Professor V.F. Voino-Yasenetsky Krasnoyarsk State Medical University
Department of Pathological Anatomy named after Professor P.G. Podzolkov

Lecture 1.
Pathological Anatomy (Pathology) as a Science. Tasks and Subject of Study. Introduction to Pathology. Pathology of metabolism.

Contents:

Part 1.

- Tasks of pathology as science, subject, specialty;
- Introduction to pathology;
- General and private pathological anatomy;
- Objects, levels and methods of pathologoanatomical investigations.

Part 2.

- Dysproteinosis;
- Dyslipidosis;
- Carbohydrates metabolism disorders;
- Morphology of pigment metabolism disorders.

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"**Pathological anatomy**" (Pathology)

- is an applied science that studies the structural basis of the diseases.

•This is the science of changes in the architecture of the cells, tissues, and organs in pathological conditions (disorders).

•Pathological anatomy is strongly associated with other medical and biological disciplines.

•**Pathology** is the science of the patterns of occurrence and development of diseases.

•Pathological anatomy has both scientific and practical significance and it is one of the clinical disciplines in healthcare.

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The scope of pathological anatomy:

- **General pathological processes** – such as injury of local and general blood circulation, inflammation, tumors and so on;
- **Etiology** (causes of the diseases);
- **Pathogenesis** (mechanism of pathological processes development);
- **Morphogenesis** (development of structural changes in diseases step by step);
- **Pathomorphosis** (changes in the diseases, morbidity, mortality, and disease course);
- **Iatrogenies** (pathology as a result of medical intervention).

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General pathological anatomy includes General pathological processes underlying all diseases:

- Alteration;
- Dystrophies (Injury);
- Blood and lymph circulation disturbances;
- Necrosis;
- Inflammation;
- Immunopathological processes;
- Regeneration;
- Adaptive and compensative processes.

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The objects of study are:

- Organs and tissues biopsies;
- Operational material;
- Cytological material;
- Postmortem material;
- Experimental material.

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Biopsy

is an intravital tissue resection from a patient for diagnostic purposes.

It serves as the main method of establishing a diagnosis.

Modern instrumental and diagnostic capabilities make it possible to obtain biological material for research from almost any part of the patient's body.

Diagnostic work of clinicians is **impossible without the participation of a pathologist.**

Any biological material obtained during a biopsy or surgical intervention is subject to mandatory histological examination, regardless of the disease for which the operation was performed.

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The main stages of pathological examination

Macroscopic (Gross) examination

- Description of external characteristics
- Discriptin of the material in the section

Material cutting

- Marking (Labeling) the margins of the resection
- Cutting small tissue fragments

Histoprocessing

- Fixation (formalin)
- Processing in chemical reagents

Preparation of histological slides and microscopic examination

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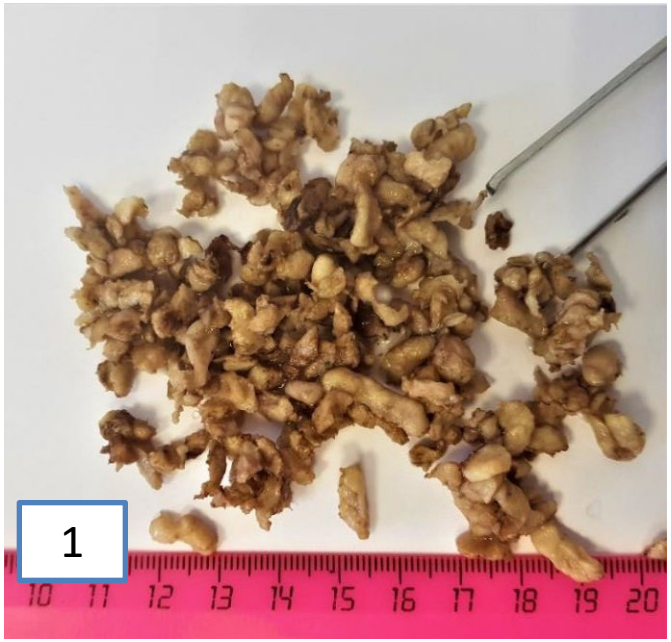
Gross examination and labeling



