumatic fatty necrosis (even if trauma is not identified as the underlying cause).

- elicits an inflammatory response characterized by the presence of numerous macrophages with foamy cytoplasm, neutrophils and lymphocytes.

- Then - fibrosis, while this process can be difficult to distinguish from a tumor.

Gangrenous Necrosis

- Most often seen on extremities, usually due to trauma or physical injury
- “Dry” gangrene – no bacterial superinfection; tissue appears dry
- “Wet” gangrene – bacterial superinfection has occurred; tissue looks wet and liquefactive

Gangrenous Necrosis

- definition= necrosis (usually ischemic) of extremities, eg digits, ear tips.
- dry gangrene= coagulation necrosis of an extremity.
- wet gangrene= when the coagulative necrosis of dry gangrene is modified by liquefactive action of saprophytic/putrefactive bacteria.


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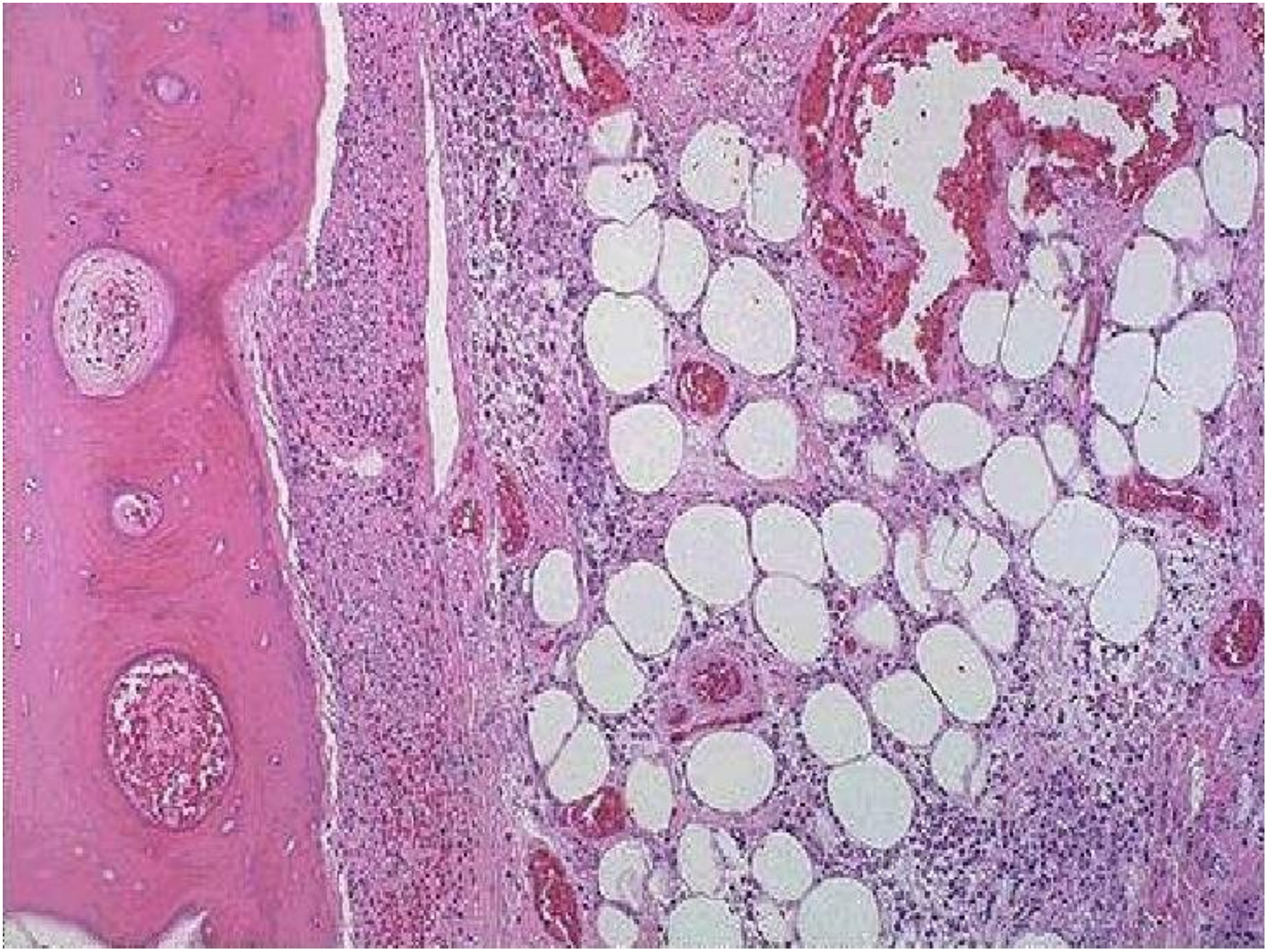
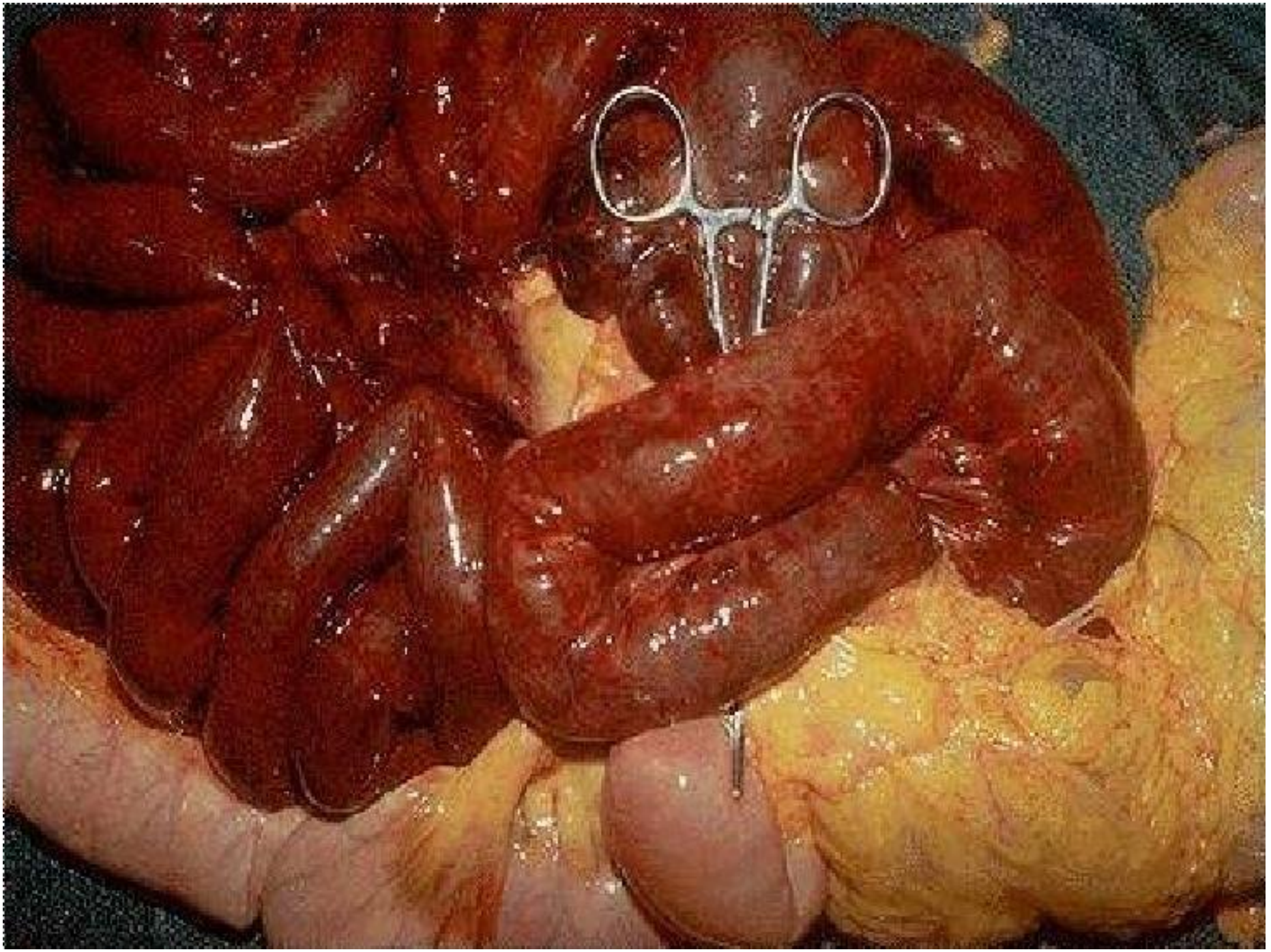


