DEGENERATION

- Defined as **deterioration** of live cells following injury, but with a possibility of the injured cells to reverse to normal when the **injury** is removed
- Deterioration of cells is evaluated in terms of **morphological changes** that occur **inside** or **outside** the cells
- **Injury** is defined as any **harmful** stimulus that induces disturbance in the **homeostasis** of cells

CLASSIFICATION OF CELL INJURIES

- Cell injuries are classified according to:
- 1. Magnitude/Severity:
  - i) Sub-lethal injury; does not kill the cells but induces morphological changes, which are compatible with life
  - ii) Lethal injury; kills the cells
- 2. Nature of injury
- **i) Physical injuries**, examples here are:

a. Mechanical trauma, cause direct rupture and death of cells

Examples of physical injuries(cont.)

b. Electrical trauma, generate great heat
c. Heat, denature enzymes, increase metabolic reactions to lethal levels
d. Cold, impair flow of blood and ice crystals rupture cell membranes
e. Radiant energy e.g. X-rays, UV light induce formation of free radicals, damage the genetic material causing genetic defects and neoplasia

ii) Chemical injuries, examples are:

a. Biological toxins, e.g. bacterial, fungal, anthropoid, snake
b. Pesticides, e.g. organophosphates, organochlorides(DDT), botanical insectcides
c. Herbicides, e.g. chlorophynoxy compounds
d. Environmental contaminants, e.g. metals, nitrates

- The mechanism of actions of chemical injuries is either through:
- Direct damage of critical molecules e.g. cytochrome oxidase in the mitochondria or
- Metabolism of harmless chemical compounds into toxic substances, e.g. when carbon tetrachloride is metabolized to give free radicals

iii) Biological injuries, examples are

a. Viruses, can redirect host cell to synthesize viral proteins harmful to the host cell
b. Bacterial, produce toxins as said earlier above
c. Fungi, in addition to possible toxin production, resist destruction of infected cells leading to chronic inflammatory reaction, e.g. granuloma formation
d. Protozoa, replicate in specific host cells resulting in destruction of infected cells

iv) Malnutrition

- Deficiencies in nutrients result in abnormal metabolic processes
- **v) Over nutrition**
- Excess of calories can be implicated in cardiovascular disease and several other diseases
- **vi) Genetic abnormalities**
- Mutation for example is incompatible with cell survival
- **vii) Immunological dysfunctions**
- Immunodeficency, autoimmunity and hypersensitivity are also incompatible with cell survival

viii) Aging

- Induces diminished capacity of cells to carry out their normal functions and are predisposed to diseases
- **ix) Hypoxia**
- This is defined as partial reduction of in oxygen concentration supplied to cells via blood
- Thus hypoxia results from ischemia(partial occlusion of arterial blood supply)
- The mechanisms of cell injury due to hypoxia follow a chain of reactions as follows:

Chain of reactions in cells exposed to hypoxia

a. Reduction in mitochondria phosphorylation
b. Reduction in ATP production in the mitochondria
c. Contraction of inner compartment of mitochondria and expansion of outer compartment of mitochondria(**low amplitude swelling)**
d. Increased activity of phosphofructokinase to promote anaerobic glycolysis
e. This leads to accumulation of lactic acid and inorganic phosphate, which lowers the intra-cellular pH
f. The low pH causes clumping of nuclear proteins

Chain of reactions in cells exposed to hypoxia(cont.)

g. If hypoxia continues, the plasma membrane is damaged leading to leaking of sodium into the cell and potassium outside the cell

h. Entry of sodium into the cell attracts entry of water into the cell leading to dilation of the endoplasmic reticulum, high amplitude swelling and formation of blebs on the plasma membrane

i. Beyond this stage, other changes start taking place in the cell, which signify a process of cell death consisting of:

Changes that occur during cell death

i. Influx of calcium ions into the cell
ii. Increased anaerobic glycolysis and further fall in intracellular pH
iii. Disruption of the skeleton, rupture of lysosomes, release of lysosomal enzymes and other degrading phospholipases, which initiate autolysis and invoke an inflammatory reaction to the surrounding normal cells
iv. An important sign of the process of cell death is that the nucleus remains intact
v. The process of cell death ends up when irreversible nuclear changes called necrosis start occurring

Classification of degenerative changes

- As stated earlier on deterioration of cells is evaluated in terms of **morphological changes** that occur **inside** or **outside** the cells
- Morphological changes that occur **inside** the cells following injury involve accumulation of **metabolites** in the **endoplasmic reticulum**
- One of these metabolites is **water** as described above following injury of cells by **hypoxia**
- Other metabolites known to accumulate inside cells following injury are:
  - Protein
  - Carbohydrates
  - Lipids

1. TYPES OF DEGENERATION INVOLVING ACCUMULATION OF WATER INSIDE CELLS

a) Cell swelling

- This is the earliest morphological change that occurs whenever the plasma membrane is injured
- It is characterized by swelling of the cell due to distention of the endoplasmic reticulum with water
- It is readily observed by light microscopy in epithelial and endothelial cells stained with H&E
- The cytoplasm of affected cells appear pale

Types of degeneration involving accumulation of water(cont.)

a) **Cloudy swelling**

- This is a synonym term to cell swelling, initially used by one of the founding fathers of Pathology Rudolph Virchow, 1821 - 1902 who described swollen unstained cells

a) **Hydropic degeneration**

- This is the advanced stage of cell swelling
- Characterized by appearance of either one large clear vacuole(**balooning degeneration**) or multiple vacuoles(**vacuolar degeneration**)

2. TYPES OF DEGENERATION INVOLVING ACCUMULATION OF CARBOHYDRATES

a) Glycogen degeneration

- Excess glucose in blood(hyperglycaemia) becomes stored in hepatocytes in the form of **glycogen** when the body is able to produce insulin
- Stored glycogen stains purple with periodic acid schiff(PAS)
- Longstanding levels of hyperglycaemia leads to over accumulation of glycogen in hepatocytes that brings about morphological change of the cells(**glycogen degeneration**)
- In situations when there is lack of insulin, cells cannot utilize glucose, thus glucose remains in the circulation
- Glucose that accumulates in blood denies the body source of energy and the glucose is excreted via urine
- This characterizes a condition called **diabetes**

Diabetes

- There are two types of diabetes:
- Type 1 usually diagnosed in children hence formally known as **juvenile diabetes** results from autoimmune destruction of insulin producing **β cells** in the pancreas
- Type 2 is the most common form in which either the body does not produce enough insulin or the cells ignore/become resistant to insulin

3. TYPES OF DEGENERATION INVOLVING ACCUMULATION OF PROTEIN INSIDE CELLS

a) Hyaline droplet degeneration

- This is defined as accumulation of excess normal protein droplets inside cells, which in H&E stained cells appear **homogeneous, glassy eosinophilic**
- Normal homogeneous, glassy eosinophilic protein is also called **hyaline**
- Hyaline droplets can be seen in the following cells:
  - Intestinal epithelial cells of neonates following excessive absorption of colostrums **immunoglobulins**
  - Epithelial cells in the renal tubules following absorption of protein leaking from damaged **glomeruli**

b) Zenkers degeneration/Waxy degeneration/Zenkers necrosis

- The condition is named after Friendrich Albert Zenker
- It is a form of severe hyaline degeneration in skeletal muscle caused by toxins in severe infections e.g. typhoid
- Grossly, the muscles appear pale and friable due to coagulation of sarcoplasm proteins
- Microscopically the muscles are swollen with loss of cross striation and show a hyaline appearance
- Zenkers degeneration is infarct a necrotic change based on:
  - Loss of cross striation
  - Condensation of nuclei
  - Loss of nuclei

4. TYPES OF DEGENERATION INVOLVING ACCUMULATION OF LIPIDS INSIDE CELLS

a) Fatty change

- Defined as accumulation of excess normal lipids inside the cells
- It occurs mostly in the liver and occasionally in the kidney and heart
- The cause of accumulation of lipids inside hepatocytes is interference in any of the metabolic pathways of lipid metabolism

Metabolic pathways of lipid metabolism that may be interfered include

I. Excessive accumulation of FFA in hepatocytes due to:
   - Over supply of FFA to the liver during starvation
   - Failure of **esterification** of FFA due to damage of mitochondria
   - Failure of use of the FFA in the **synthesis** of lipoproteins due to damage of RER

II. Excessive accumulation of lipoproteins due to:
   - Failure of **packing** of lipoproteins due to damage of golgi apparatus
   - Failure of **release** of lipoproteins from the cell due to damage of the plasma membrane

Morphological changes of livers with fatty change

- Grossly, fatty livers are enlarged(round edges), yellow, greasy texture on the cut surface
- Histopathologically, **conventionally** processed tissue livers and stained with H&E show hepatocytes with empty **discrete vacuoles** of variable sizes
- The empty vacuoles represent areas initially occupied by lipids, that have been dissolved in alcohol used in the conventional tissue processing
- In order to preserve the lipid for subsequent demonstration, tissues have to be processed differently

Steps involved in tissue processing for demonstration of lipids

i. Preserve the tissues by freezing instead of using 10% neutral buffered formalin
ii. Section the frozen tissues in a cryostat
iii. Stain the sections with special stain for lipids e.g. Sudan III, or Oil red

- Deposits of lipids will appear red
- **Fatty infiltration/Fatty replacement/Steatosis**
- These are synonym terms, which do not refer to degeneration
- They refer to accumulation of adipose cells in tissues that do not contain lipids e.g. muscles

Classification of degeneration based on accumulation of metabolites outside the cells

- The metabolites that accumulate outside the cells and induce degenerative changes are:
- 1. Metabolites that are protein in nature : hyaline, fibrinoid, amyloid, uric acid
- The corresponding degenerative changes resulting from each are:
  - **Hyalinization**
  - **Fibrinoid/fibrinous degeneration**
  - **Amyloidosis**
  - **Gout**
- 2. A metabolite of lipid nature commonly deposited outside cells is cholesterol
- The degenerative change arising from cholesterol deposition is **atherosclerosis**

3. Minerals

- The mineral commonly deposited outside cells is calcium
- The degenerative change arising from deposition of calcium is **metastatic calcification**
- **1. Hyalinization**

➢ This is defined as deposition of hyaline outside the cells

➢ In H&E stained tissues, the material appears homogenous, translucent, eosinophilic

➢ Common sites where hyalinization occurs include:

  ➢ Walls of arteries, following damage of endothelial cells

  ➢ Glomeruli, following damage of glomeruli and Bowman`s capsule

  ➢ Renal tubules, in the form of **hyaline casts** formed due to crystallization of leaking protein on the renal tubules walls

2. Fibrinoid/Fibrinous degeneration

- This is a degenerative change in which there is accumulation in walls of arteries or connective tissue of a homogeneous eosinophilic material that resembles fibrin
- The fibrinoid/fibrinous material defers from hyaline in the following ways:
  - It is **more red** than hyaline
  - It is composed of **fibrin** and **immunoglobulins**
  - It occurs following severe damage of endothelial cells by **immune-complexes**

3. Amyloidosis

- Amyloidosis is a term used to refer to extracellular deposition of a group of **chemically abnormal** proteins, which are arranged in specific **beta-pleated fibrils** as seen by electron microscope
- Such chemically abnormal proteins are also called `**bad proteins**` or **amyloid proteins** and are produced by the body when the body is suffering from a variety of conditions
- The term **amyloid** was first introduced by **Virchow** in 1853

Gross and microscopic appearance of amyloid

- Grossly, organs containing large amount of amyloid have a **pale, grayish waxy** appearance to their cut surface
- In H&E stained paraffin-embedded tissue sections, deposits of amyloid appear **homogeneous, dull** and **eosinophilic** in the interstitium and walls of blood vessels
- **Confirmation of amyloid in tissues**
- Grossly, fresh tissues containing amyloid stain **blue** if immersed in **iodine** solution, followed by dilute **sulphuric acid**

Confirmation of amyloid in tissues(cont.)

- Histologically, 10% neutral buffered formalin fixed, paraffin embedded sections, cut between 6-12µm and stained with 11% **Congo red** solution give a characteristic **apple-green birefringence** appearance
- **Classification of amyloidosis**
- There are many often overlapping ways of classification
- However, the different classifications are based on:
  - Clinical
  - Chemical composition

Clinical classification of amyloidosis

- This is the simplest way, under which the following types are recognized:

a. **Primary amyloidosis**, affecting the tongue and heart
b. **Secondary amyloidosis**, affecting mainly the spleen, liver and other organs, associated with chronic inflammatory conditions e.g. ostiomyelitis, tuberculosis and rheumatoid arthritis
c. **Familial amyloidosis**
d. **Isolated organ amyloidosis**

Chemical composition classification of amyloidosis

- Immunohistochemical analysis of amyloid proteins has shown the following chemical composition:
  - Apolipoprotrein, an α-globulin protein
  - Macroglobulin, a β2 globulin protein
  - Transthyretin
  - Light chains of immunoglobulins
- Classification of amyloidosis based on chemical composition e.g. AA amyloidosis and AL amyloidosis has become obsolete
- Instead **Glenner**, in1980 introduced the following types of amyloidosis:

Glenner`s classification of amyloidosis

a. **Acquired systemic amyloidosis**, this is associated with either:

- Deposition of serum A protein, (AA amyloid), which is an **acute phase reactant** or
- Deposition of fibril proteins, from either:
- **Immunocyte dyscrasias** e.g. myeloma (AL amyloid) as in heredofamilial disorders or
- **Prealbumin** as in aged individuals over 60 years of age)

Glenner`s classification of amyloidosis(cont.)

- **b. Localized amyloidosis**

➢ In this type, amyloid has a predilection for a particular organ or tissue for example lungs, urinary bladder, heart(cardiac amyloid), skin, joints, and brain(cerebral amyloid)

➢ Localized amyloidosis may occur in the form of nodular amyloidosis

➢ **Consequences of deposition of amyloid in different organs:**

- Glomelular basement-uremia
- Islets of Langerhans-dibetes mellitus
- White pulps of the spleen –immunodefficiency
- Sinusoids of liver –liver dysfunction
- Wall of arteries –high blood pressure

4. Gout

- Gout is defined as accumulation of **urate crystals**(**white chalky masses**) in joints causing inflammation and intense pain of gout attack
- Urate crystals can form when there is high levels of uric acid in blood
- Uric acid is derived from break down of **purines**
- Purines are natural substances found in all body cells and in all foods
- However, there are some foods contain high concentrations of purines; these include
  - Organ meat like kidney
  - Fish e.g. herring, sardines

Causes of gout

- There are two main causes of gout:
  - Kidney damage, which leads to impaired excretion of uric acid
  - High protein intake that leads to increased production of uric acid
- **Classification of gout**
- In human the white chalky masses are found in joint spaces, hence the type of gout here is **articular gout**
- In birds, the white masses can be deposited both in joint spaces and on visceral organs, leading to a second type of gout called **visceral gout**

5. Atherosclerosis

- This is defined as deposition of lipids(mainly cholesterol) in the form of cholesterol crystals on the inner most layer of arteries(**intima**)
- Cholesterol may also be deposited in the anterior chamber of the eye, causing disappearance of the lens a condition termed(**aphakia**)
- Cholesterol becomes deposited when there is longstanding high levels of cholesterol in blood(**cholesterolemia**)
- Deposition of cholesterol crystals in the intima causes inflammation of the walls of arteries **vasculitis**

Atherosclerosis(cont.)

- Vasculitis heals by fibrosis and deposition of calcium(calcification)
- Deposition of cholesterol, vasculitis, fibrosis and calcification in the arteries walls leads to **hardening** of the walls and **reduction** in their lumen
- These events predispose to occurrence of **high blood pressure**, formation of **thrombi** on the endothelium and **ischemia** responsible for myocardial **infarction**, **stroke** and **gangrene** in the lower extremities
- In H&E stained sections, clefts/fissures/slits that represent spaces initially occupied by cholesterol are seen in the walls of arteries

6. Metastatic calcification

- This is defined as deposition of calcium in **normal**(non-injured) **soft tissues**
- It occurs when there is long standing high levels of calcium in blood(**hypercalcaemia**)
- Long standing hypercalcaemia may occur due to:
  a. Excessive intake of vitamin D, which enhances absorption of calcium from the intestine
  b. Increased resorption of calcium from bones due to increased secretion parathormone(**hyperparathyroidism**)

Metastatic calcification(cont.)