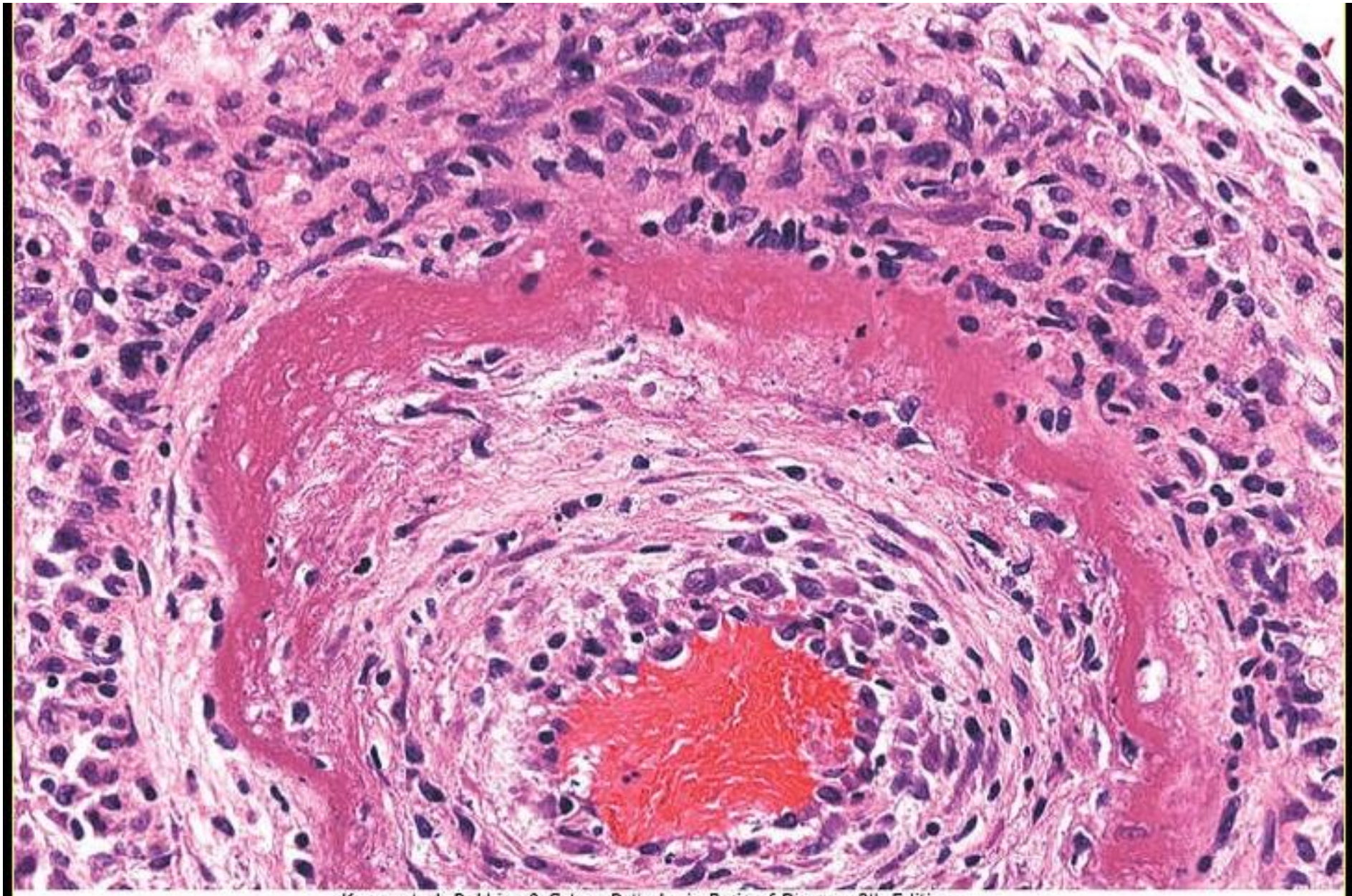


Fibrinoid necrosis

- is a special form of necrosis usually seen in immune reactions involving blood vessels. This pattern of necrosis typically occurs when complexes of antigens and antibodies are deposited in the walls of arteries.

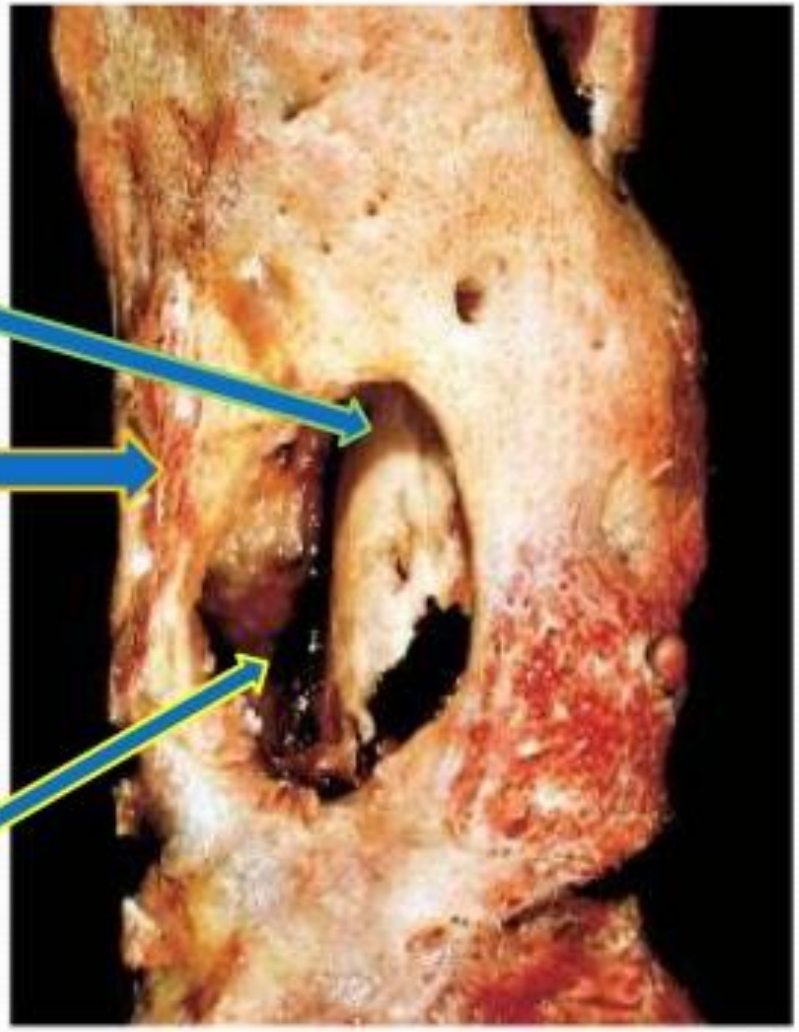
Fibrinoid Necrosis

- Usually seen in the walls of blood vessels (e.g., in vasculitides)
- Glassy, eosinophilic fibrin-like material is deposited within the vascular walls