- Metastatic calcification occurs mostly on basement membranes in different organs e.g. of glomeruli in kidneys leading to **uremia**
- In H&E stained sections, deposits of calcium appear **reddish, purple-blue**
- The common special stain for confirming calcium is **von Kossa**
- The principle of this method involves use of **silver nitrate**, which reacts with **calcium phosphates** present in tissues
- In the reaction, there is **substitution** of phosphate and nitrate such that calcium nitrate and silver phosphates are formed
- Silver phosphate is then reduced to metallic silver by strong light to **black** deposits of silver phosphates

Dystrophic calcification

- This is defined as deposition of calcium in **necrotic** soft tissues
- It occurs mostly in tissues with **caseous necrosis**, where the calcium salts induce a **gritty** texture when a knife is used to cut through the lesion
- Dystrophic calcification is **not** a degenerative change as it occurs in dead tissue

Necrosis

- Necrosis is commonly defined as death of a group of cells in a living animal
- However, as stated earlier on, the process of necrosis starts after the process of cell death is over
- The process of necrosis is characterized by irreversible nuclear changes, which include:
  - **Pykinosis**-condensation of the nucleus into a small body
  - **Karyorehexis**-splitting of the nucleus into fragments
  - **Karyolysis**- dissolution of the nucleus

Necrosis(cont.)

- Other microscopic changes associated with necrosis include:
  - Increased eosinophilia of the cytoplasm
  - Loss of outlines of plasma membrane
- Grossly, the necrotic tissue presents the following changes:

➢ **Paleness** of the area, corresponding to a **white infarct** caused by occlusion of arterial blood supply(ischemia), in organs with dual blood supply e.g. kidney, heart, hence the infarct also known as an area of **ischemic necrosis**

➢ **Red** circumscribed area, corresponding to a **red infarct** caused by occlusion of arterial blood supply in organs with collateral blood supply e.g. the spleen

Necrosis(cont.)

- **Softening** of the area due to rupture lysosomes and release of their hydrolytic enzymes, which induce **autolysis**
- Demarcation of the area by a **hyperemic zone**, corresponding to vascular response of the viable tissue adjacent to the dead
- Drying of the area, corresponding to evaporation and healing of the area

Classification of necrosis

There are **three** main types of necrosis classified according to **gross** and **histological** changes as well as **causes**

The major types of necrosis

1. Coagulation necrosis

- **Grossly**, the area with coagulation necrosis appears pale, therefore, being a **white infarct** and, as expected caused by **ischemia**
- **Histologically,** two features are used in characterizing coagulation necrosis:
  i. Recognition of the morphological organization of the affected organ
  ii. Presence of irreversible nuclei change(s)

2. Liquefaction necrosis

- **Grossly**, the area with liquefaction necrosis presents as an **abscess** i.e. encapsulated whitish yellow fluid(**puss**), which is an inflammatory **exudates** caused by puss forming(**pyogenic**) bacteria

Liquefaction necrosis(cont.)

- Histologically, two features are used in characterizing liquefaction necrosis:
  - i. **Non-recognition** of the morphological organization of the affected organ
  - ii. Presence of **homogeneous** fluid, consisting of fragmented residential and inflammatory cells

3. Caseous necrosis

- **Grossly**, the area with caseous necrosis presents circumscribed cheese-like solid material, which gives a gritty texture when a knife is cut through the lesion, indicating calcification
- The **cause** of caseous necrosis are resistant bacteria such as *mycobacterium tuberculosis* and *corynebacterium pseudotuberculosis*

Caseous necrosis(cont.)

- Histologically, two features are used in characterizing caseous necrosis:
  - i **Non-recognition** of the morphological organization of the affected organ
  - ii Presence of **homogeneous solid** mass, consisting of inflammatory cells arranged in a granuloma form or scattered

Sub types of necrosis

1. **Fat necrosis**
2. **Gangrene**

Fat necrosis

- This is defined as accumulation of solid lipid material in connective tissue following traumatic injury of the tissues
- Traumatic injury of tissues induces death of resident cells and spill of lipids into the dead tissues
- **Grossly** the solid lipid material appears white and firm, and gritty texture is felt when a knife is cut through the lesion
- **Histologically,** the solid material is composed of fibrous tissue(**fibrosis**), macrophages and giant cells(**granulomatous**)
- **Chemically**, the mass is composed of cholesterol and calcium phosphate(**calcification**)

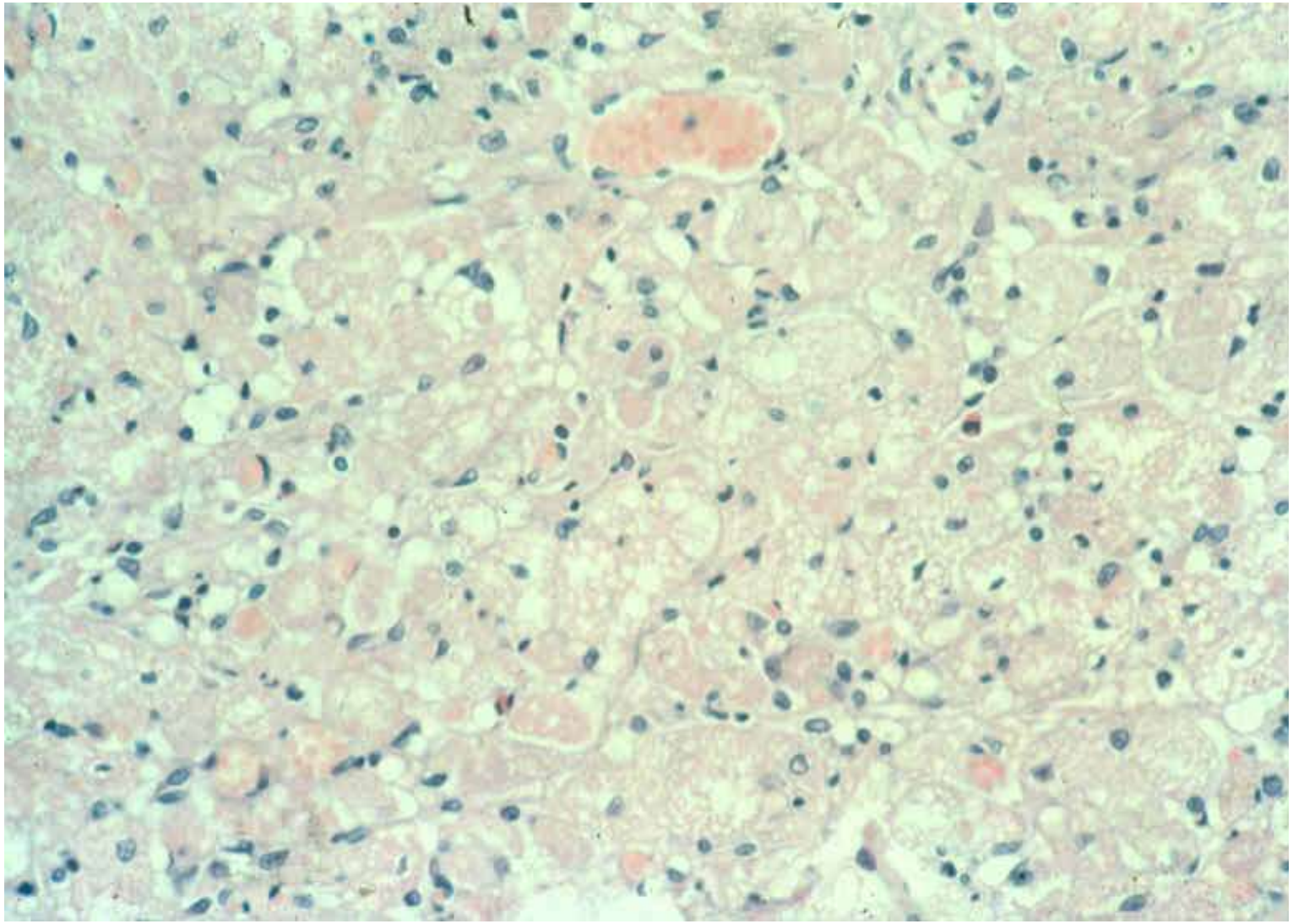
Gangrene

- This is defined as necrosis associated with growth of **saprophytic** bacteria in the dead tissue
- Growth of the saprophytes on dead tissues is favoured by fluid environment
- Dead tissues in fluid environment, therefore, progress to liquefaction of the tissues
- The type of gangrene in a fluid/wet environment is, therefore, termed **wet gangrene** a sub type of **liquefaction necrosis**
- One of the saprophytes associated with wet gangrene is *clostridium perfregens*
- Its growth in tissues is also associated with gas production, hence also referred to as **gas gangrene**

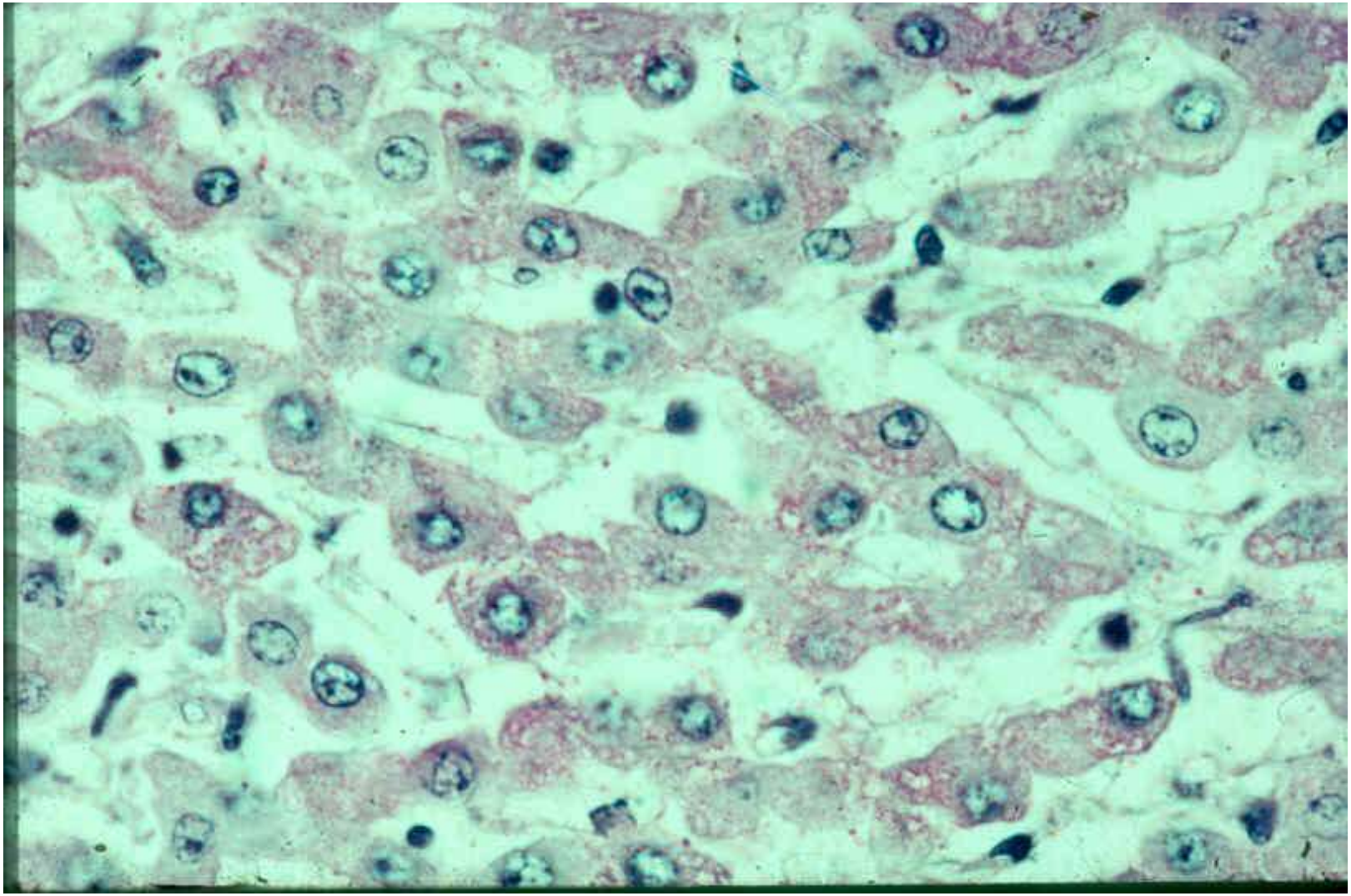
Gangrene(cont.)

- In dry tissues e.g. the skin and body extremities, there is limited growth of saprophytes and, the dead cells, therefore, maintain their morphology as in coagulation necrosis
- The type of gangrene that occurs in a dry environment is, therefore, termed **dry gangrene**, a sub type of **coagulation necrosis**

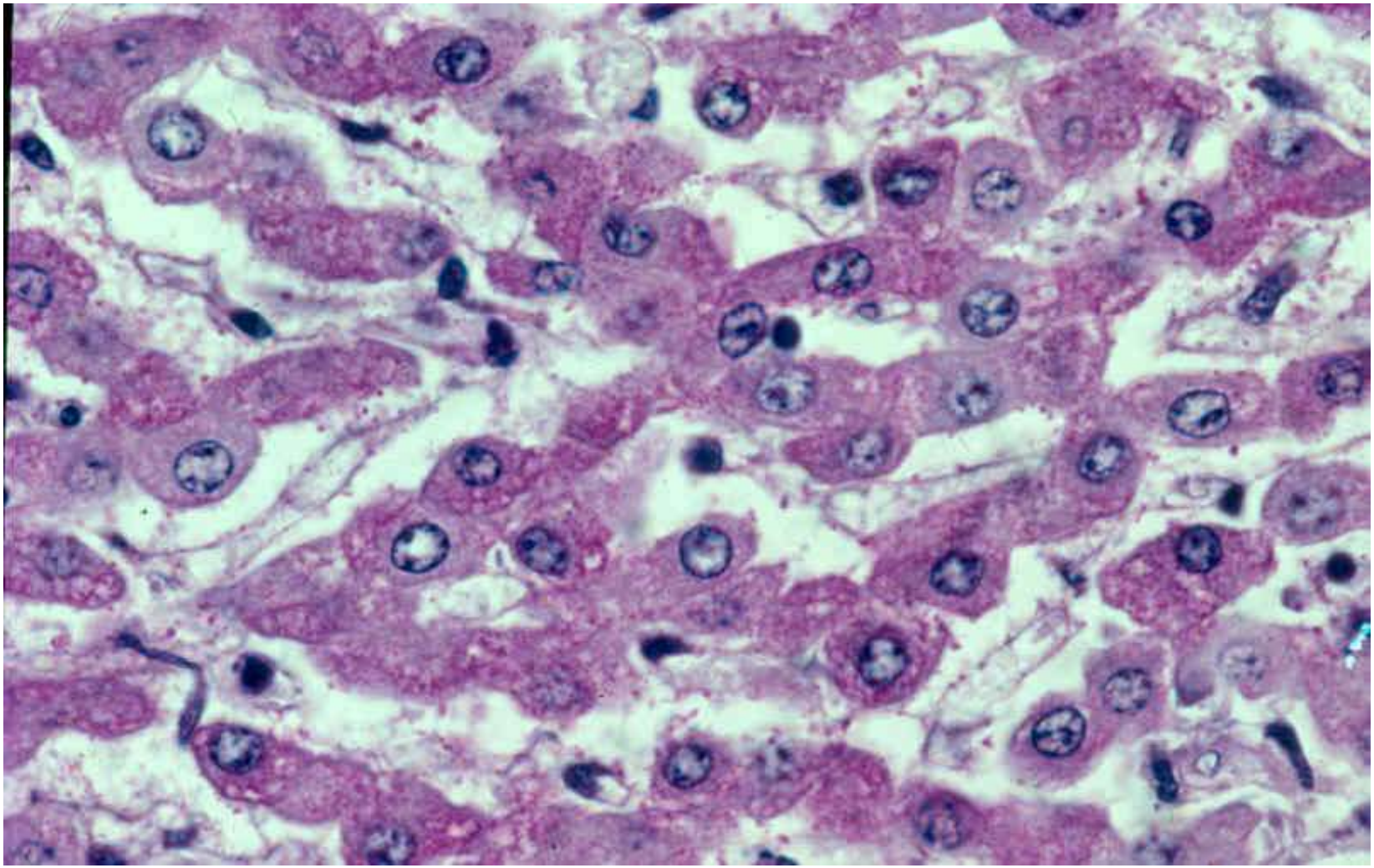
CELL SWELLING



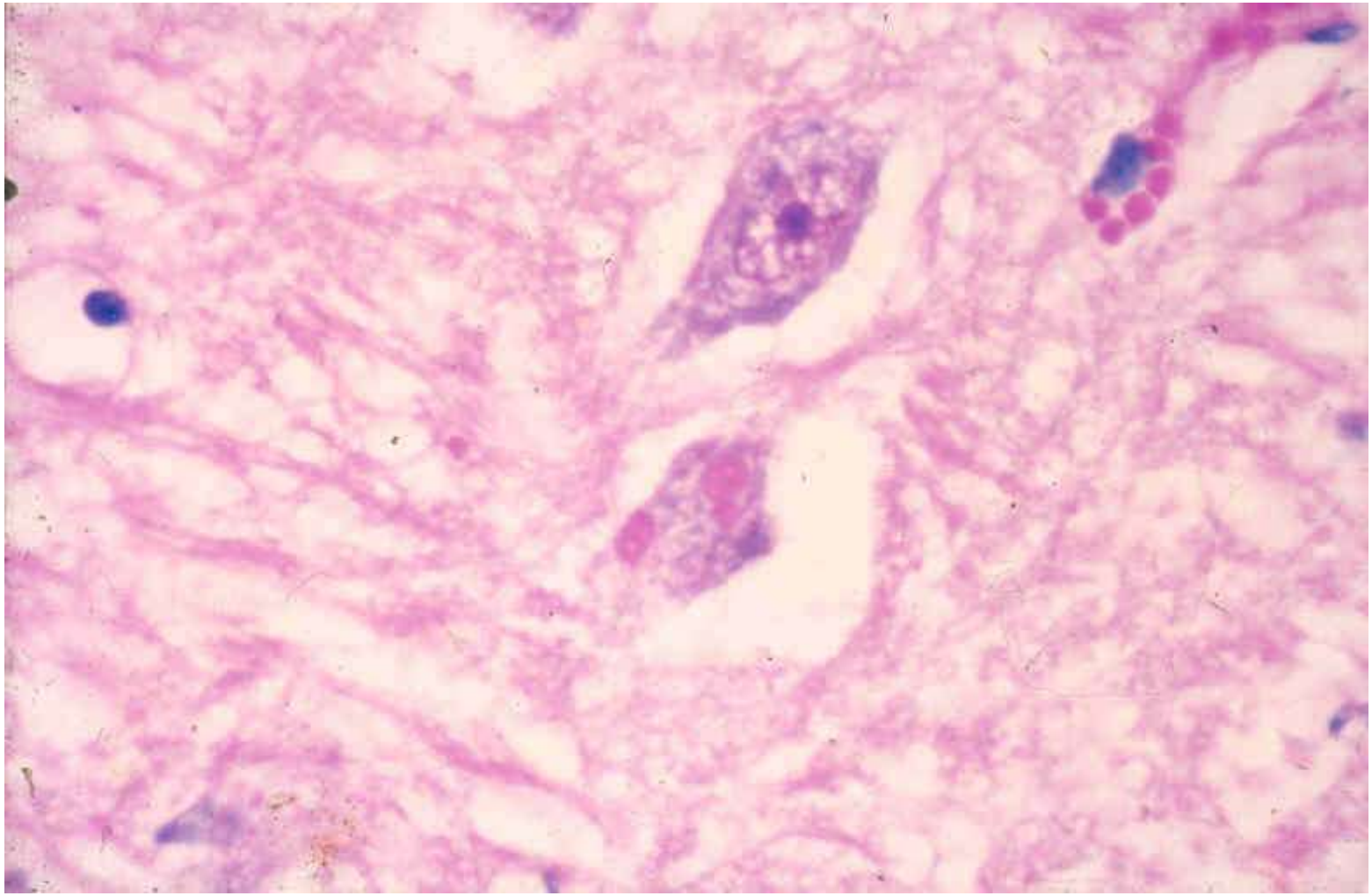
MILD GLYCOGEN DEGENERATION



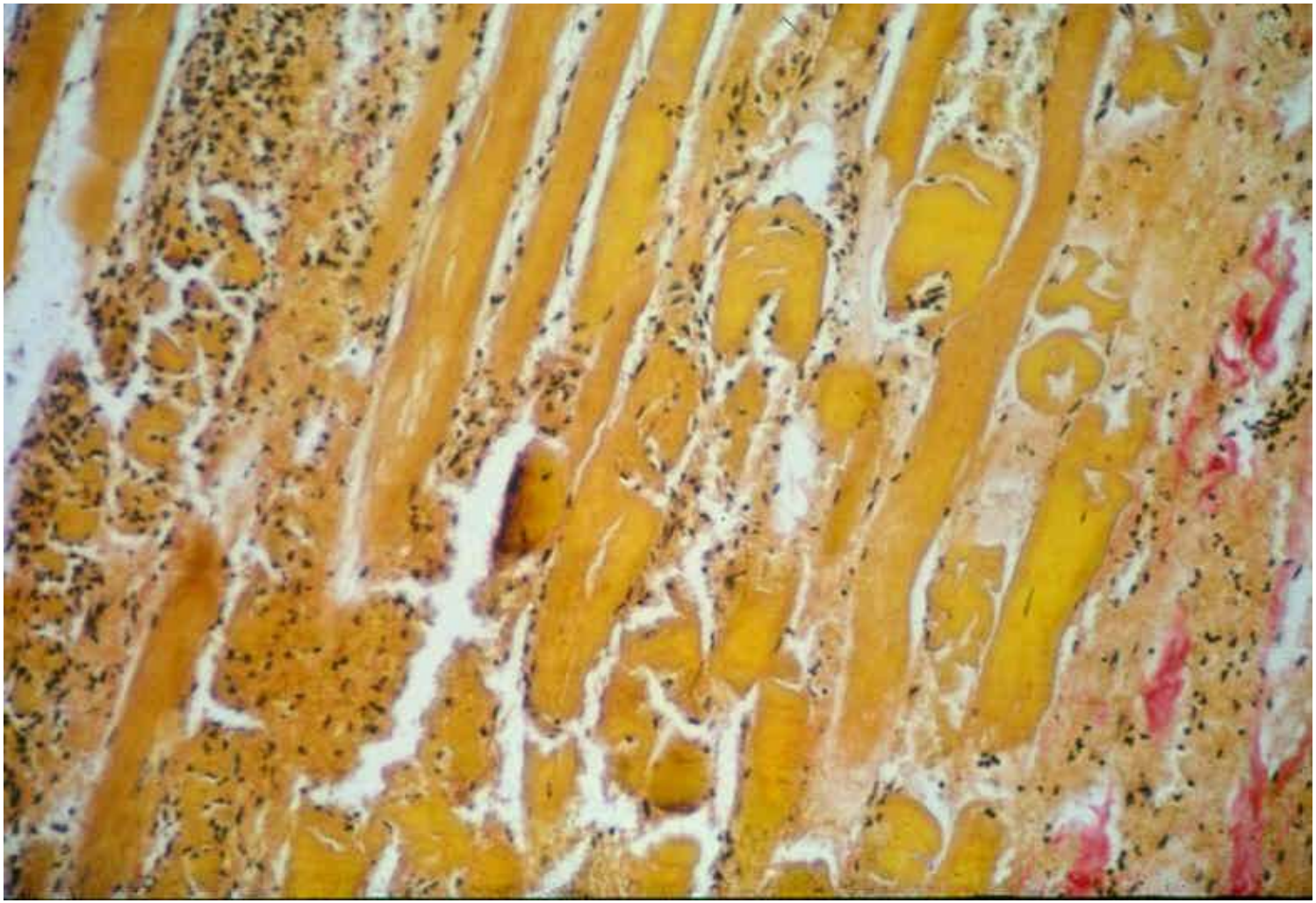
SEVERE GLYCOGEN DEGENERATION



INTRA-CYTOPLASMIC EOSINOPHILIC BODIES



ZENKERS DEGENERATION
(At low magnification)



ZENKERS DEGENERATION
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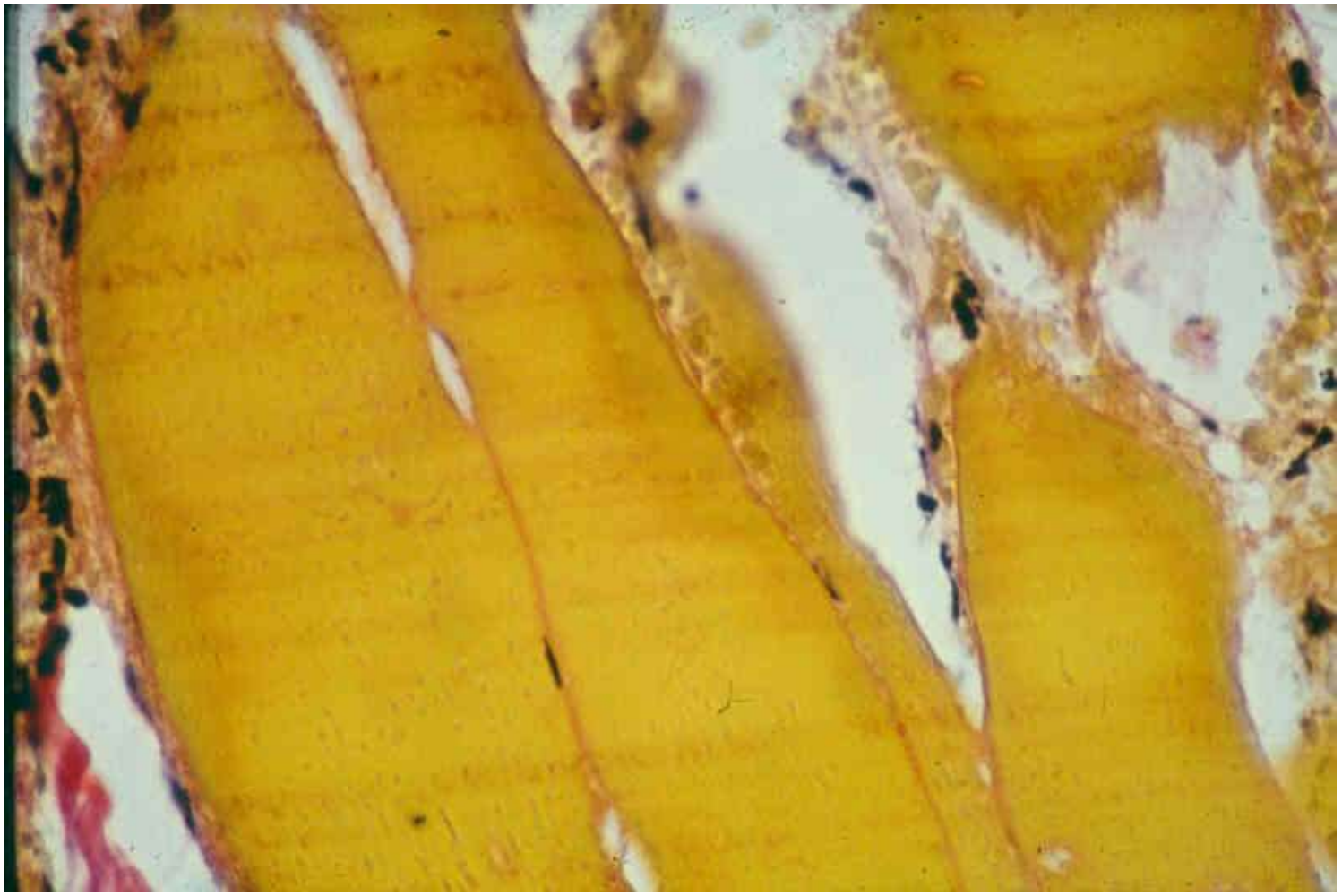
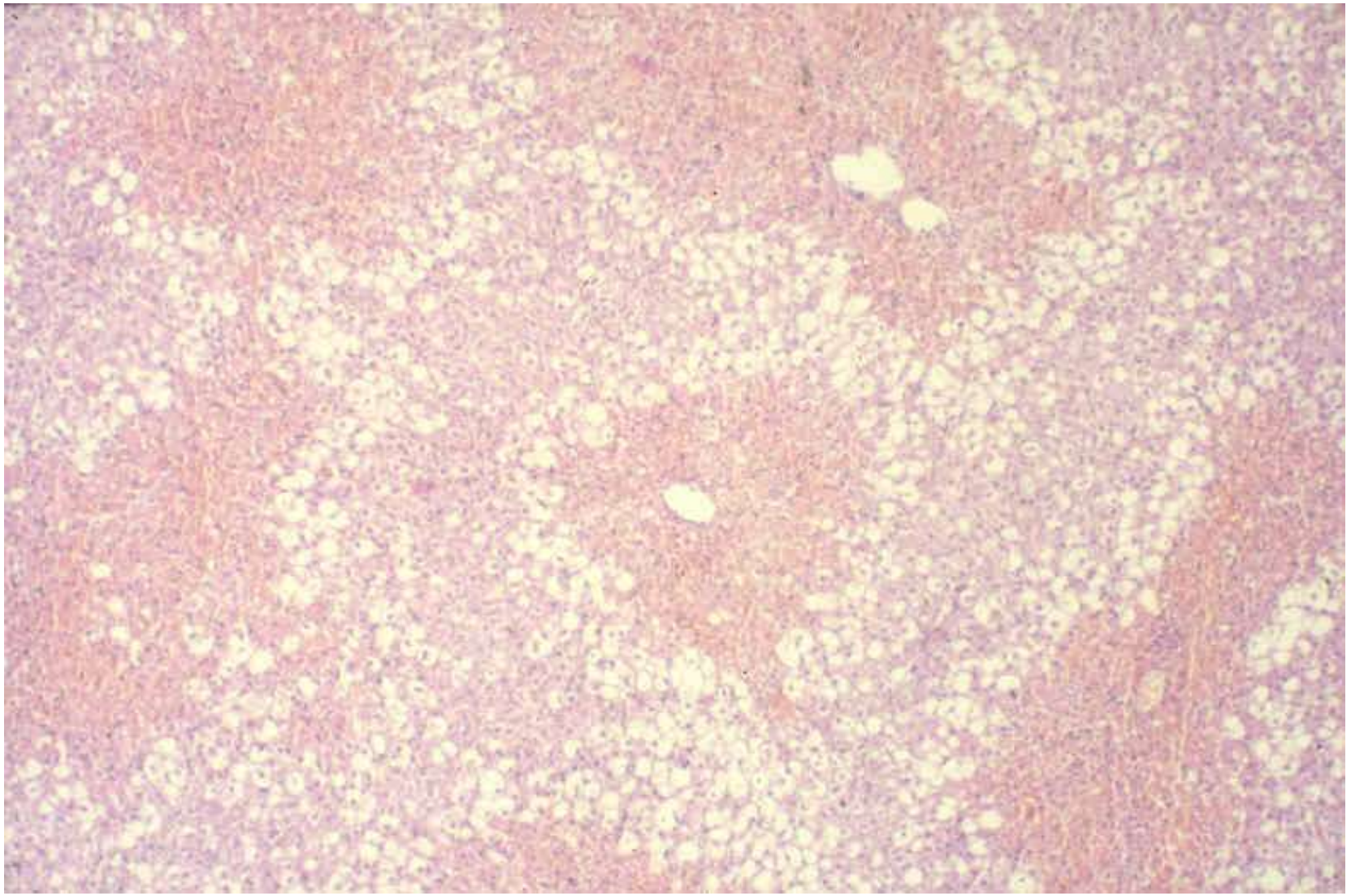
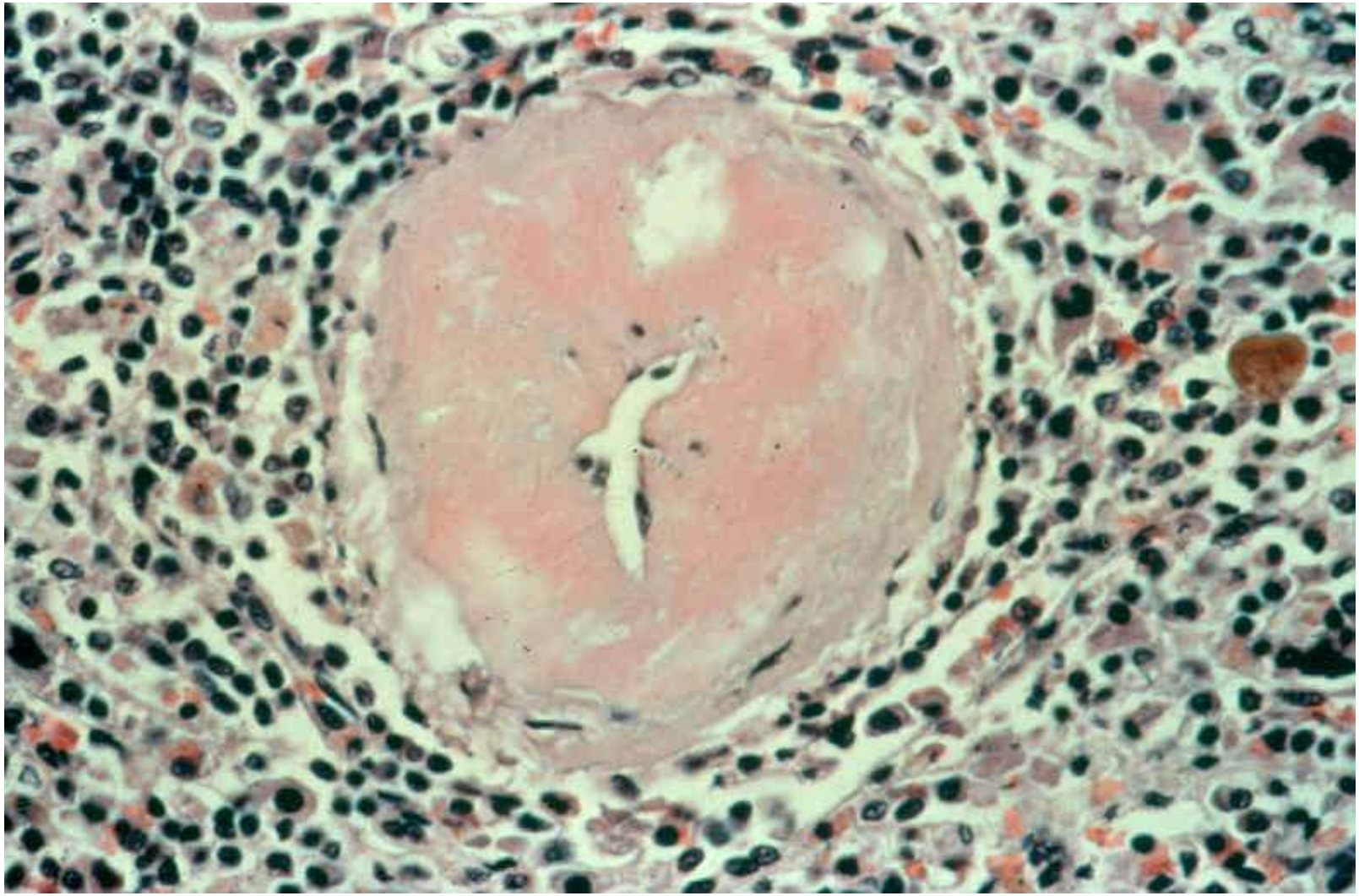