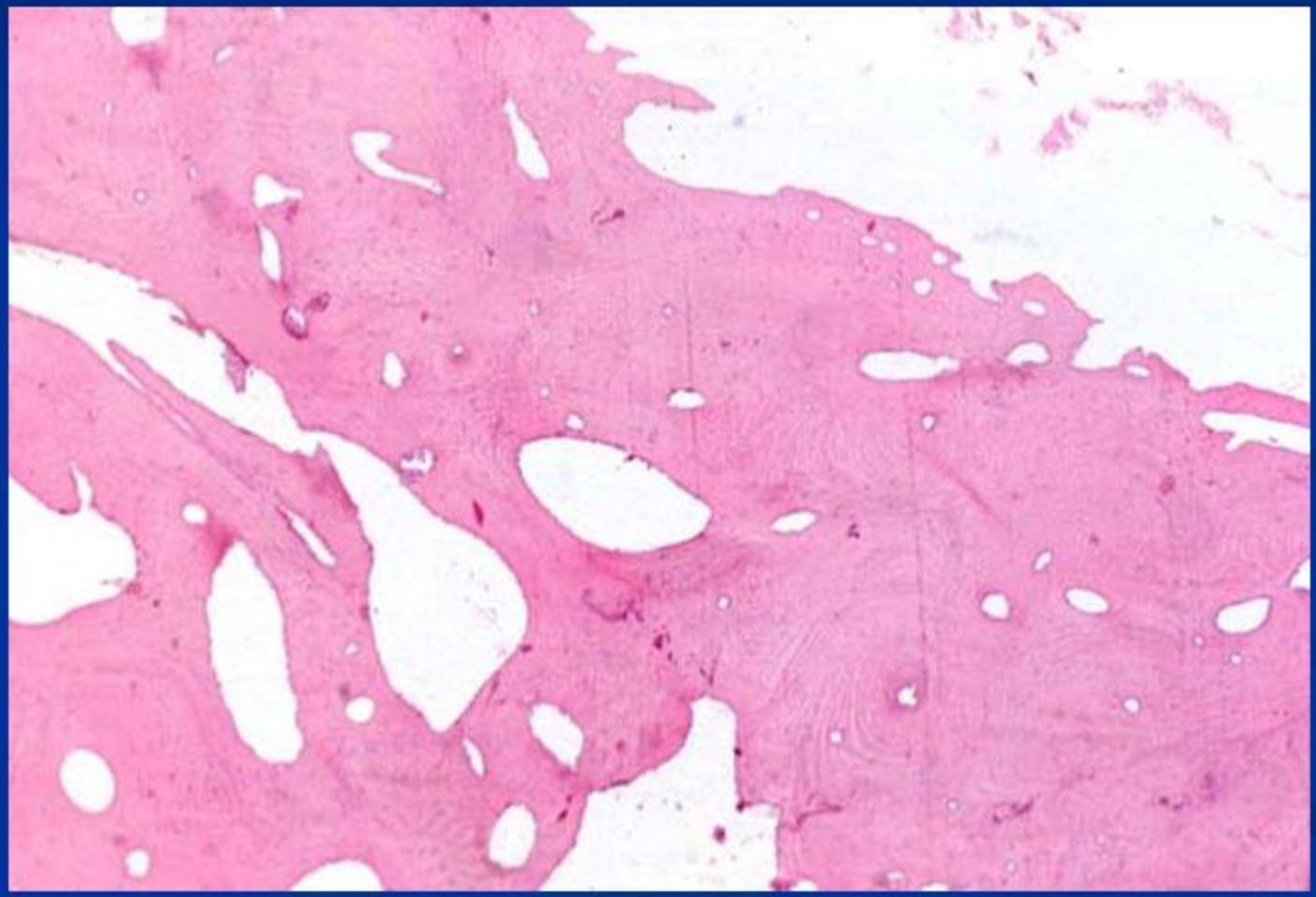


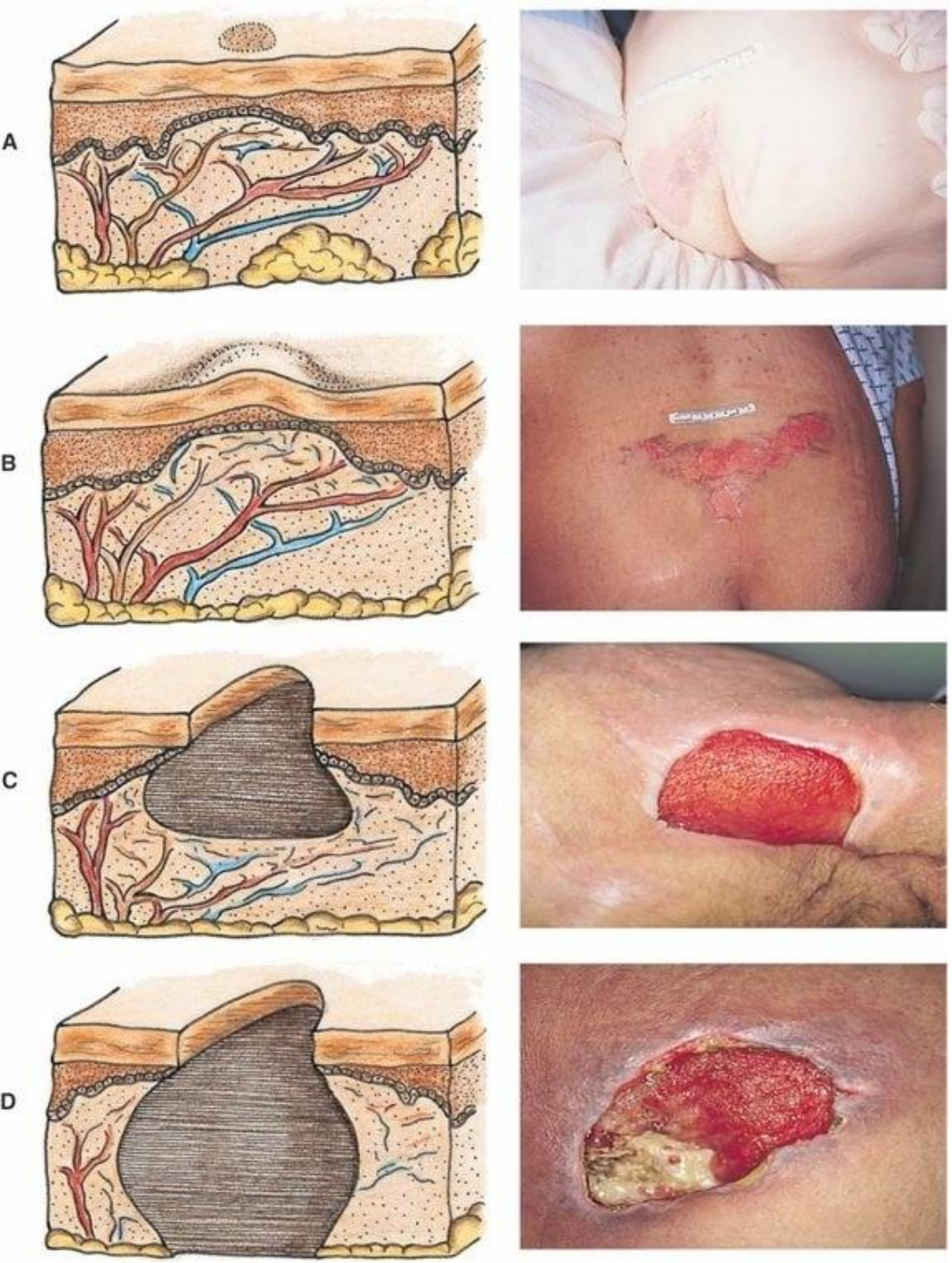
CHRONIC OSTEOMYELITIS

- **Sequestrum** is the necrotic bone that is embedded in the pus/infected granulation tissue.
- **Involucrum** is the new bone laid down by the periosteum that surrounds the sequestra.
- **Cloaca** is the opening in the involucrum through which pus & sequestra make their way out.



Sequestrum (necrotic bone)





Decubitus

Infarction

- This is necrosis resulting from the cessation of blood supply to a site of tissue or organ.

- A heart attack is also called circulatory necrosis.

Types of infarction

1. ***white (ischemic) infarction***,
2. ***red (hemorrhagic) infarction***,
3. ***white (ischemic) infarction with a hemorrhagic rim (corolla).***

- The difference in the morphology of the types of infarction is due to the unequal mechanisms of their development.
- Macroscopically infarction of any type can be either conical or irregular.
- A conical shape is usually a heart attack that develops in the pool of arteries with a main branching type,
- irregular shape - with loose type.

White (ischemic) infarction

- his type of infarction occurs in the spleen, liver.
- Its development is preceded by the ischemic stage.
- Formed ischemic infarction becomes visible to the naked eye after about 1 day.
- ***Microscopically*** in the infarction zone: necrosis is more often coagulative, less often colliquation type (brain).
- On the periphery, the zone of necrosis is limited by the inflammatory demarcation shaft.

White (ischemic) infarction

- *Infarction of the spleen*

Red (hemorrhagic) infarction

- the area of necrosis is saturated with blood. due to which the site of infarction acquires a dark red color.

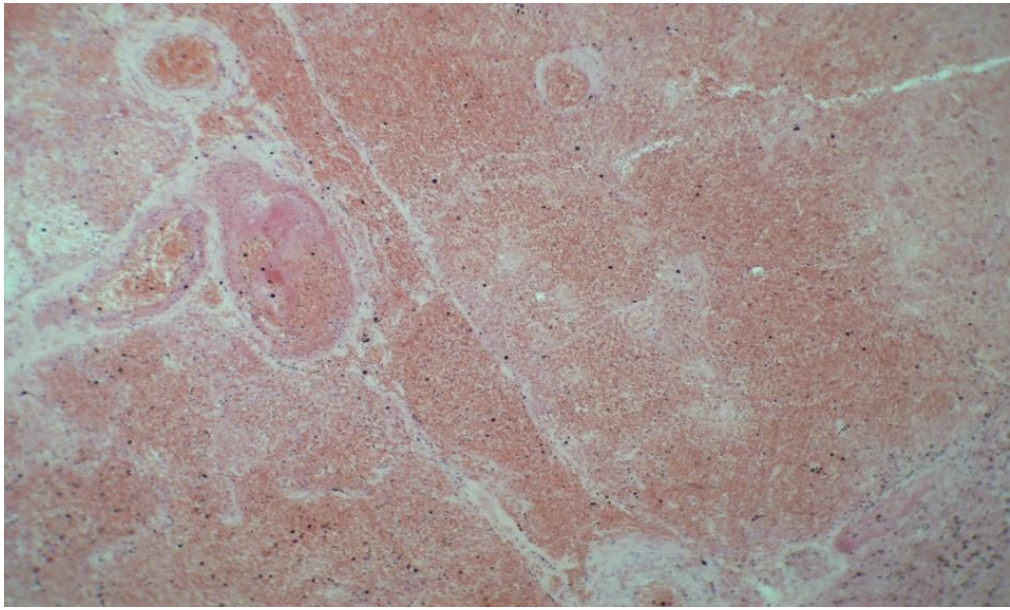
- more often in the lung, brain, intestines.

- Conditions contributing to its development: venous stasis and double blood supply to the organ (from different vascular systems).

- ***Microscopically:*** masses of agglutinated and hemolyzed erythrocytes replacing the destroyed structures of the organ.
- A feature of the perifocal reaction is the presence of a large number of siderophages and hemosiderin lumps.

Red (hemorrhagic) infarction

- ***hemorrhagic infarction of the lung***

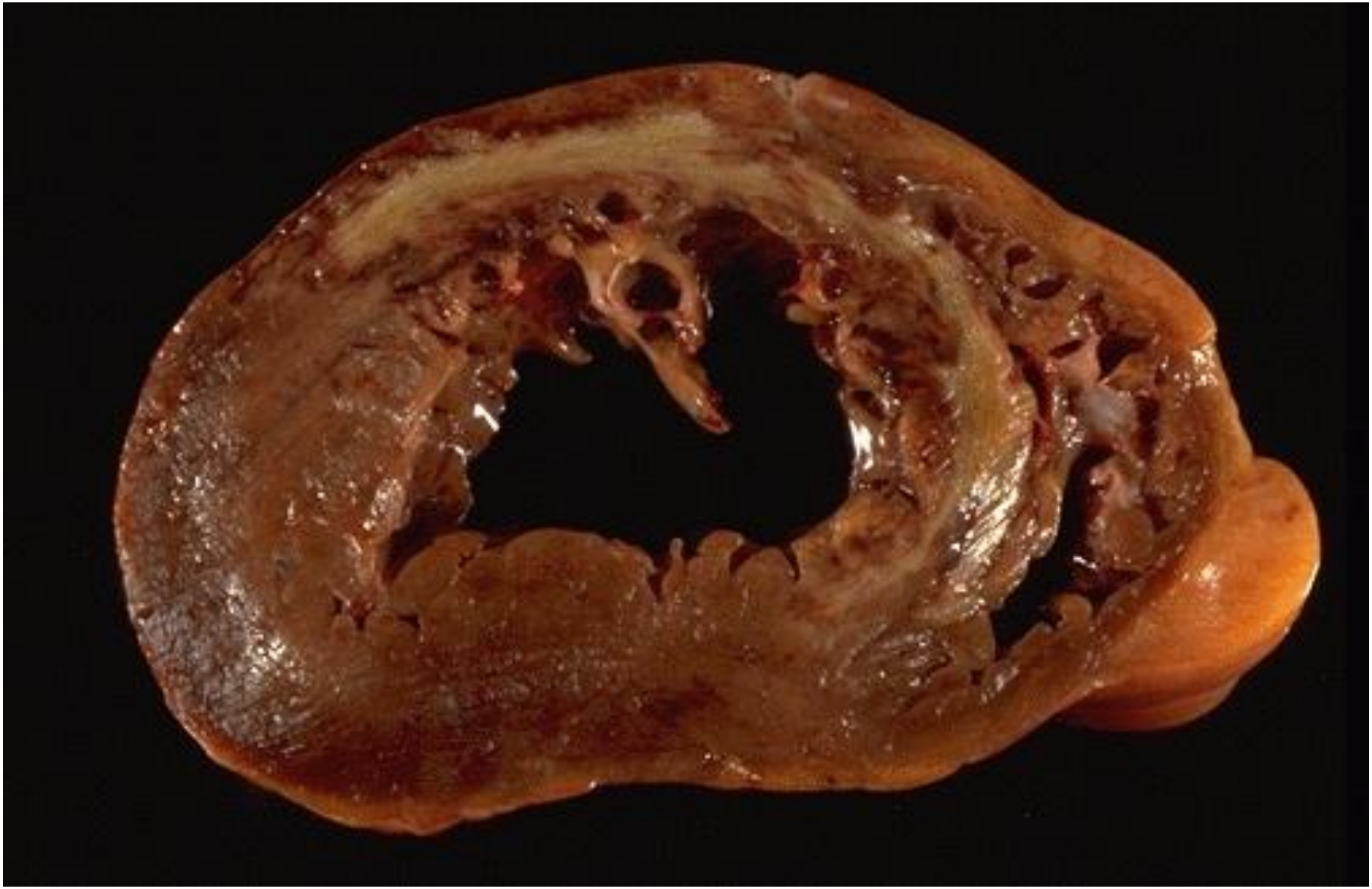


White (ischemic) infarction with a hemorrhagic rim (corolla)