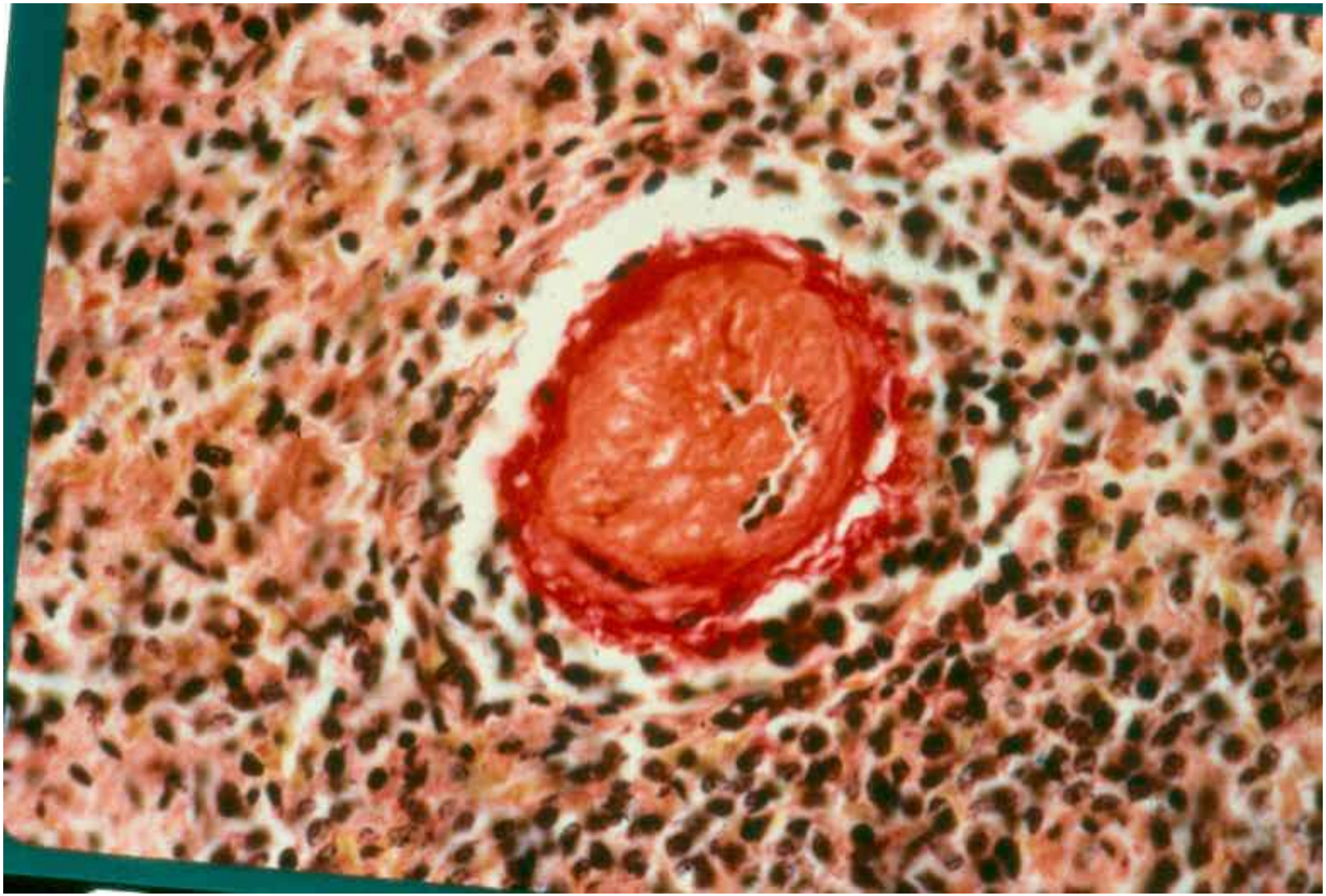
FATTY CHANGE



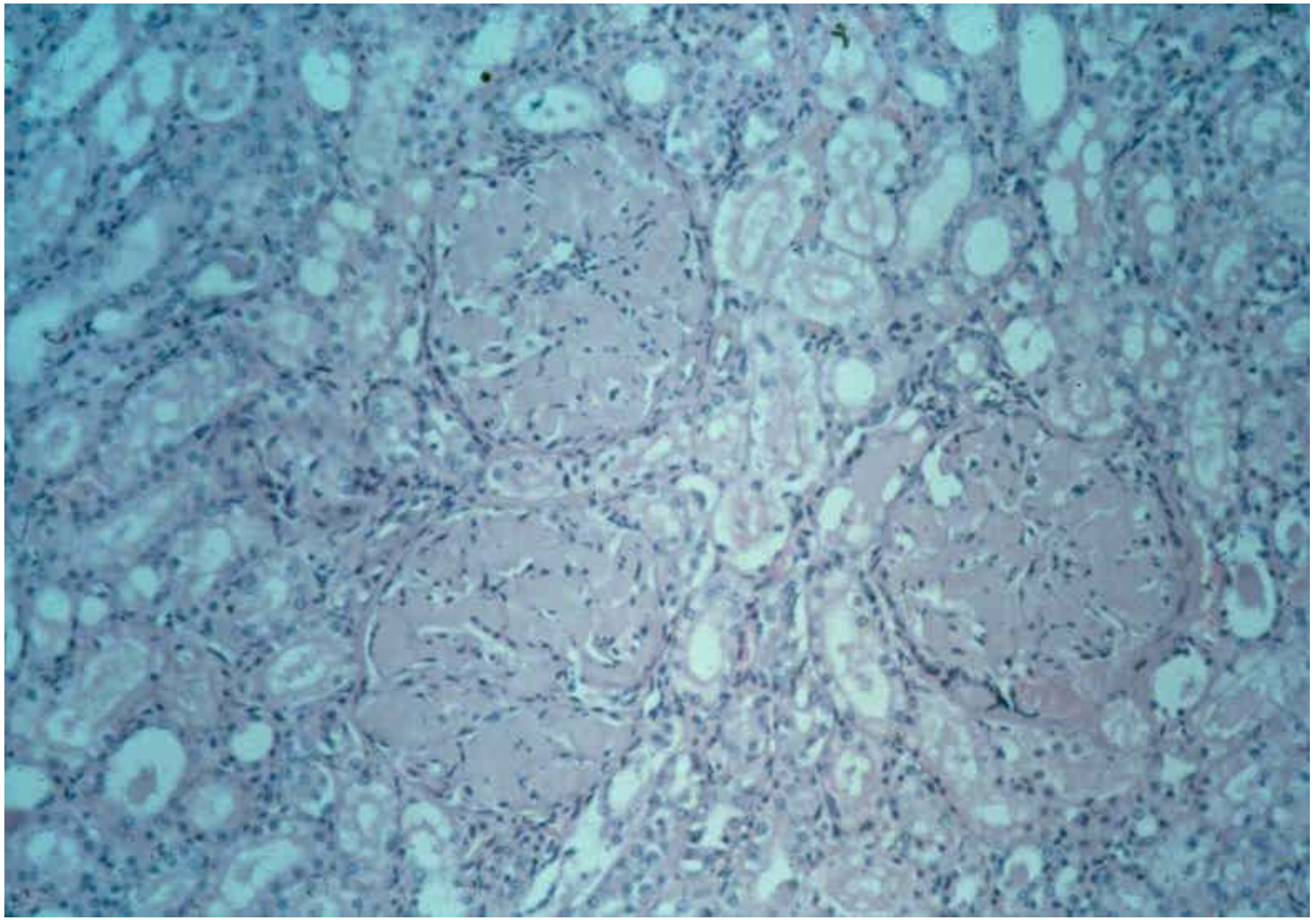
HYALINIZATION



FIBRINOID DEGENERATION



AMYLOIDOSIS
(At low magnification)



AMYLOIDOSIS
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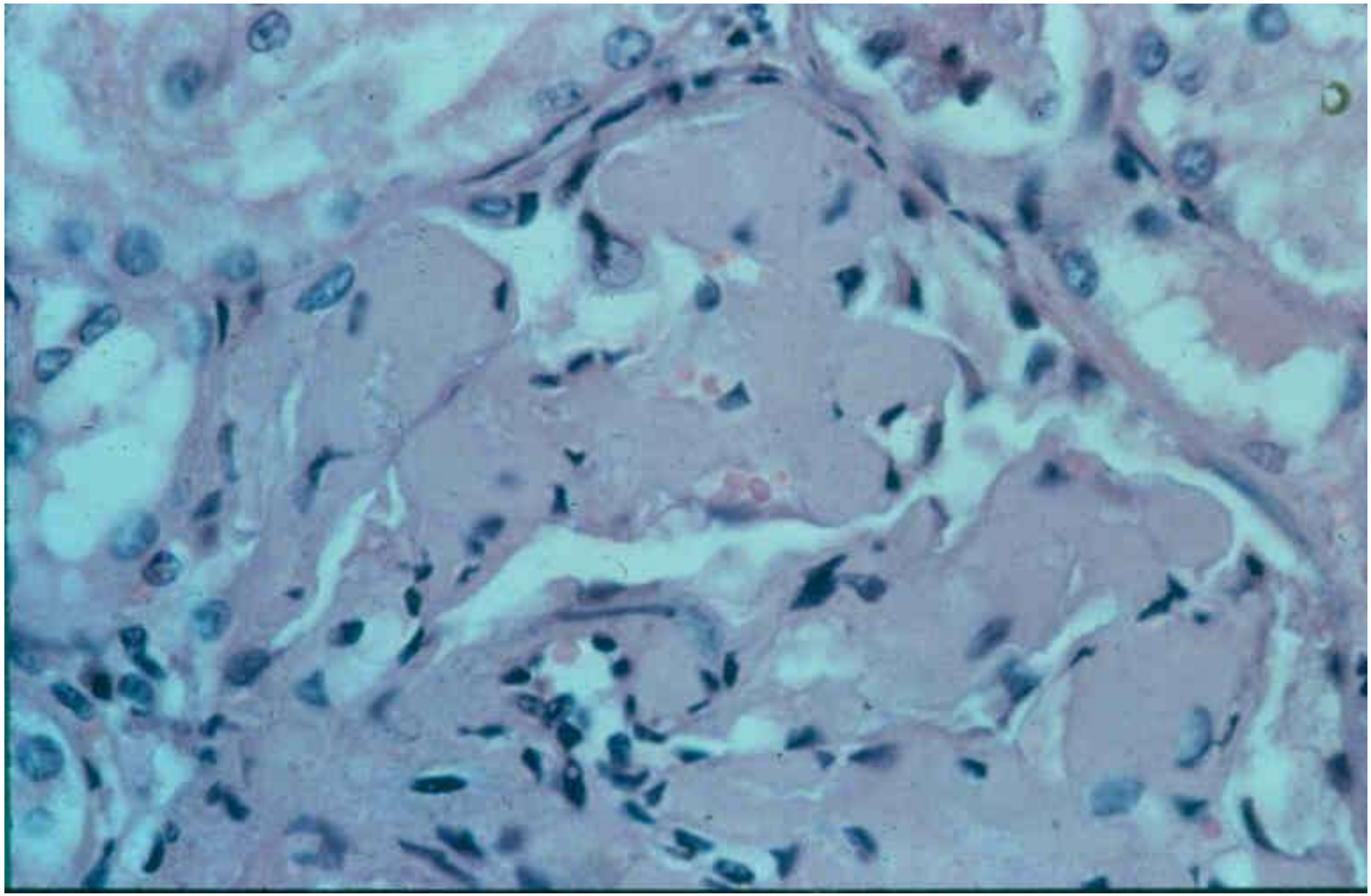
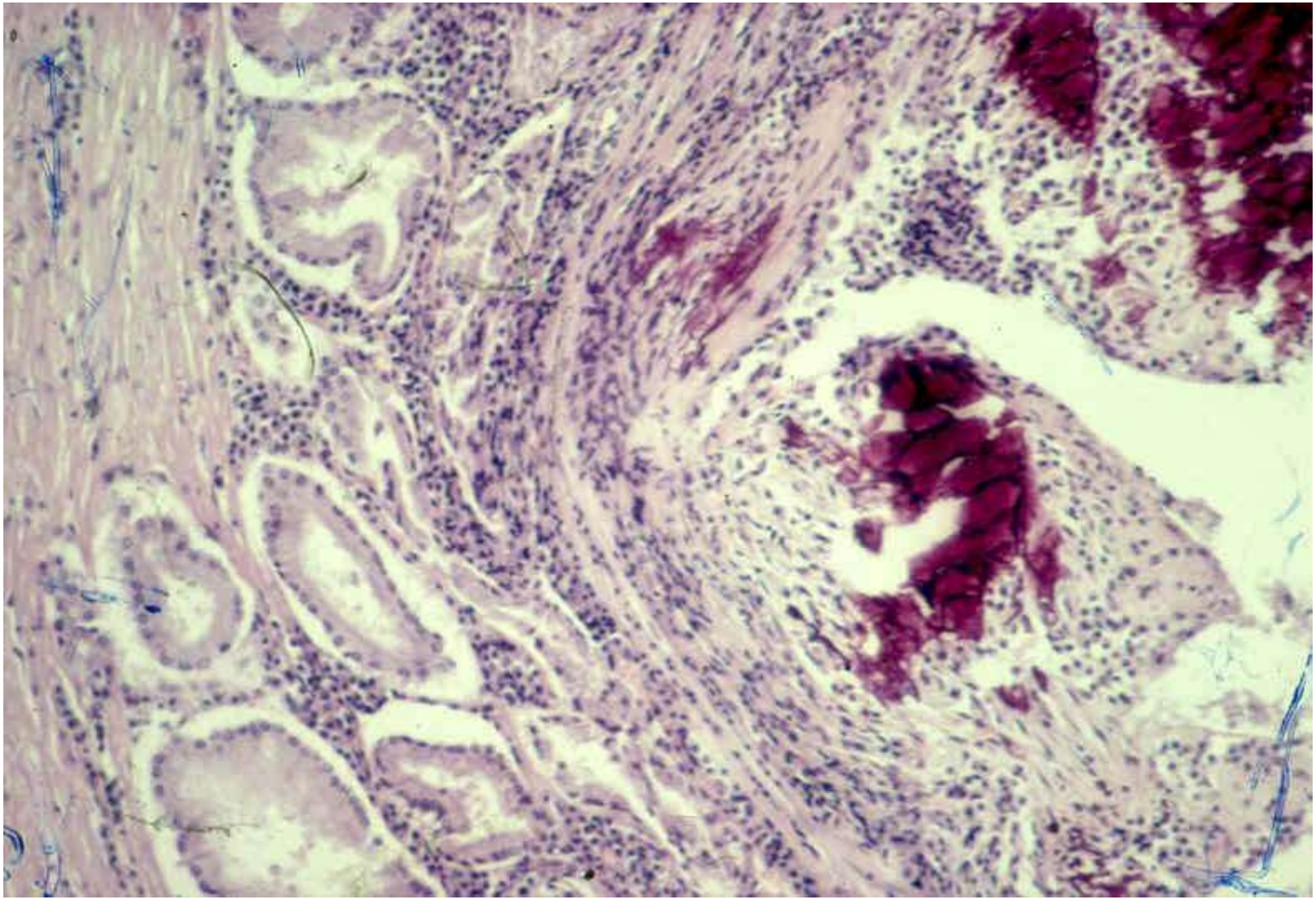
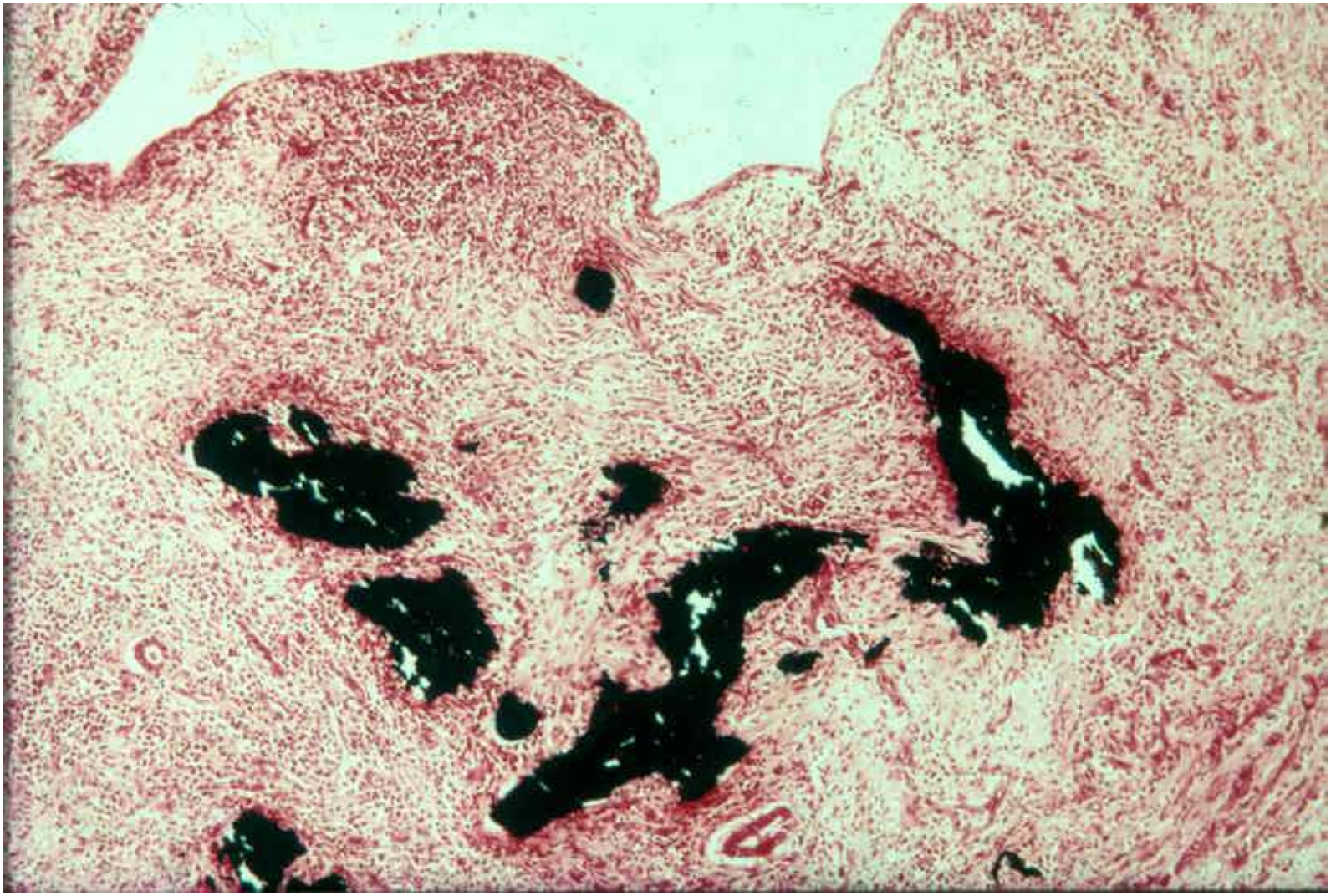