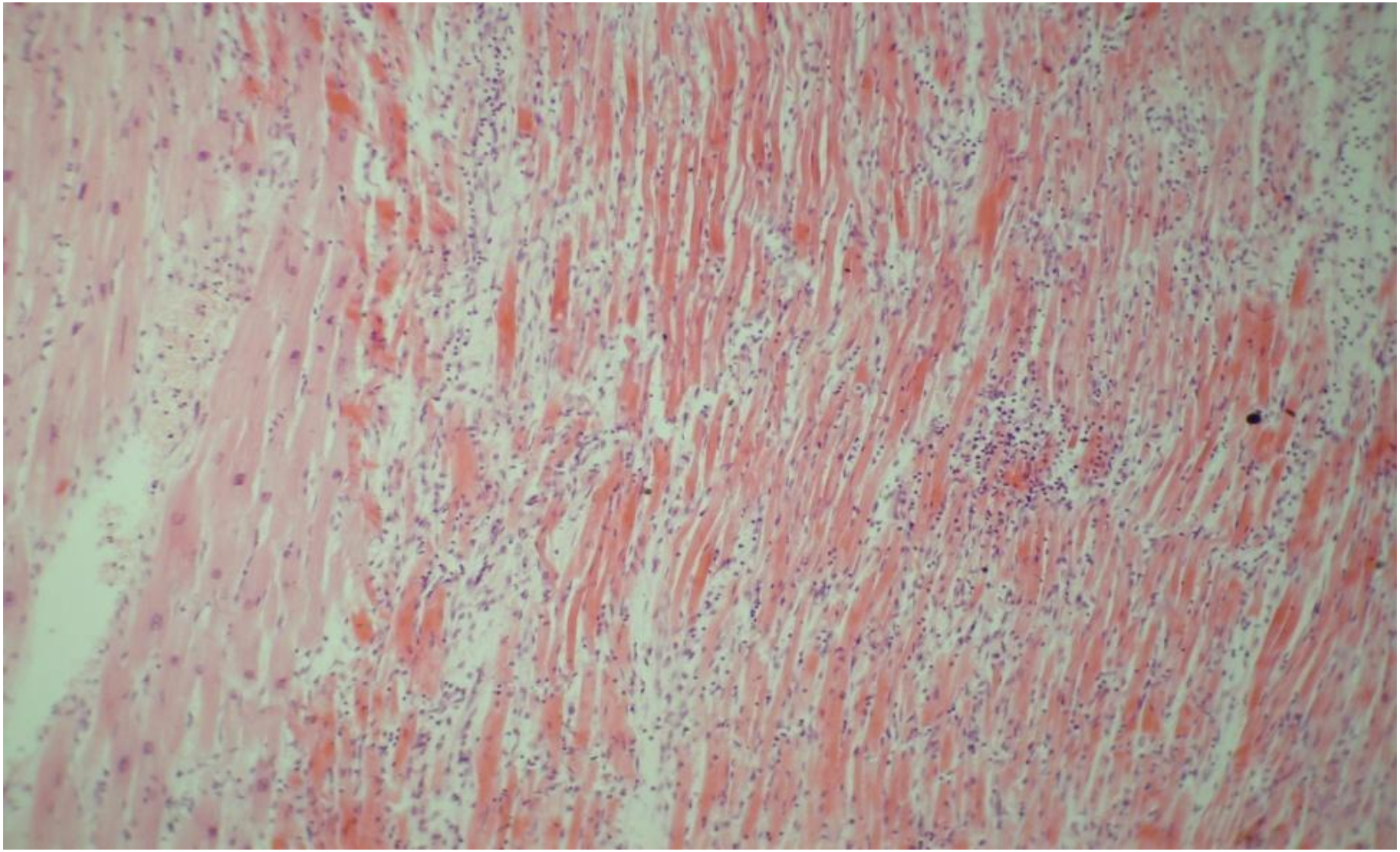
- develops in cases when, during the formation of ischemic necrosis, collaterals and vessels of the marginal zone of the infarction turn on with a delay after their prolonged spasm.

- in the vessels of the marginal zone - paralytic expansion.
- sharp plethora, stasis + outpouring of blood into necrotic tissue.
- ***This type of infarction is a combination of red and white infarction:***
- in the center - white type infarction,
- on the periphery - red.
- often in the heart and kidneys.

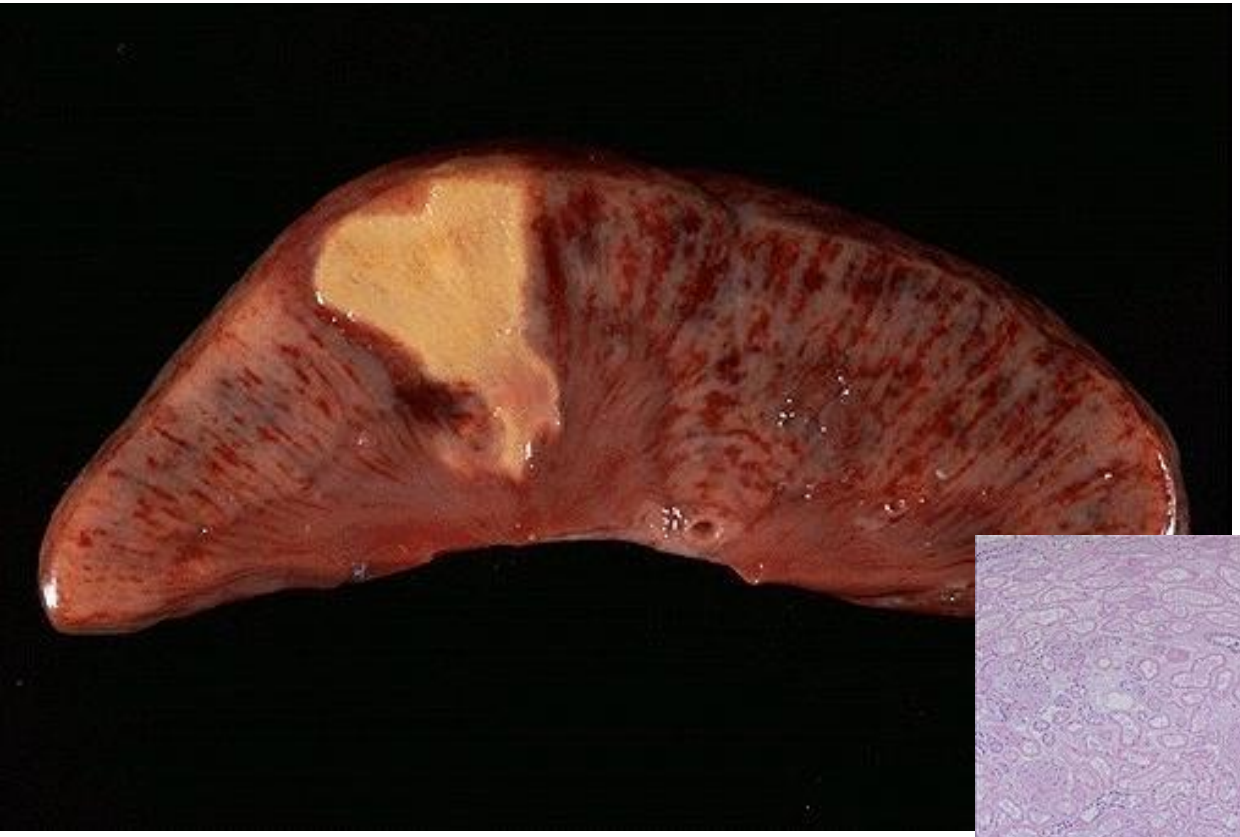
White (ischemic) infarction with a hemorrhagic rim (corolla):myocardial infarction



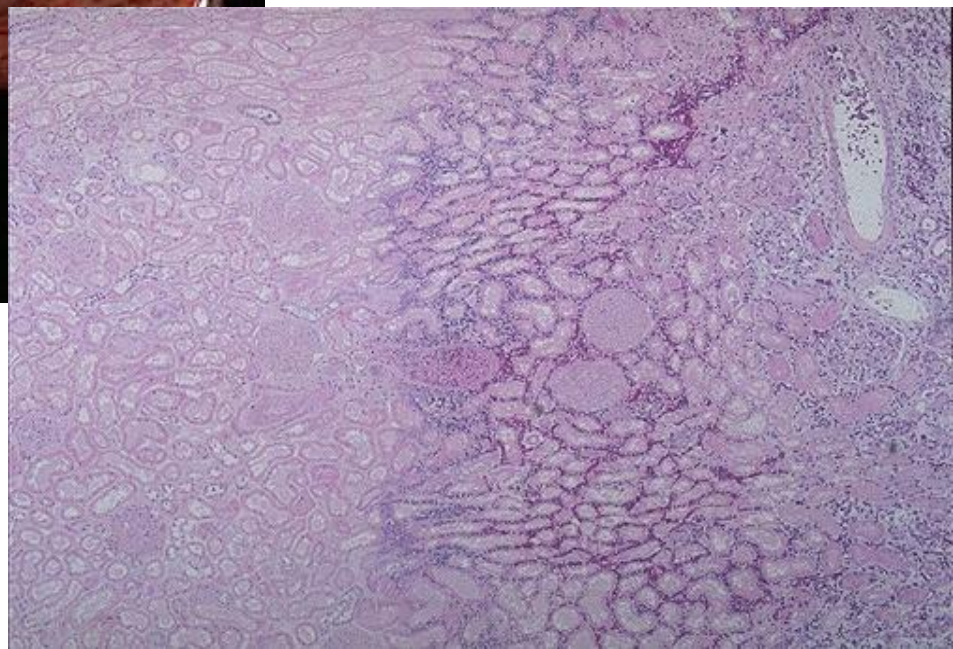
White (ischemic) infarction with a hemorrhagic rim (corolla):myocardial infarction



White (ischemic) infarction with a hemorrhagic rim (corolla)



- *Infarction of the kidney*



Evolution of infarction

- In the course of the evolution of infarction, following the formation of necrosis, there is a stage of reparative changes:
- First, a perifocal inflammatory response.
- Microscopically, this reaction is already in a few hours; maximum - after 3-5 days.
- the influence of proteolytic enzymes of neutrophilic leukocytes - lysis of necrotic masses, their resorption by lymphatic drainage and phagocytosis.