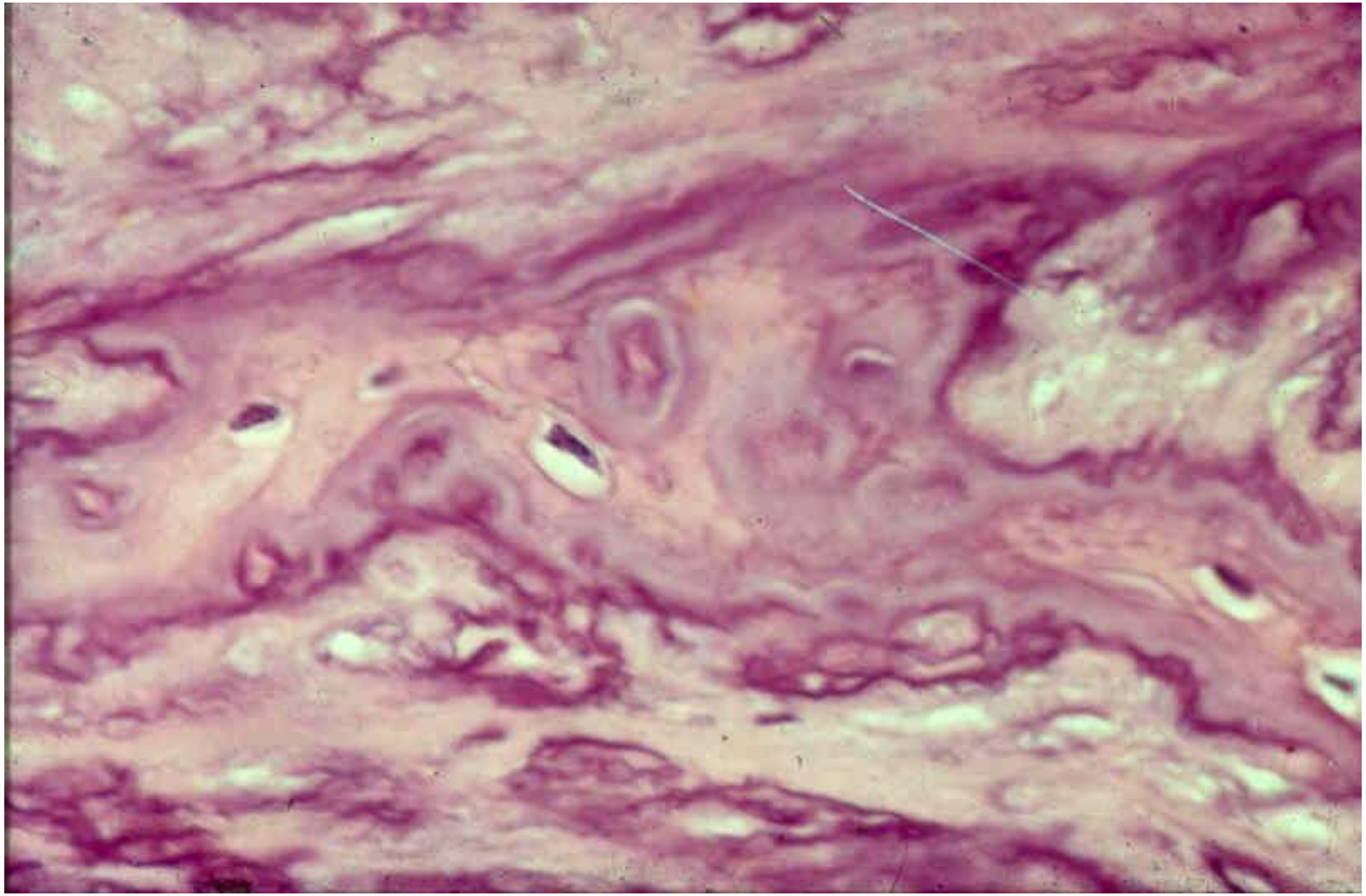
CALCIFICATION



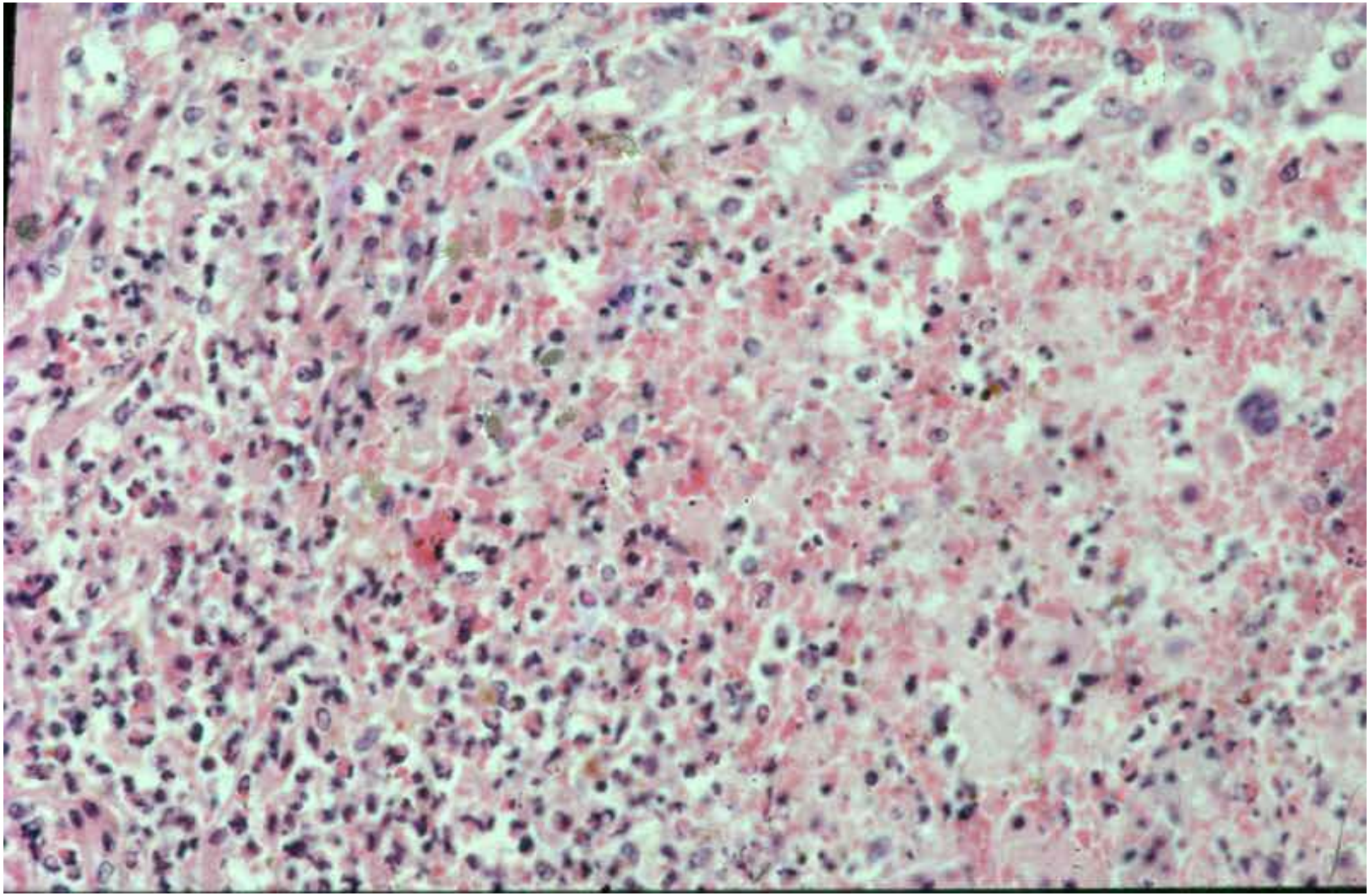
CONFIRMATION OF CALCIFICATION



METAPLASIA RESULTING FROM CALCIFICATION



COAGULATION NECROSIS



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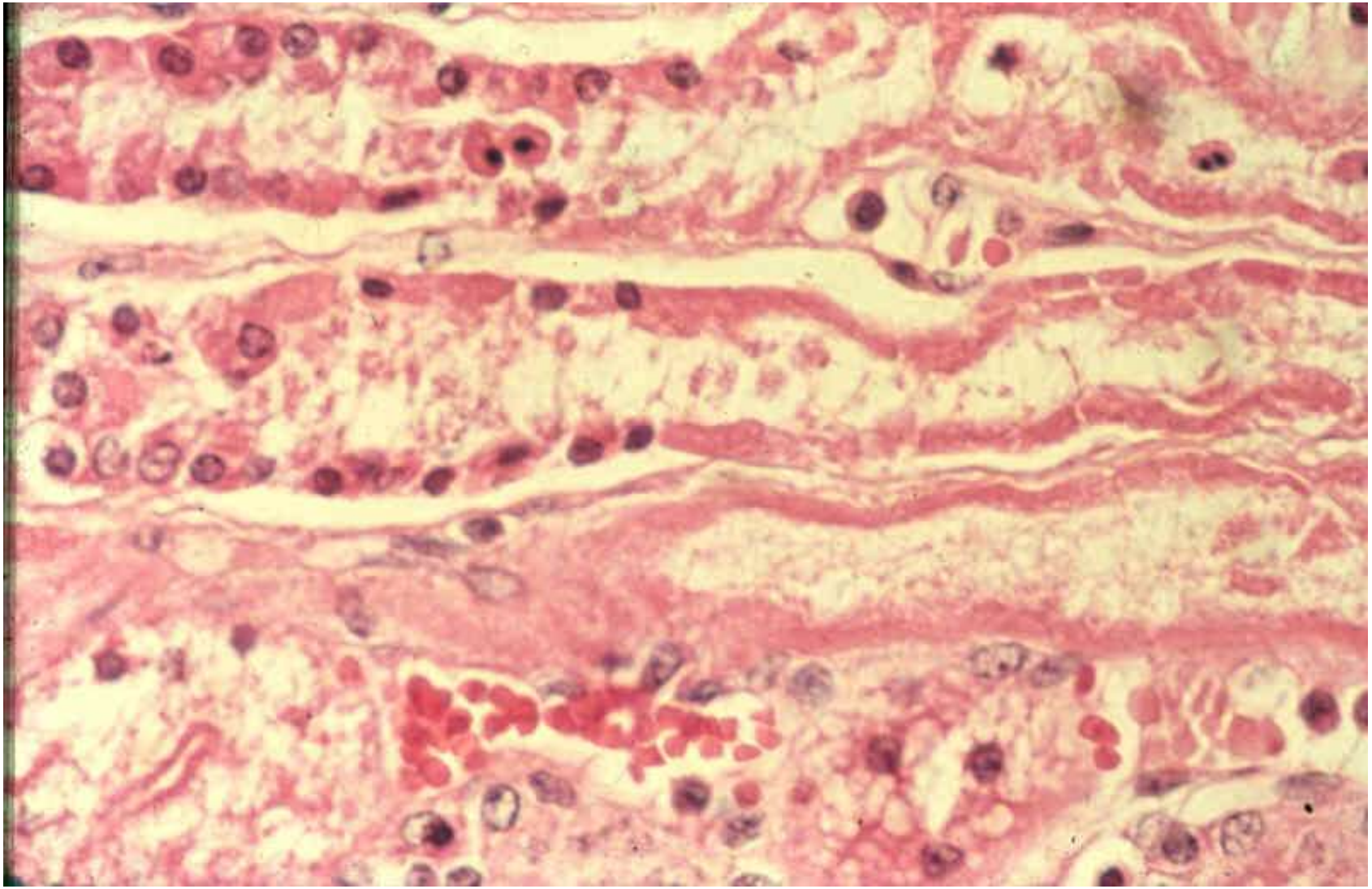
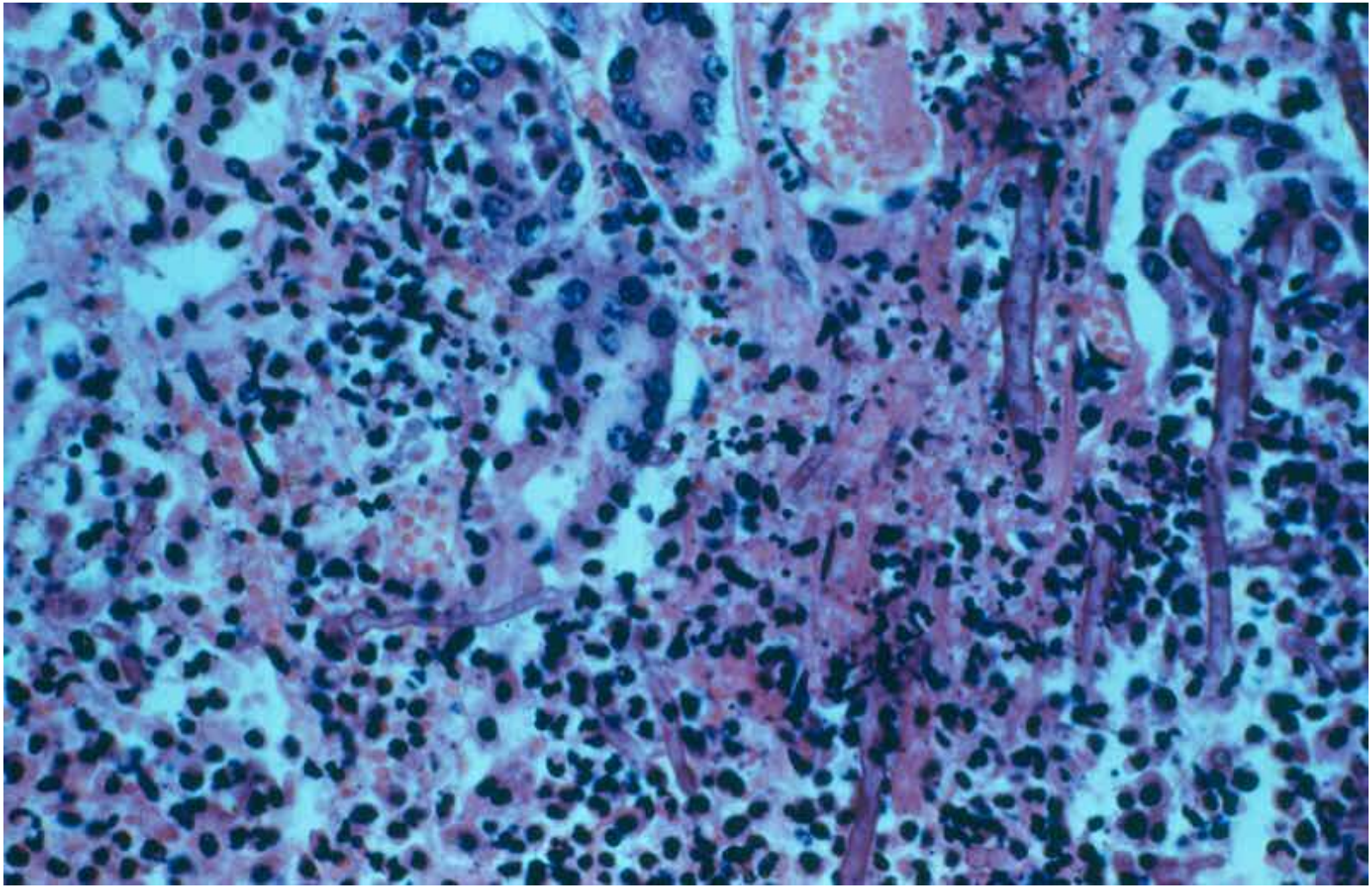
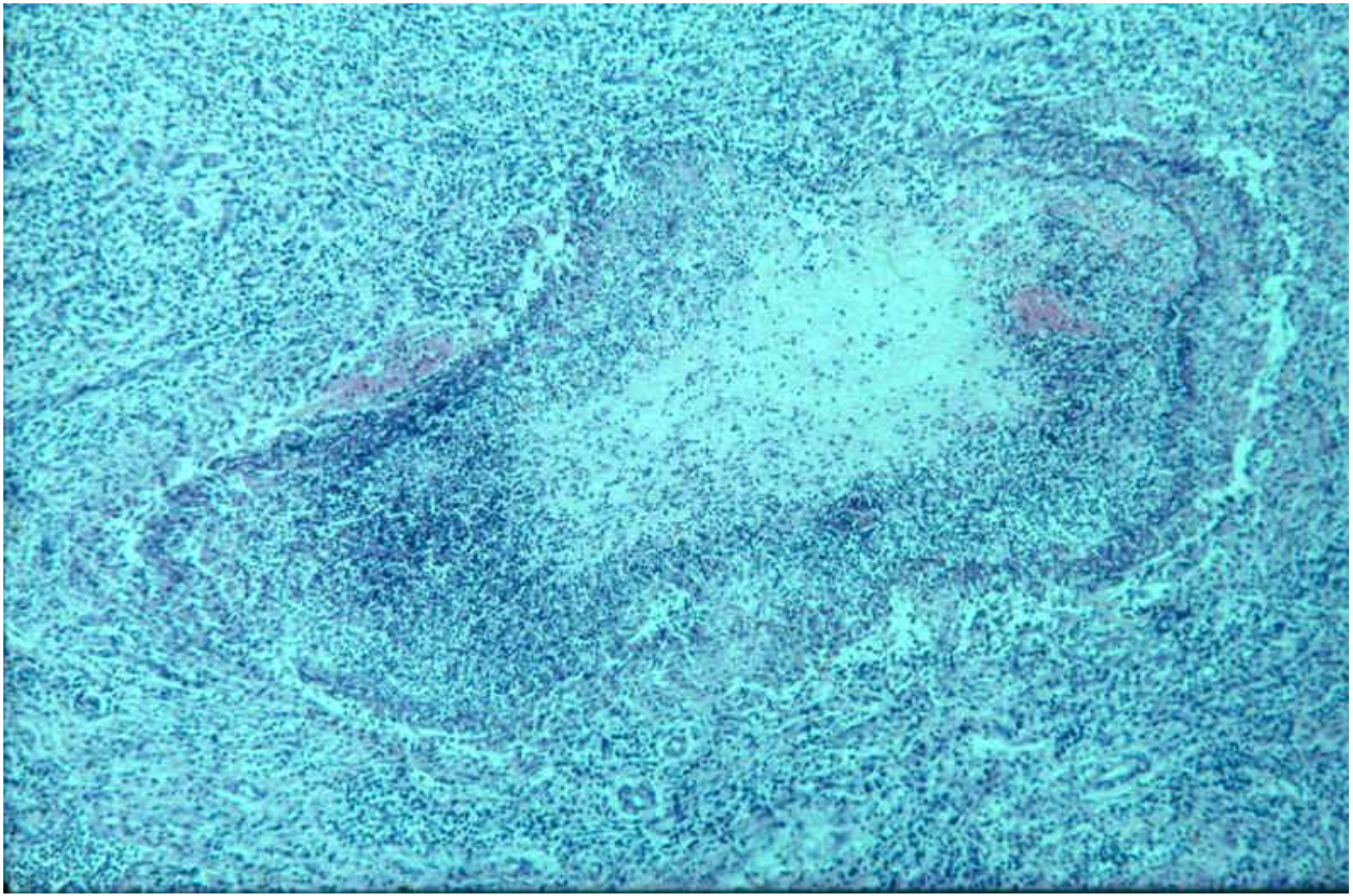


COAGULATION NECROSIS



LIQUEFACTION NECROSIS



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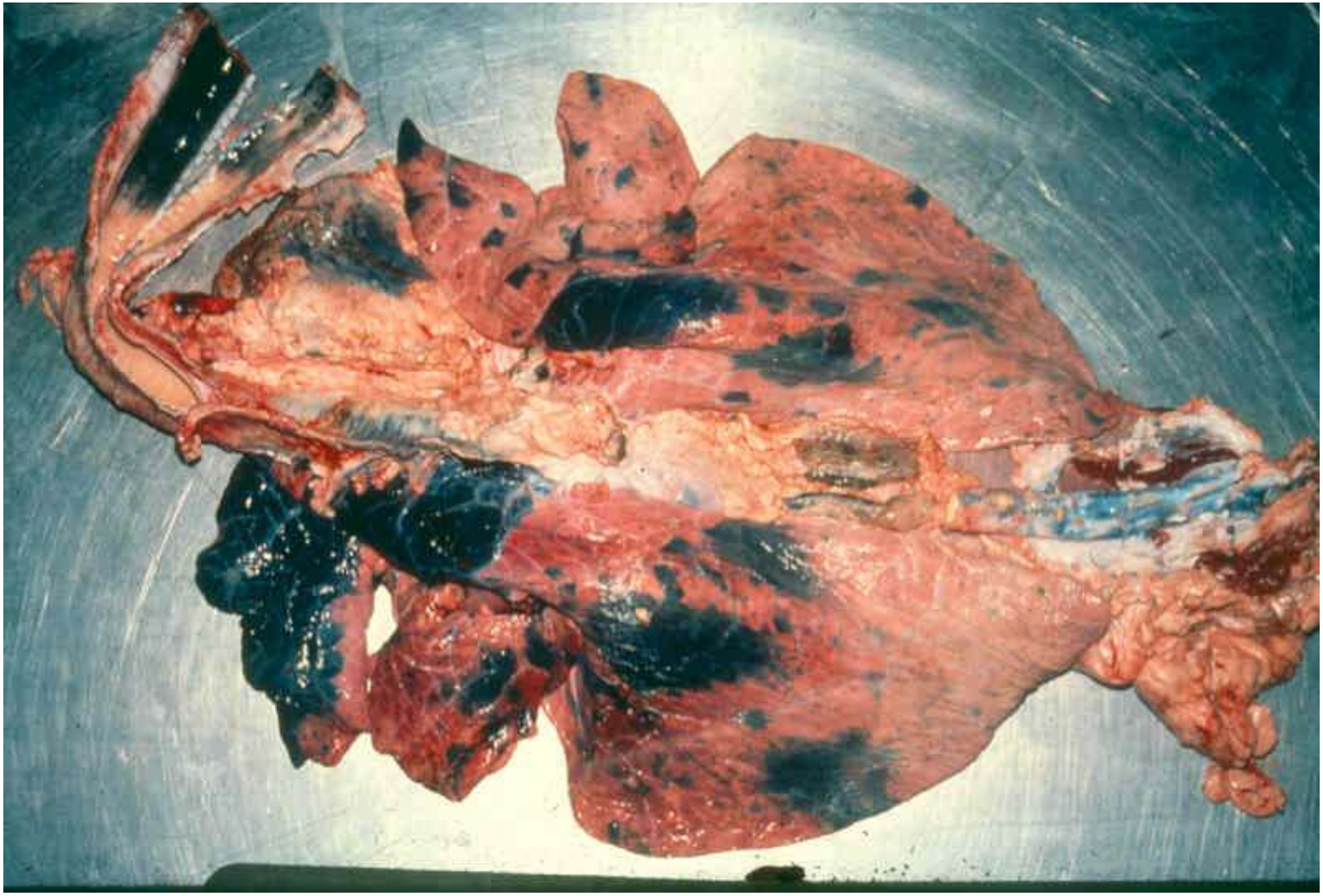