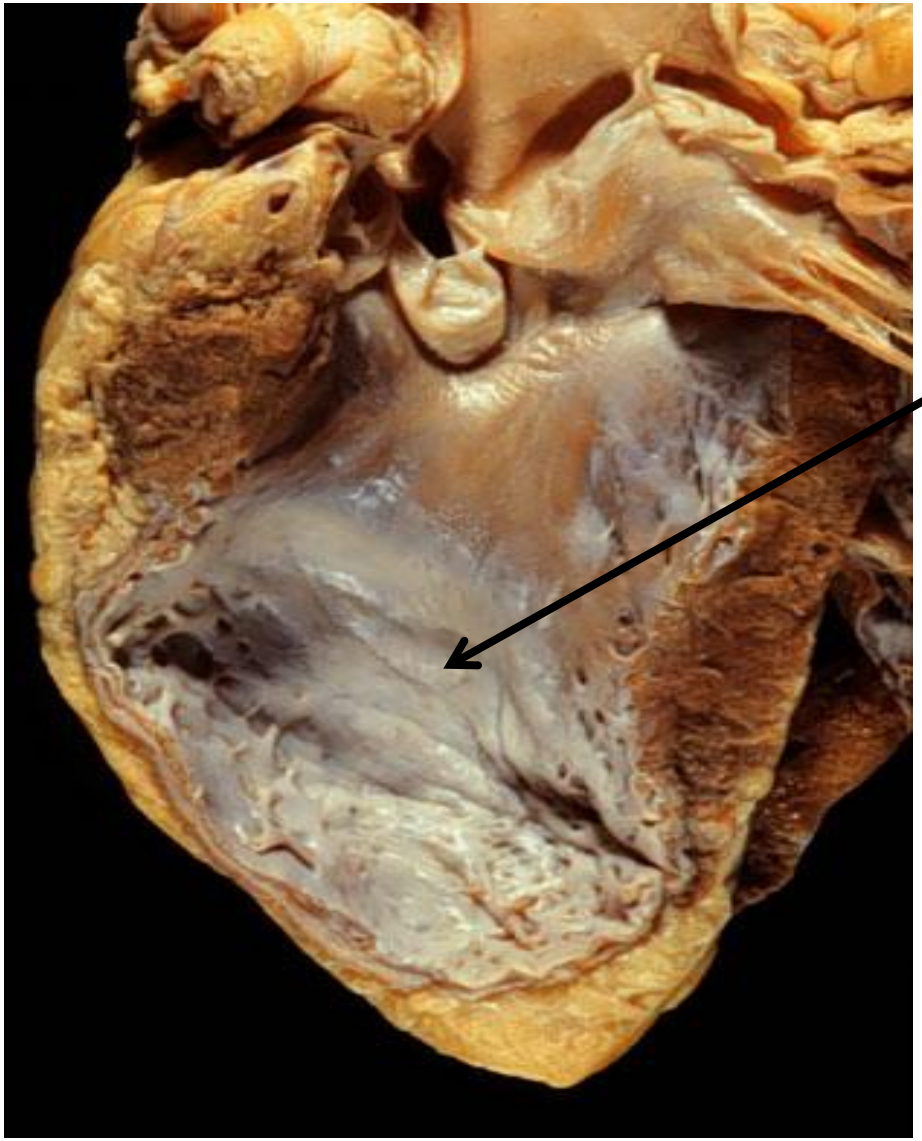
- After 7-10 days, the demarcation shaft is transformed into granulation tissue, which gradually replaces the necrotic masses.

- The outcome of infarction is the formation of a scar (heart, kidney) or cyst (brain).

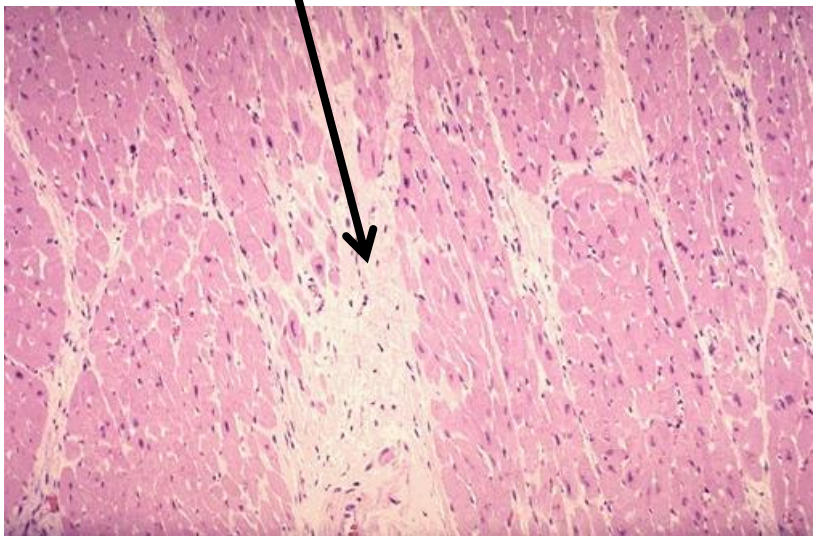
Outcomes of necrosis

1. Tissue regeneration;
2. Organization (formation of coarse fibrous connective tissue = scar);
3. Encapsulation (the formation of a capsule around the focus of necrosis);
4. Calcification (calcification, petrification);
5. Ossification (bone formation);
6. Inlay (deposits of uric acid salts);
7. Hyalinosis;
8. Cyst formation (cavity - brain);
9. Sequestration;
10. Mutilation (spontaneous rejection of dead parts - a finger);
11. Mummification (fetus)
12. Death from damage of vital organs.