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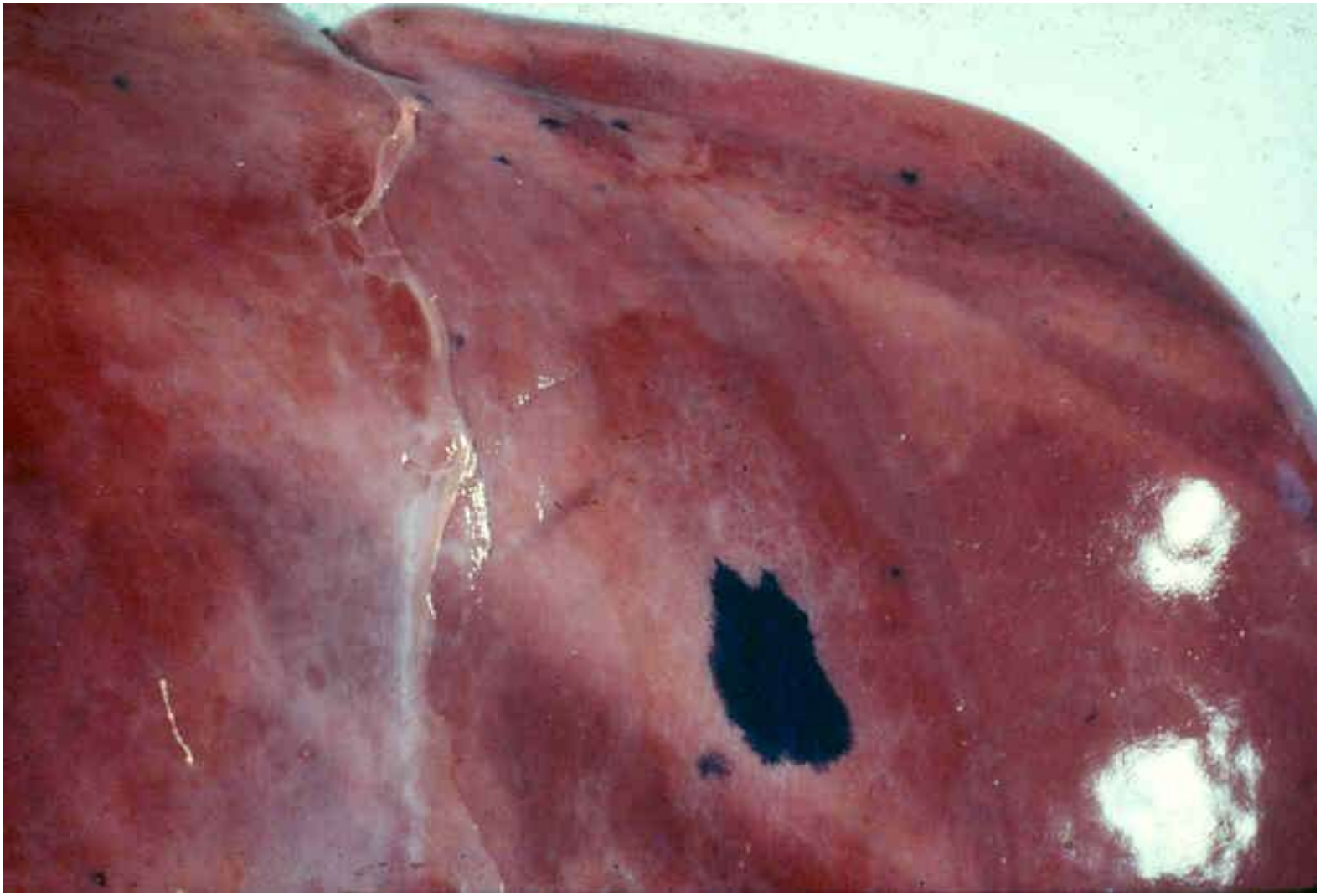
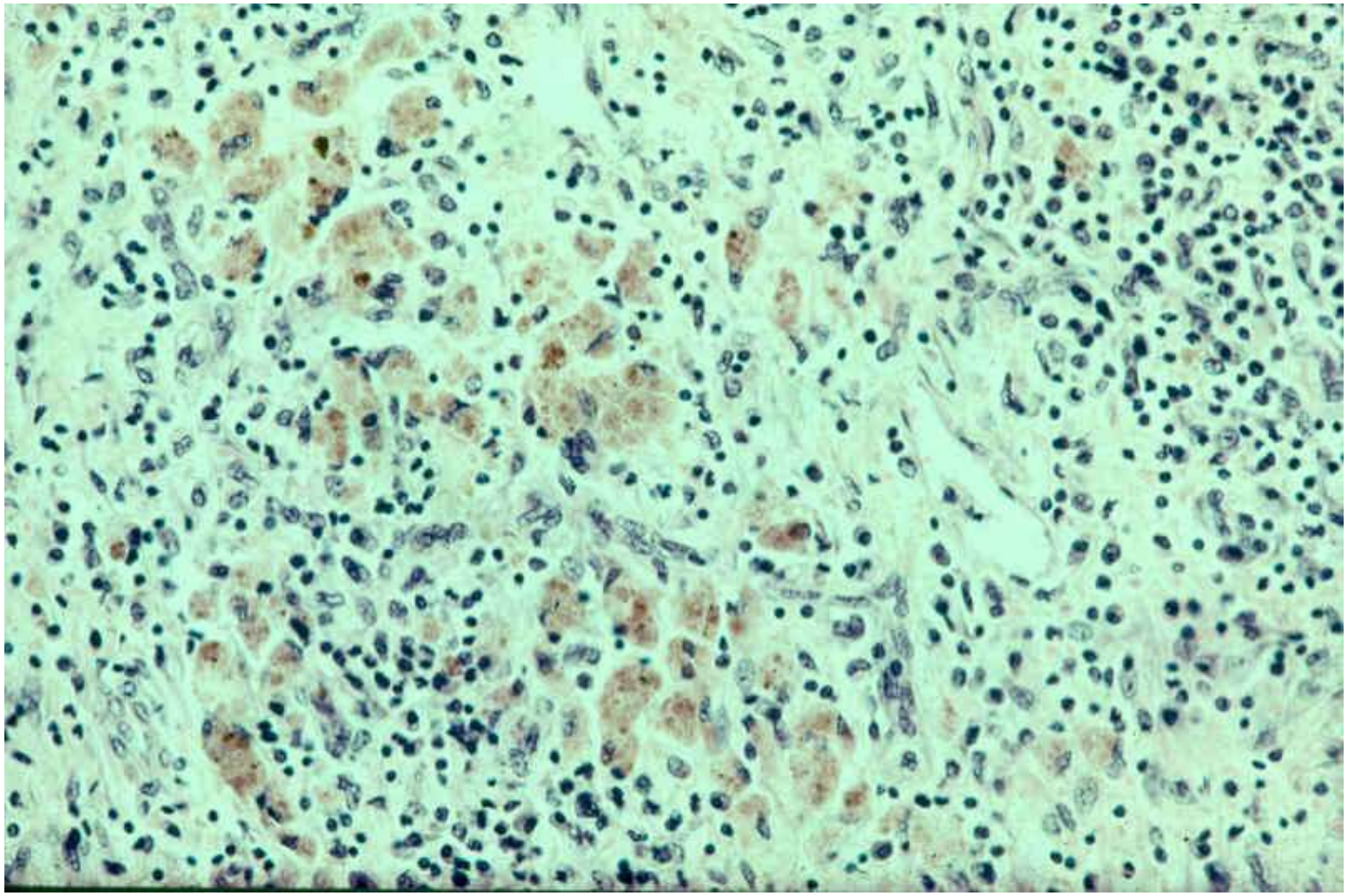
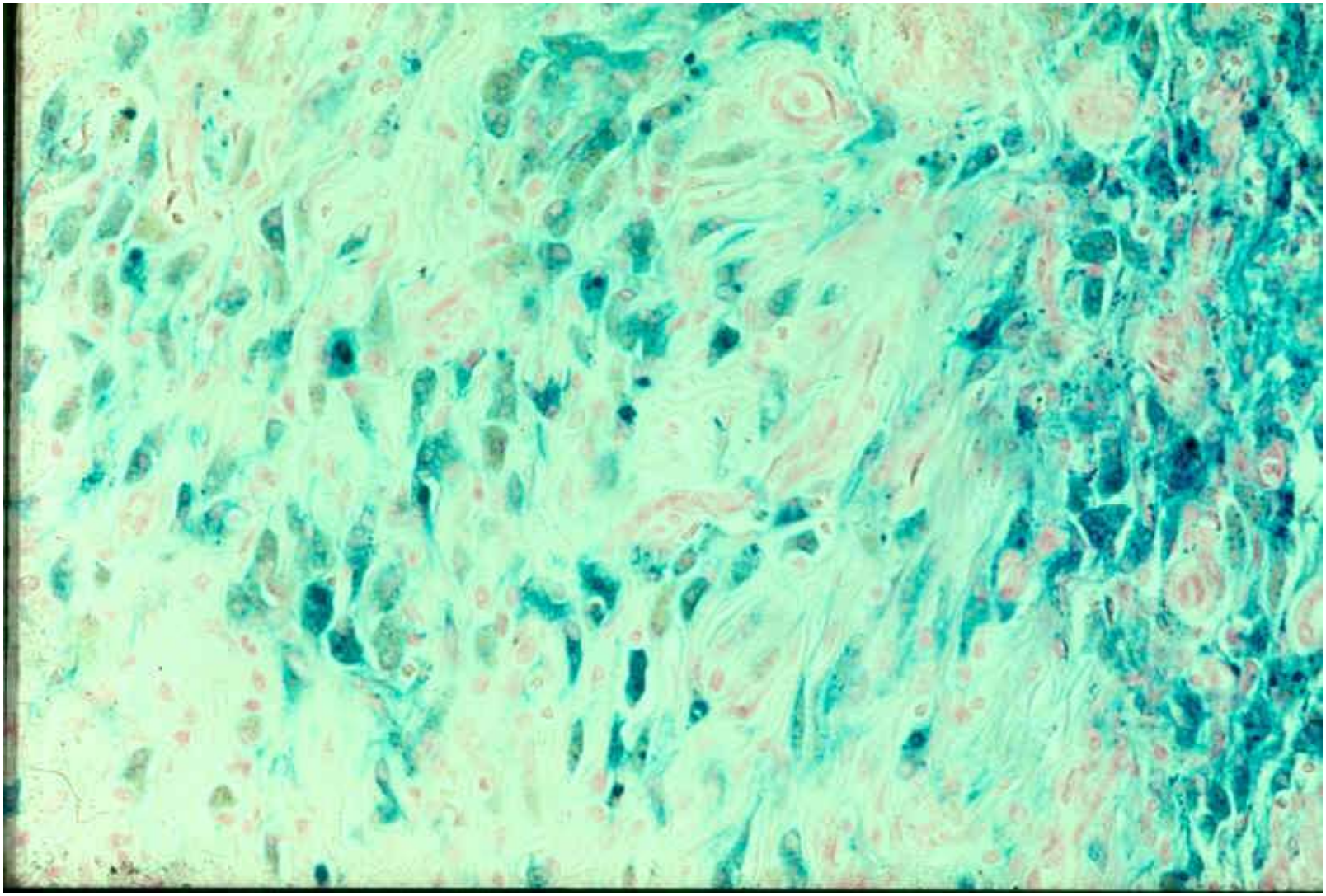


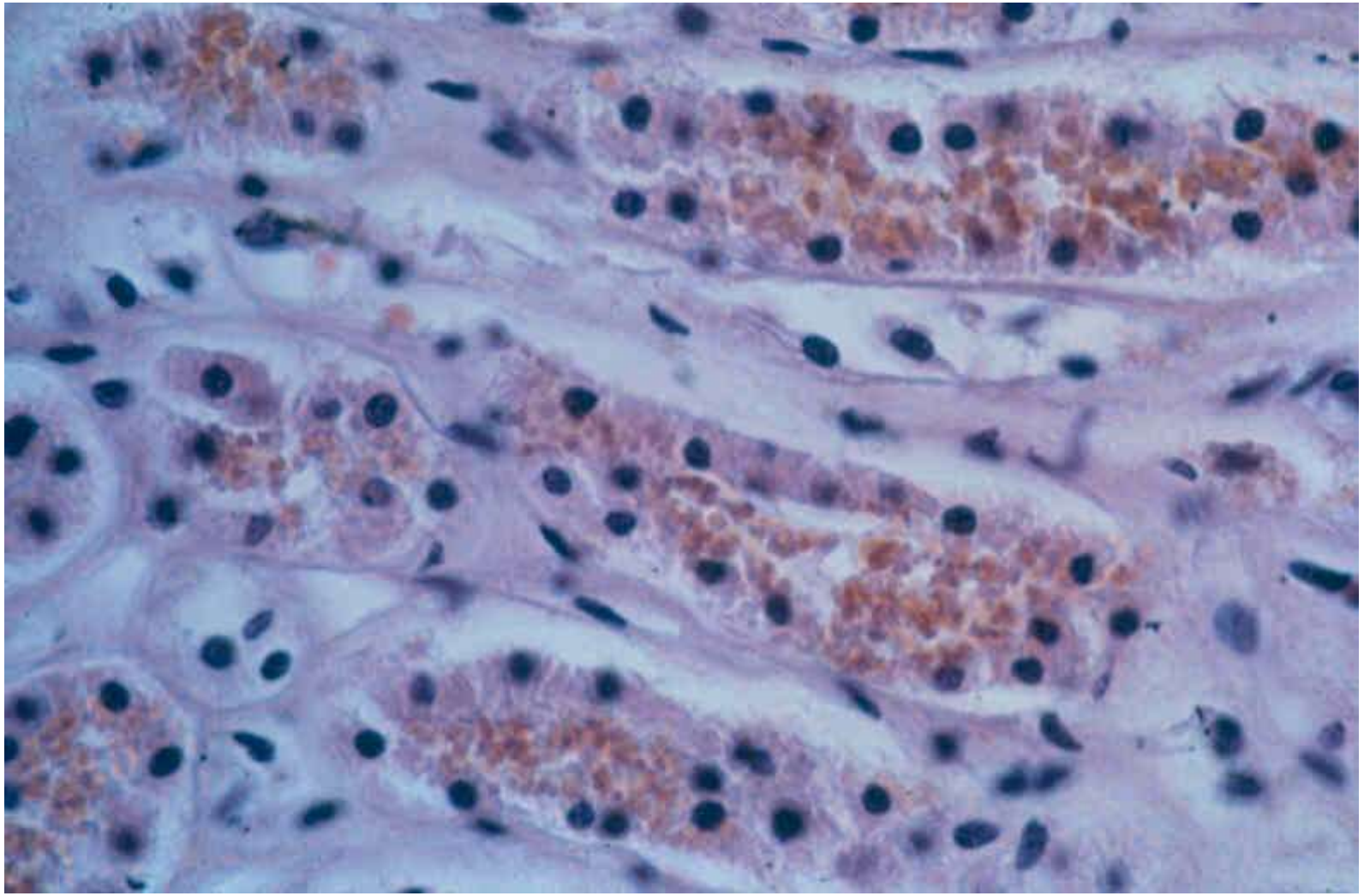
HEMOSIDERIN IN MAGROPHAGES



CONFIRMATION OF HEMOSIDERIN



HEMATIN



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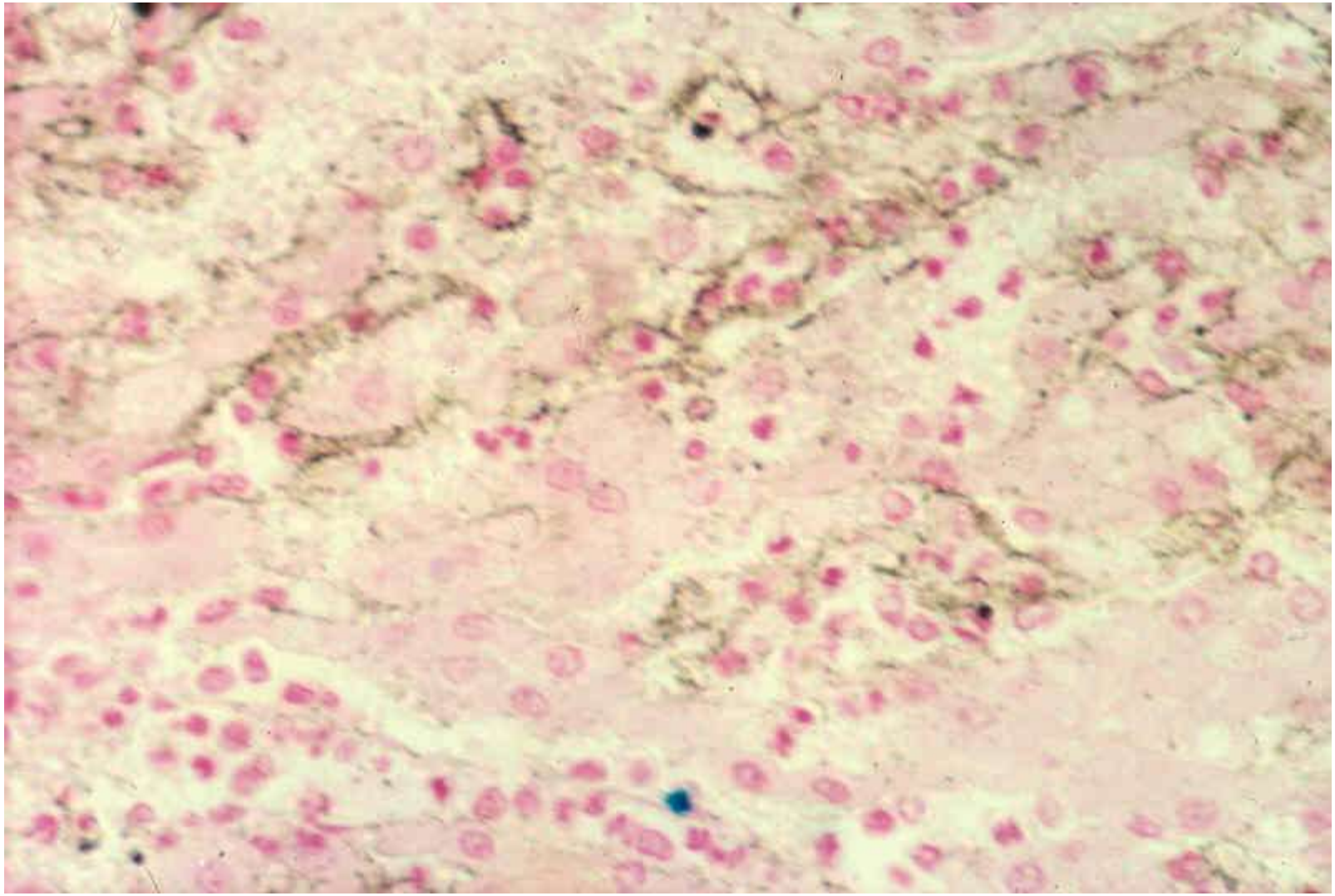
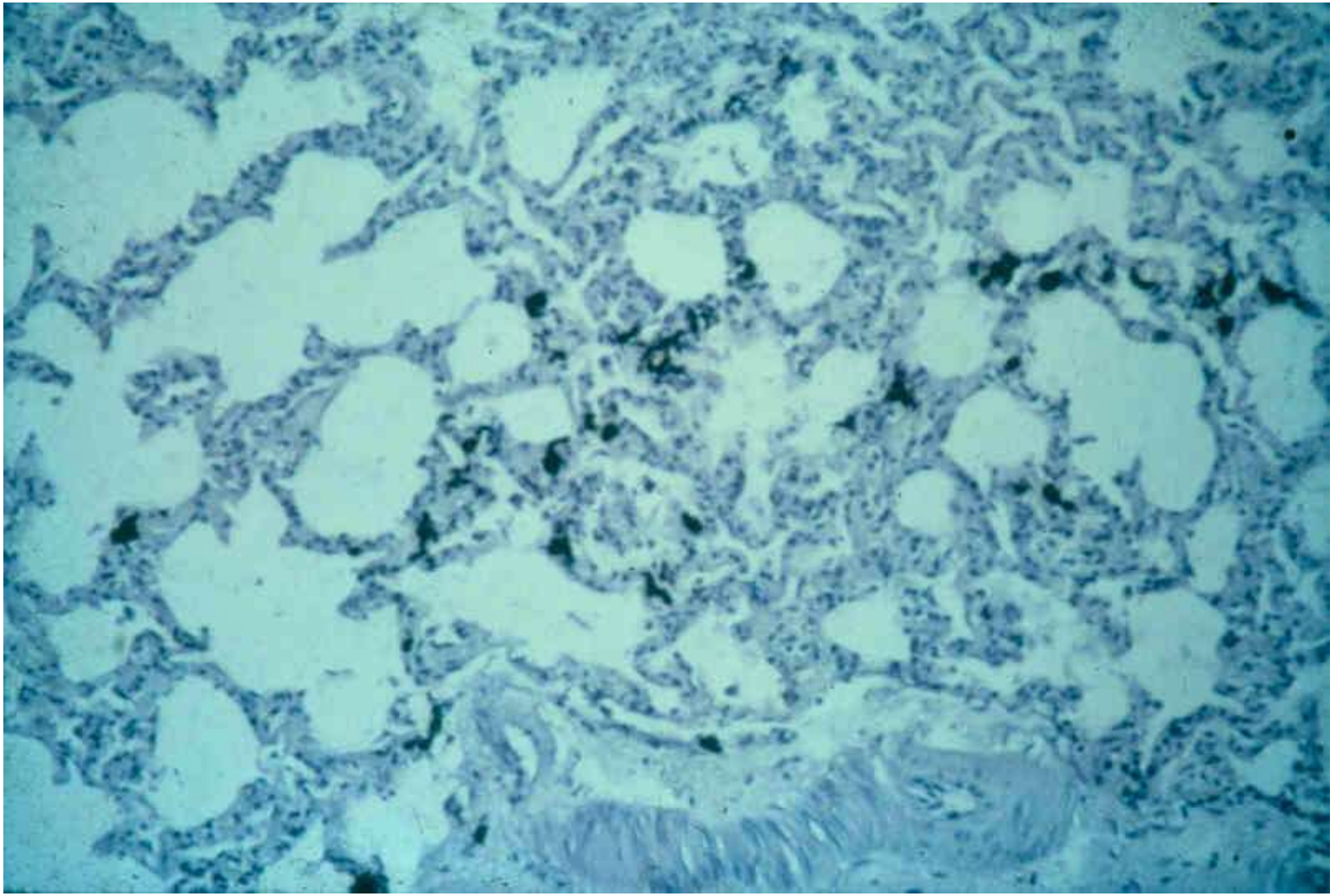


ANTHRACOSIS



PATHOLOGICAL PIGMENTATION

- This is defined as deposition of inherently coloured substances(pigments) in aberrant locations or in excess in locations normally found
- **Classification of pigments**
- Pigments are classified according to their origin as:
  - Exogenous and
  - Endogenous
- **1. Exogenous pigments**
- Originate from **outside** the body, entering the body through the skin, lungs or intestines and being deposited in macrophages of regional lymph nodes
- Some of the exogenous pigments undergo **modification** in the tissues e.g. mercury, which is converted to mercuric sulphide responsible for mercurial pigmentation

Effects of exogenous pigments

- Some exogenous pigments like **carbon dust** cause little or no harm in the tissues
- Others like silica, asbestos and iron produce tissue injury associated with extensive inflammatory reaction and healing by fibrosis
- The general pathological term for such lesion in the lungs due to inhalation of irritant elements is **pneumoconiosis**
- **2. Endogenous pigments**
- Endogenous pigments are further classified into four types based on their source of formation in the body:

Source of endogenous pigments in the body and the specific pigments derived from each source

- **Melanocytes**
  - Melanin
- **Haemoglobin**
  - Hemosiderin
  - Hematin
  - Bilirubin
- **Porphyrins**
  - Porphyrin
- **Lipids**
  - Lipofuscin
  - Ceroid

Melanin

- The pigment is produced in the skin by **melanocytes** located at the junction between the epidermis and the dermis
- The melanocytes have branching processes through which melanin granules(**melanosomes**) are transferred to **keratinocytes** in the epidermis
- The process of formation of melanin is called **melanization**
- The concentration of melanin in tissues determines the colour of the tissue

Melanin(cont.)

- In low concentrations, melanin appears **yellow-brown**, whereas in large quantities **black**
- In other situation, its dispersion may be that it appears **blue** or **green** e.g. in the sexual skin of some baboons
- Fish have additional reflecting cells called **iridocytes**, which contain **guanin** instead of melanin responsible for beautiful metallic sheen of many fish
- There are two types of animal melanin: brown to black insoluble called **eumelanin** and yellow to reddish brown soluble called **pheomelanin**
- The function of melanin is largely protective e.g. in chameleons, and in man against sunlight

Melanization

- **Melanoblasts** in the skin differentiate into:
- Melanocytes, which contain an amino acid **tyrosine**
- Tyrosine is oxidized to **dioxyphenylalanine**(DOPA) under the catalytic activity of enzyme tyrosinase
- DOPA is changed into **melanosomes**
- Melanosomes are sent to **keratinocytes**

Pathological conditions resulting from abnormal melanization

1. **Albinism** defined as lack of melanin in the entire skin, caused by lack of tyrosine
2. **Focal congenital hypomelanosis** defined as lack of melanin in some parts of the body, caused by congenital lack of melanoblasts in the affected areas
3. **Chronic dermatitis** defined as inflammation of the skin caused by lack of melanin in kerationcytes
4. **Chediak-Higash syndrome** defined as excessive accumulation of melanin in some parts of the skin, caused by proliferation of neoplastic melanocytes(melanoma)

Hemosiderin

- Hemosiderin is the insoluble stored form of iron in macrophages bound to ferritin
- The source of iron is usually haemoglobin arising from hemolysis
- Destruction of erythrocytes occurs in different haemoparastic **diseases** or when blood escapes from the blood vessels(**haemorrhage**) or when blood stagnates in the blood vessels(**congestion**)
- Following hemolysis, haemoglobin dissociates into hem and globin and it is the hem part that is taken by the macrophages in which iron still remains in its **ferrous** state

Hemosiderin(cont.)

- In H&E stained sections, deposits of hemosiderin appear **golden brown**
- A special stain for the ferrous iron in hemosiderin is **Perl`s**, which stains the deposits **blue**
- **Hematin**
- Hematin is insoluble deposits of iron in the ferric state that accumulate outside cells
- As for hemosiderin, hematin originates from haemoglobin arising from hemolysis
- Under normal conditions, haemoglobin that is released into the circulation, is excreted via urine

Hematin(cont.)

- However, under conditions involving excessive release of haemoglobin, the renal threshold for haemoglobin excretion is exceeded
- Some of the haemoglobin in the circulation is **oxidized** to methemoglobin i.e. the ferrous iron becomes ferric iron
- **Methemoglobin** in turn dissociates into hematin(**ferriheme**)
- Hematin is then bound to hemopexin to form **hematin-hemopexin** complex
- Any remaining unbound hematin is bound to albumin to form **methemalbumin**
- The two compound hematin-hemopexin and methemalbumin are converted to **bilirubin** in the liver

Identification of hematin

- In H&E stained sections, hematin appears brown, hence can be confused with hemosiderin
- Distinction between hematin and hemosiderin in histological section is based on the facts that:;
  - Hematin is found outside cells
  - Hematin does not stain positive with Perl`s stain
- NB haemorrhages and use of non-buffered formalin induce formation of hematin artifacts in tissues

Bilirubin

- Bilirubin is the **yellow** bile pigment formed from haemoglobin
- Following hemolysis of senescent erythrocytes, the released haemoglobin(Hb) is normally taken by macrophages of the reticuloendothelial system(RES)
- In the RES, the Hb dissociates into **heme** and **globin**
- The amino acids of globin are **recycled**, whereas the heme, **a cyclic** molecule made up of four **pyrole** rings and centrally placed ferrous iron is opened
- Oxidation of the heme is catalysed by **heme oxygenase** and leads to its opening into a linear molecule called **biliverdin** and carbon monoxide

Bilirubin(cont.)

- In the next reaction, biliverdin is reduced to **unconjugated bilirubin** under the catalytic activity of biliverdin reductase
- The unconjugated bilirubin is then released from the RES into the circulation, where it is **bound** to albumin
- The albumin-bound unconjugated bilirubin is transferred into hepatocytes
- The rate of transfer is dependent on:
  - The concentration of bilirubin in blood
  - The concentration of albumin in blood
  - The blood flow to the liver
  - The concentration of gamma-protein in hepatocytes
  - The concentration of unconjugated bilirubin on the gamma-protein

Bilirubin(cont.)

- The gamma-protein releases the unconjugated bilirubin at the smooth endoplasmic reticulum adjacent to bile canaliculi
- At these sites, there is a conjugation enzyme called **glucuronyl transferase**, which activates two molecules of **glucuronic acids**, to enable them combine with the unconjugated bilirubin to produce **conjugated bilirubin(**bilirubin diglucuronide)
- The conjugation enzyme is not fully developed until the new born is about one month, thus premature infants have impaired conjugation
- Thus, abrupt hemolysis that occurs at the time of birth leads to uconjugated bilirubin load
- When the levels exceed 200mg/100ml,the possibility of entry into the central nervous tissue(brain) and subsequent brain damage is quite high
- Such brain damage resulting from unconjugated bilirubin is called **kernicterus**

Bilirubin(cont.)

- The conjugated bilirubin then passes through the biliary tree to the gall bladder
- Conjugated bilirubin is converted to urobilinogen in the large intestine by the action of bacterial glucuronidases
- 10% of urobilinogen are reabsorbed and passes unchanged in the liver and enters the circulation from where it is excreted through urine
- Liver damage may lead to significant amounts of urobilinogen in the urine
- 90% of urobilinogen is reduced to give stercobilinogen that is finaly oxidized to stercobilin, which imparts the brown colour of feces

Jaundice/Icterus

- These are synonymous terms, which mean yellow colouration of tissues due to **hyperbilirubinaemia**
- Hyperbilirubinaemia may occur due to:
  - Excessive hemolysis
  - Damage of hepatocytes
  - Blockage of flow of bile/blokage of bile ducts
- **Types of jaundice**
  - Pre-hepatic
  - Hepatic
  - Post-hepatic

Porphyrins

- Porphyrins are organic compounds made up of four pyrrole rings(pentagon-shaped rings of four carbon atoms with a nitrogen) and a centred bound metal ion
- Porphyrins differ in terms of:
  - Metal ion e.g. Fe- heme, Mg – chlorophyll,
  - Eight side chains, two on each pyrrole ring
- In the course of formation of heme, deficiencies in some enzymes have been reported to result into occurrence of abnormal intermediate metabolites of porphyrins

Porphyrins(cont.)

- For example, lack of uroporphyrinogen decarboxylase(**UPG decarboxylase**), prevents uroporphyrinogen III to progress into the subsequent stage, hence being oxidized into a porphyrin isomer
- Similarly, lack of corproporphyrinogen oxidase(**CPG oxidase**), prevents corproporphyrinogen III to progress to protoporphyrinogen III
- Excess formation of abnormal porphyrin isomers leads to their deposition in tissues

Effects of deposition of porphyrin isomers in tissues

- **Porphyria** is a pathological state characterized by accumulation of abnormal porphyrins in tissues
- The disease is also known as **osteohemochromatosis** due to reddish brown bone pigmentation or **pink tooth** due to colour of teeth
- Deposition of abnormal porphyrins results into photodynamic dermatitis in cattle with sloughing of the affected areas
- Photodynamic dermatitis occurs due to the fact that the porphyrin isomers are **fluorescent**, hence they become activated by sunrays and produce free radicals that induce **photosensitization**

Lipofuscin

- Lipofuscin is a golden brown granular pigment derived from break down of lipid membranes, commonly observed in myocardial and nerve cells of old animals
- Hence the pigment is regarded as an aging pigment, and the pathological term referring to deposition of lipofuscin is **lipofuscinosis**
- Factors associated with lipofuscinosis include:
  - Chronic tissue injury
  - Vitamin E and selenium deficiencies
  - Increased intake of diets reach in unsaturated fatty acids

Ceroid

- Ceroid is a golden yellow brown pigment believed to be a **variant** of lipofuscin
- It differs from lipofuscin by being acid-fast and **autofluorescent**