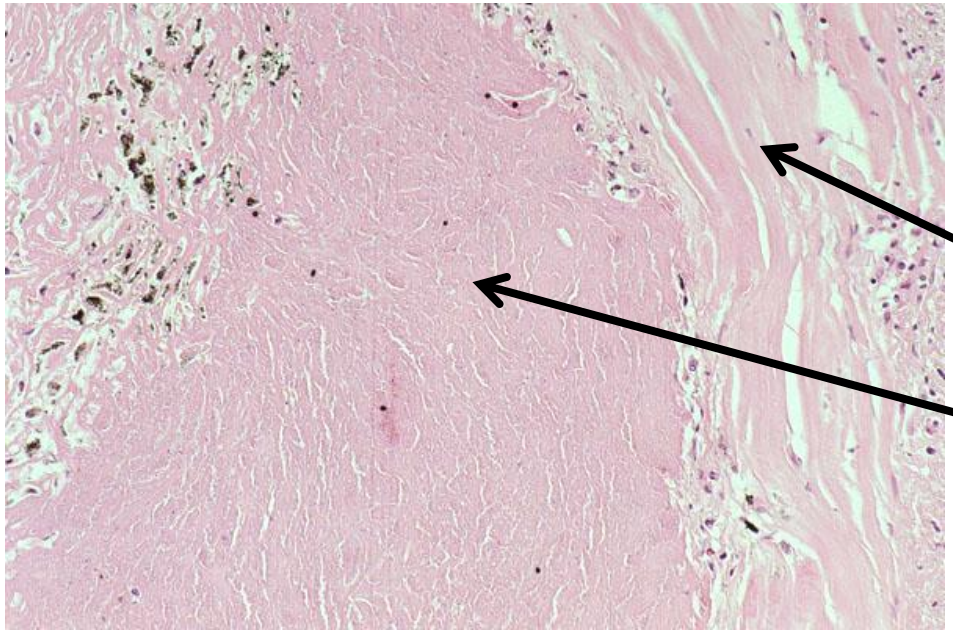
Outcomes of necrosis



•Organization

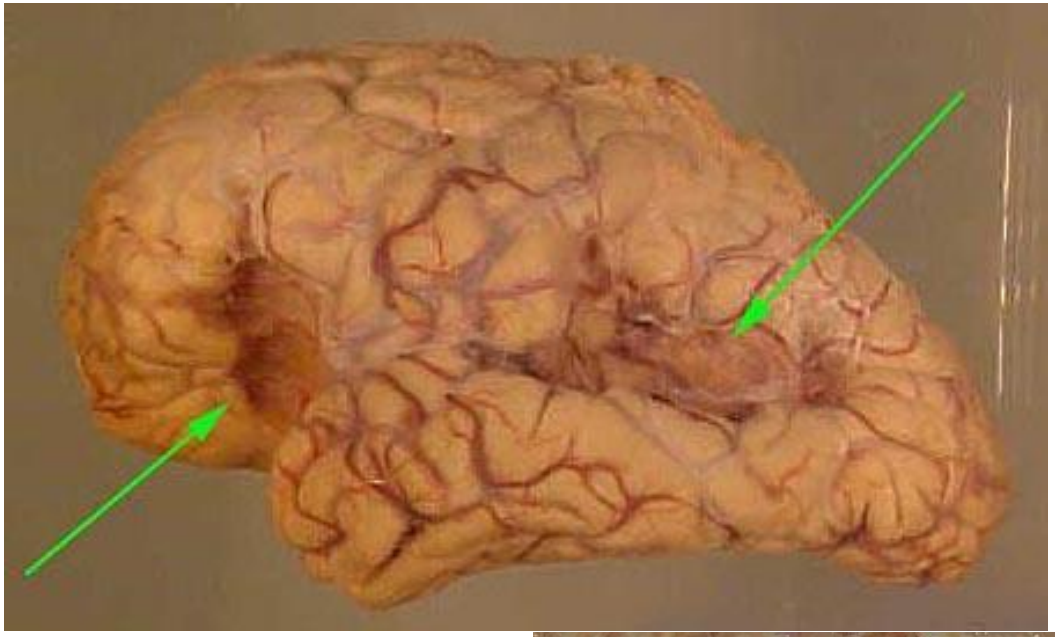


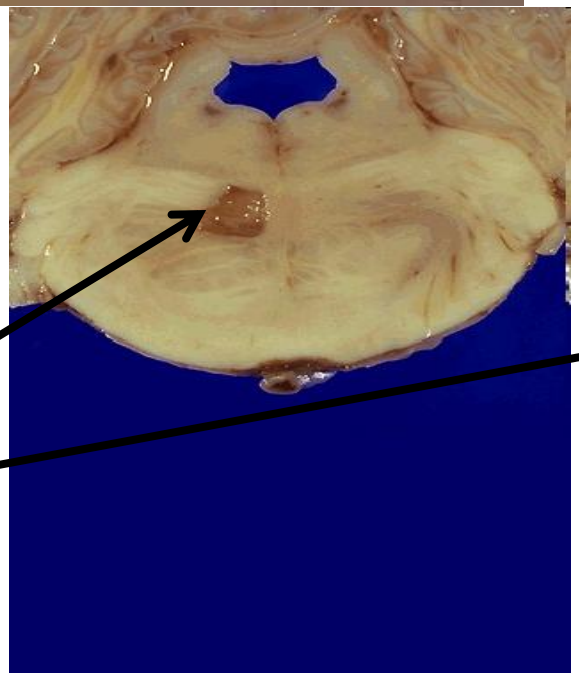
Outcomes of necrosis

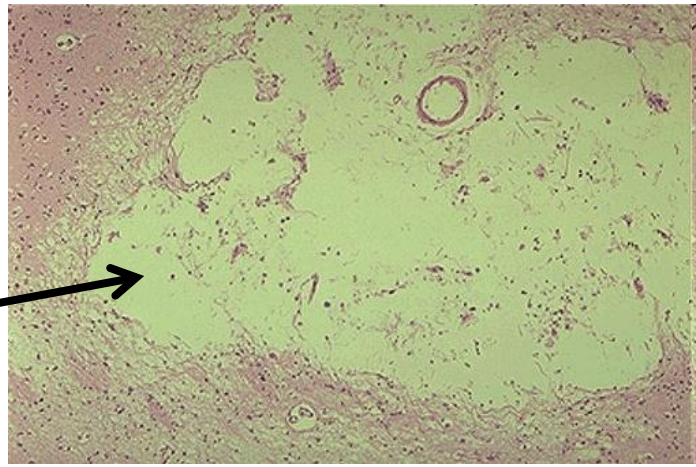


- ***Encapsulation***
- Capsule
- Caseous necrosis (tuberculosis)

Outcomes of necrosis







- Cyst formation

Outcomes of necrosis

- Mummification (fetus)

Outcomes of necrosis

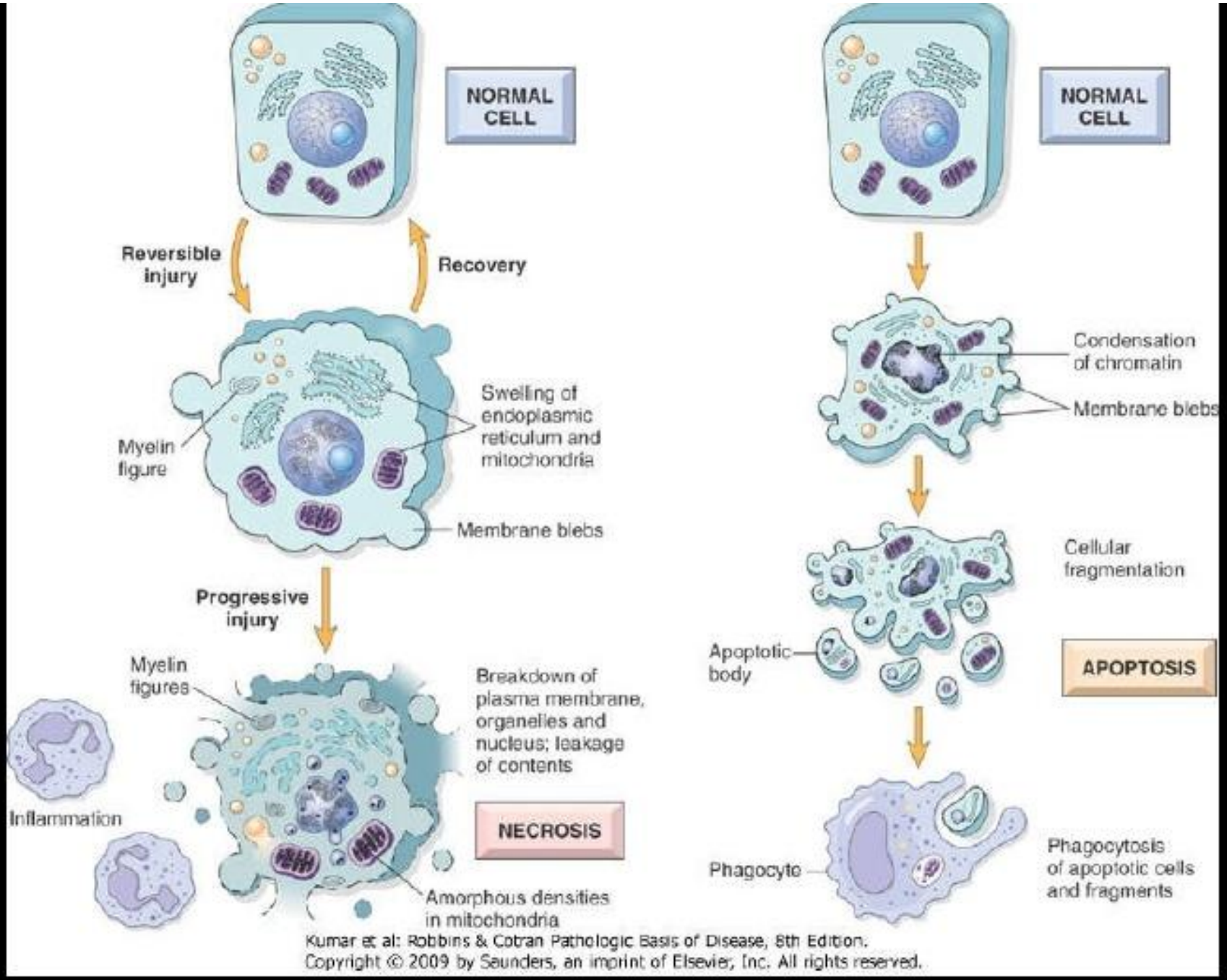
- Mutilation

Apoptosis

- Involved in many processes, some physiologic, some pathologic
  - Programmed cell death during embryogenesis
  - Hormone-dependent involution of organs in the adult (e.g., thymus)
  - Cell deletion in proliferating cell populations
  - Cell death in tumors
  - Cell injury in some viral diseases (e.g., hepatitis)

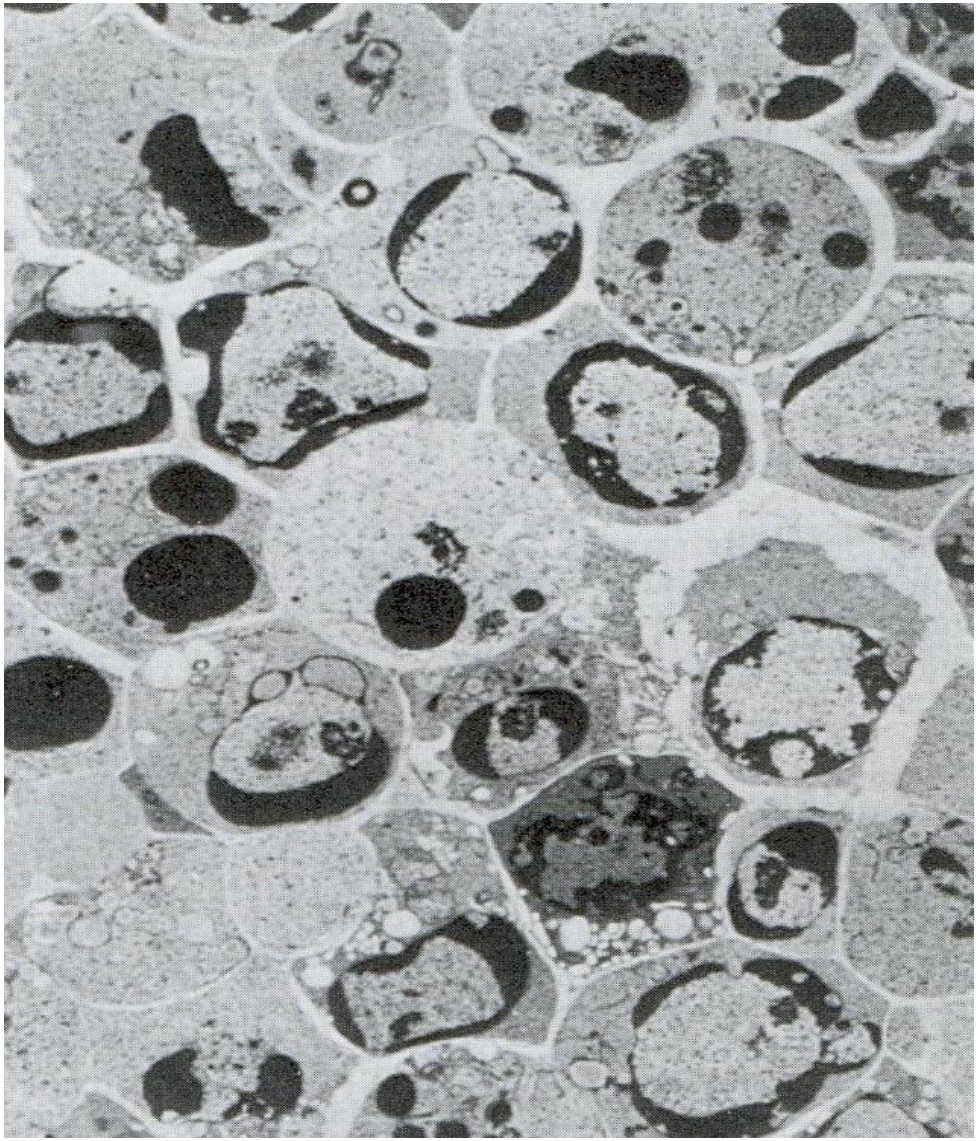
Apoptosis – Morphologic Features

- Cell shrinkage with increased cytoplasmic density
- Chromatin condensation
- Formation of cytoplasmic blebs and apoptotic bodies
- Phagocytosis of apoptotic cells by adjacent healthy cells



Kumar et al: Robbins & Cotran Pathologic Basis of Disease, 8th Edition.

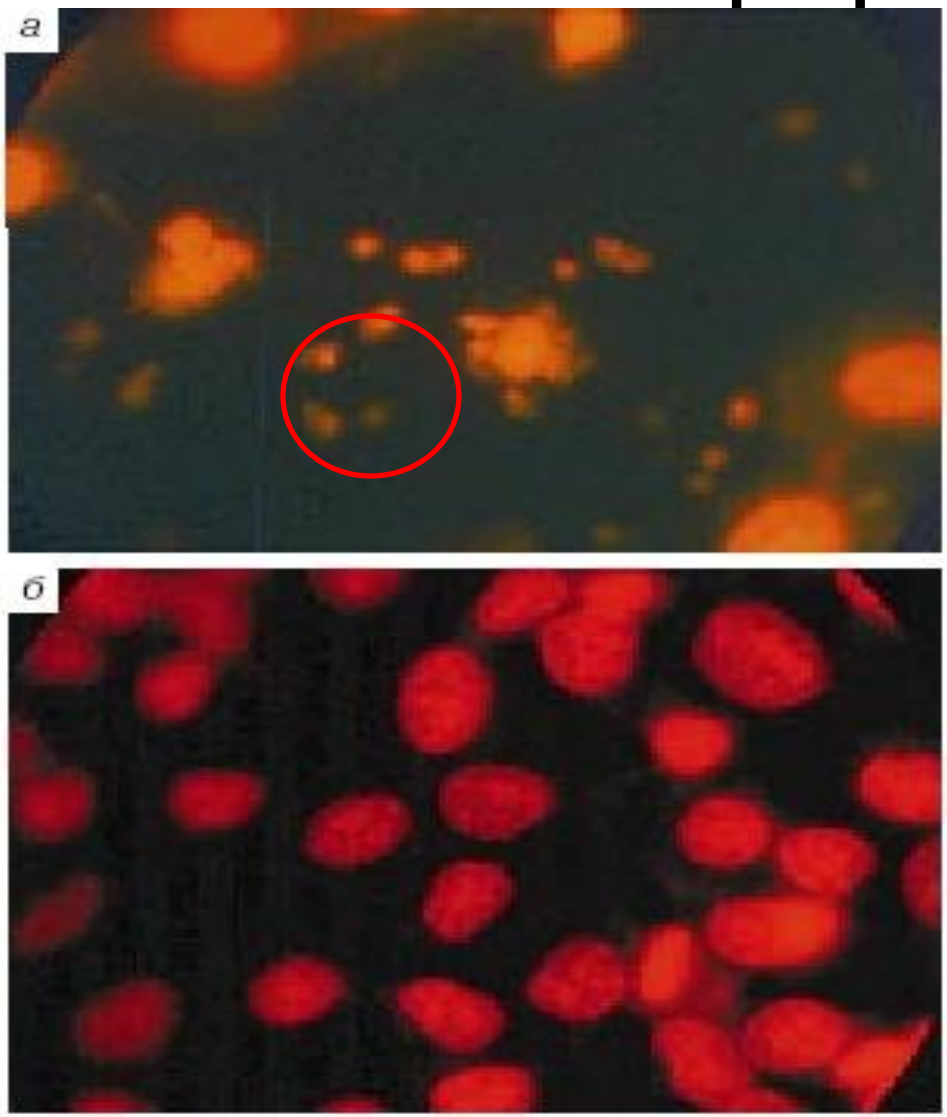
Morphology of apoptosis



Ultrastructural manifestations of apoptosis:

- Condensation of chromatin at the periphery of the nuclei;
- Fragmentation of chromatin;
- Formation of apoptotic bodies.

Apoptosis



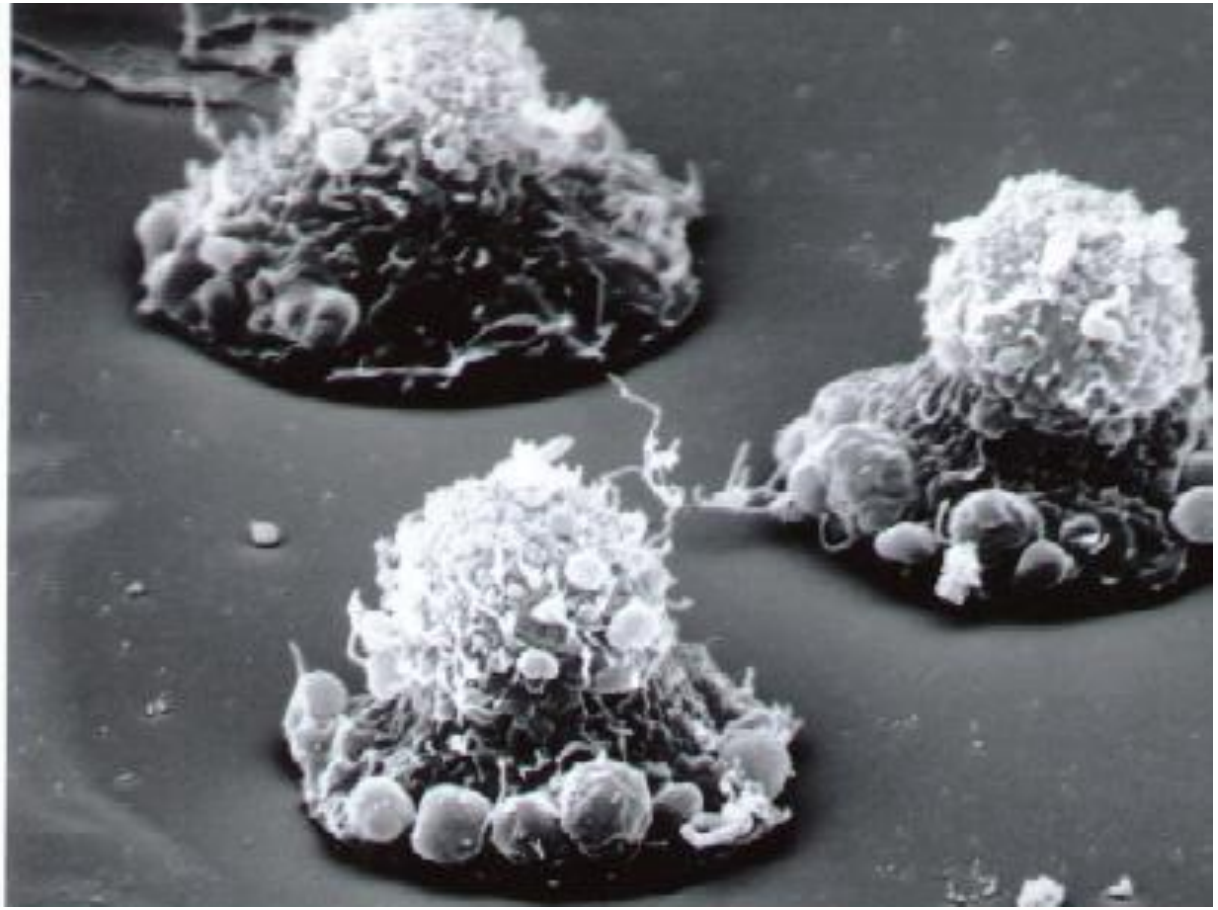
Flow cytometry:

a - cells in a state of apoptosis

b - control cells

A dye that fluoresces under ultraviolet light

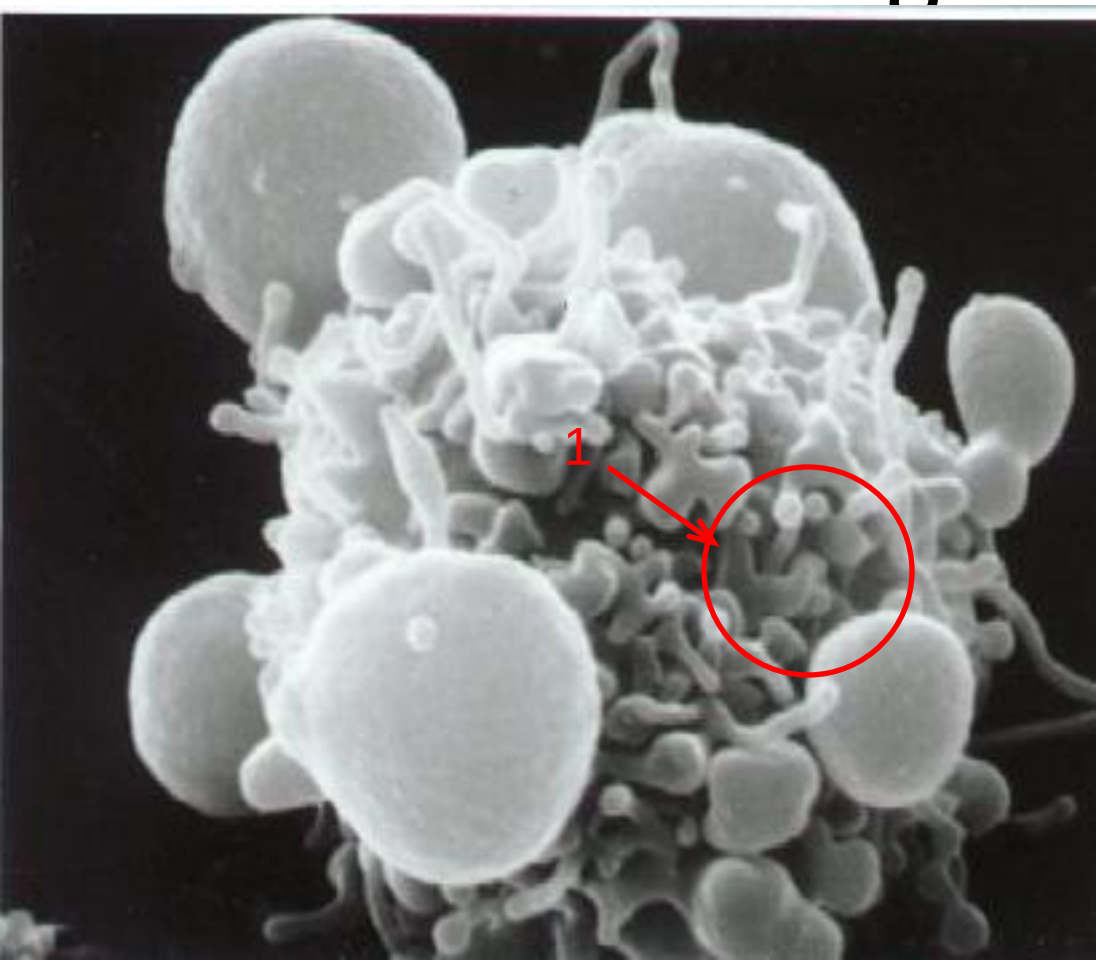
Apoptosis of epithelial cell.



Scanning Electron Microscopy:

- Formation of apoptotic bodies

The final stage of apoptosis



Scanning Electron Microscopy:

1- apoptotic bodies

Cells: apoptosis, necrosis, mitosis

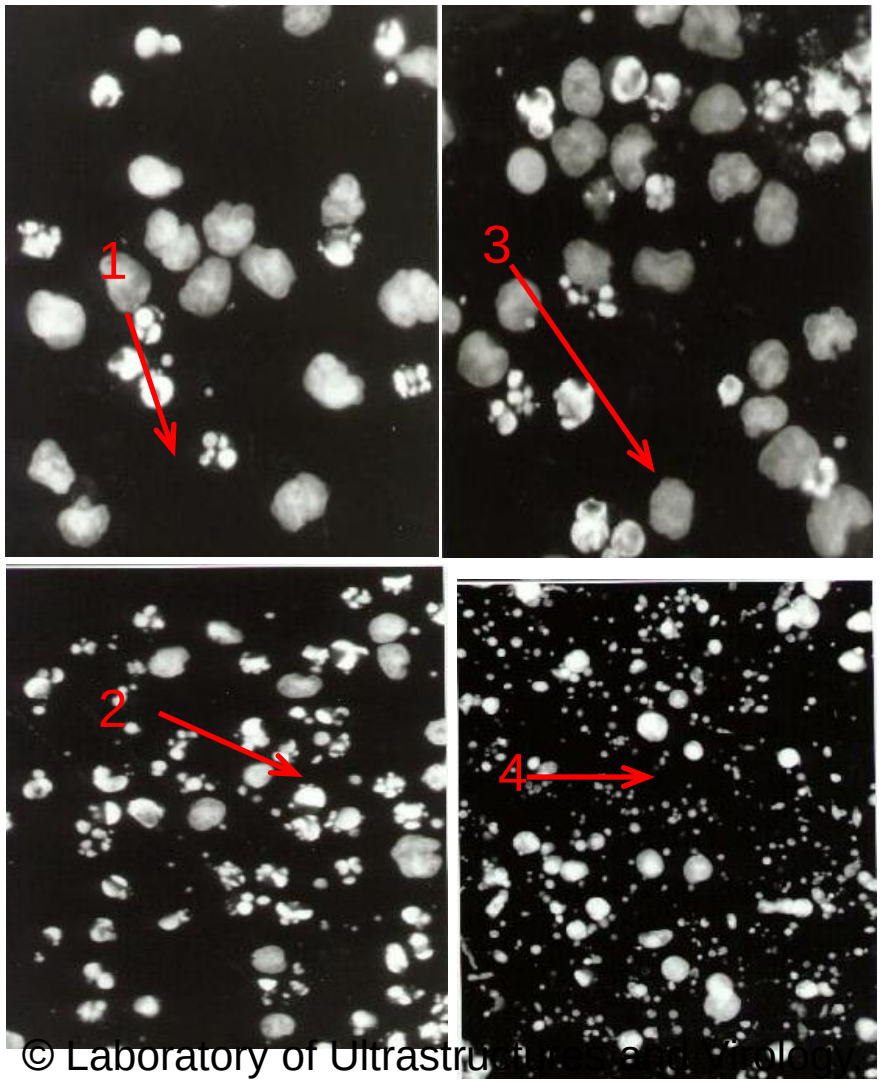
Flow cytometry:

1- norm

2- mitosis

3- apoptosis

Chromatin condensation



Flow cytometry:

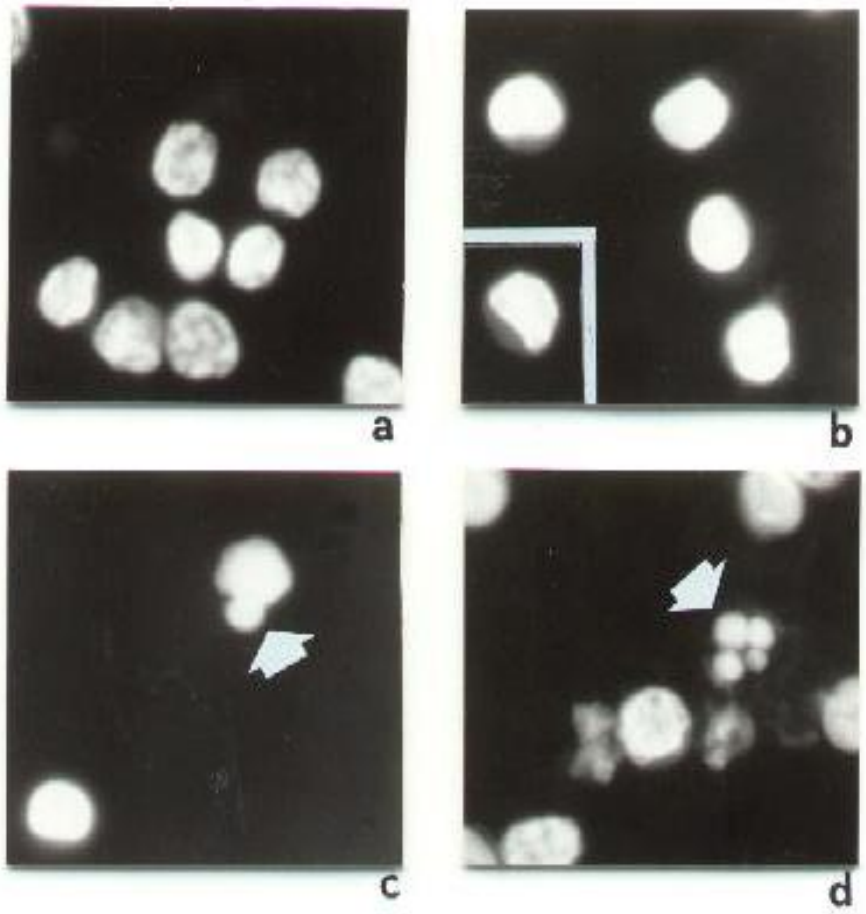
1- loss of connection with the karyolemma

2- chromatin aggregation

3-chromatin dissolution

4- apoptotic bodies

Chromatin condensation



Flow cytometry:

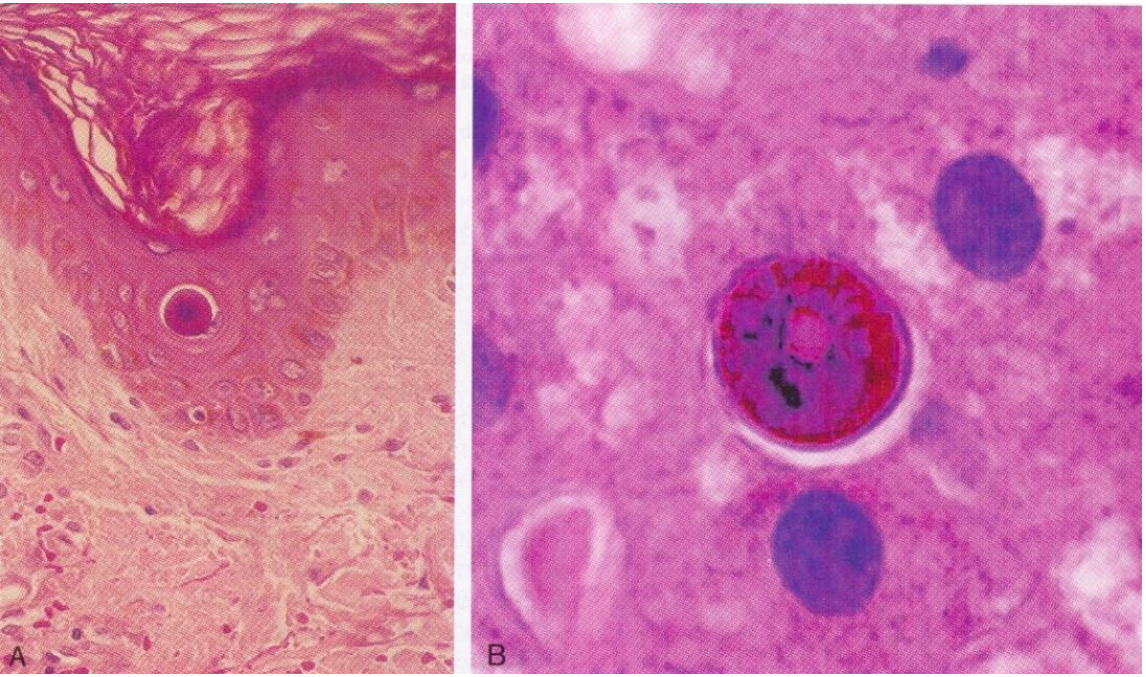
A- norm

B- loss of connection with the karyolemma

C- nuclear decay

D- formation of apoptotic bodies

Morphology of apoptosis



Light microscopy:

- intensely eosinophilic cytoplasm and a small, dark nucleus
- A) Apoptotic cell in the skin with an immunologically mediated reaction;
- B) an apoptotic cell in the liver.

THANK’S