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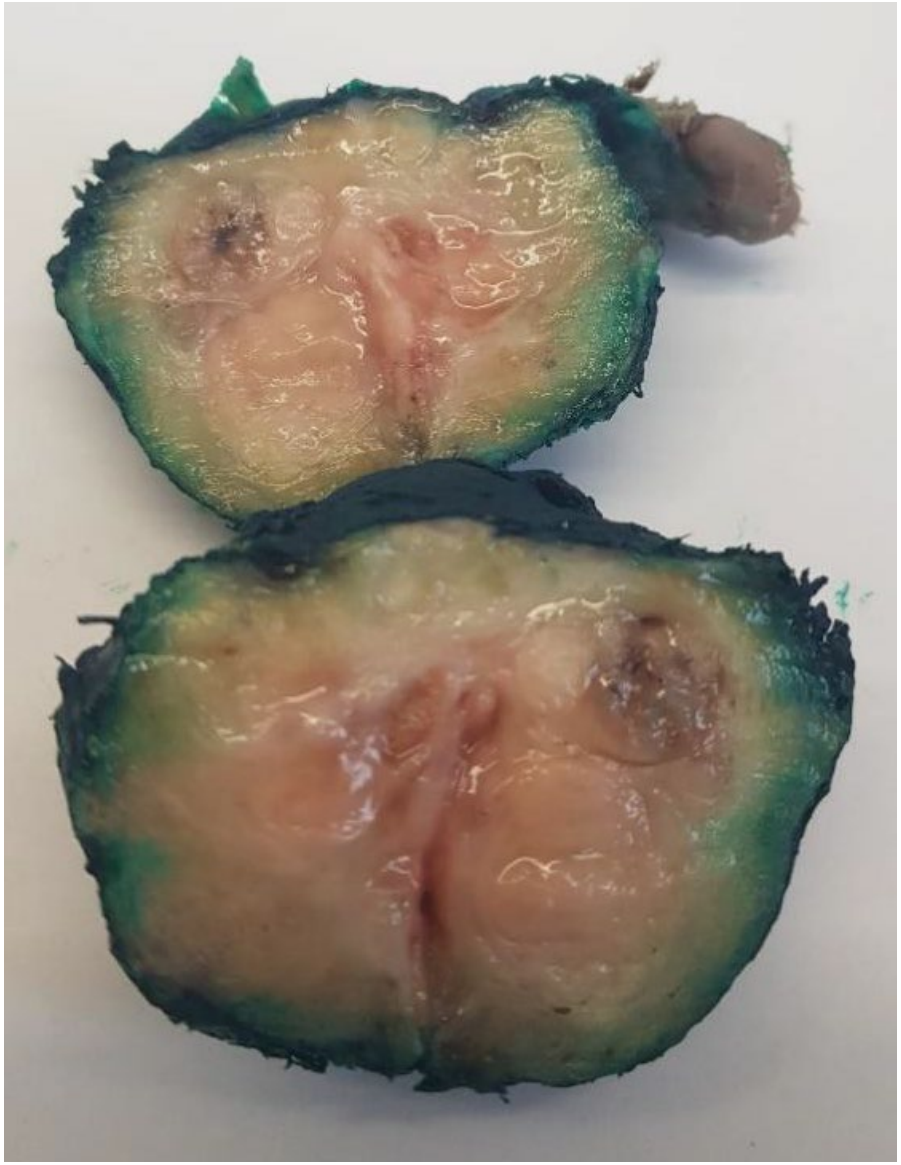
Cutting



- For microscopic examination, a pathologist cuts out individual small fragments.
- There are special protocols and recommendations governing the rules of the gross examination of organs and tissues.

Own data

An example of a macroscopic examination of the prostate gland

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Standard stages of histological processing

STAGE 1

- **Fixation in formalin** (at least 24 hours).
- This is the most critical stage, violation of which leads to irreversible damage of the tissue.
- Haste at this stage is unacceptable.

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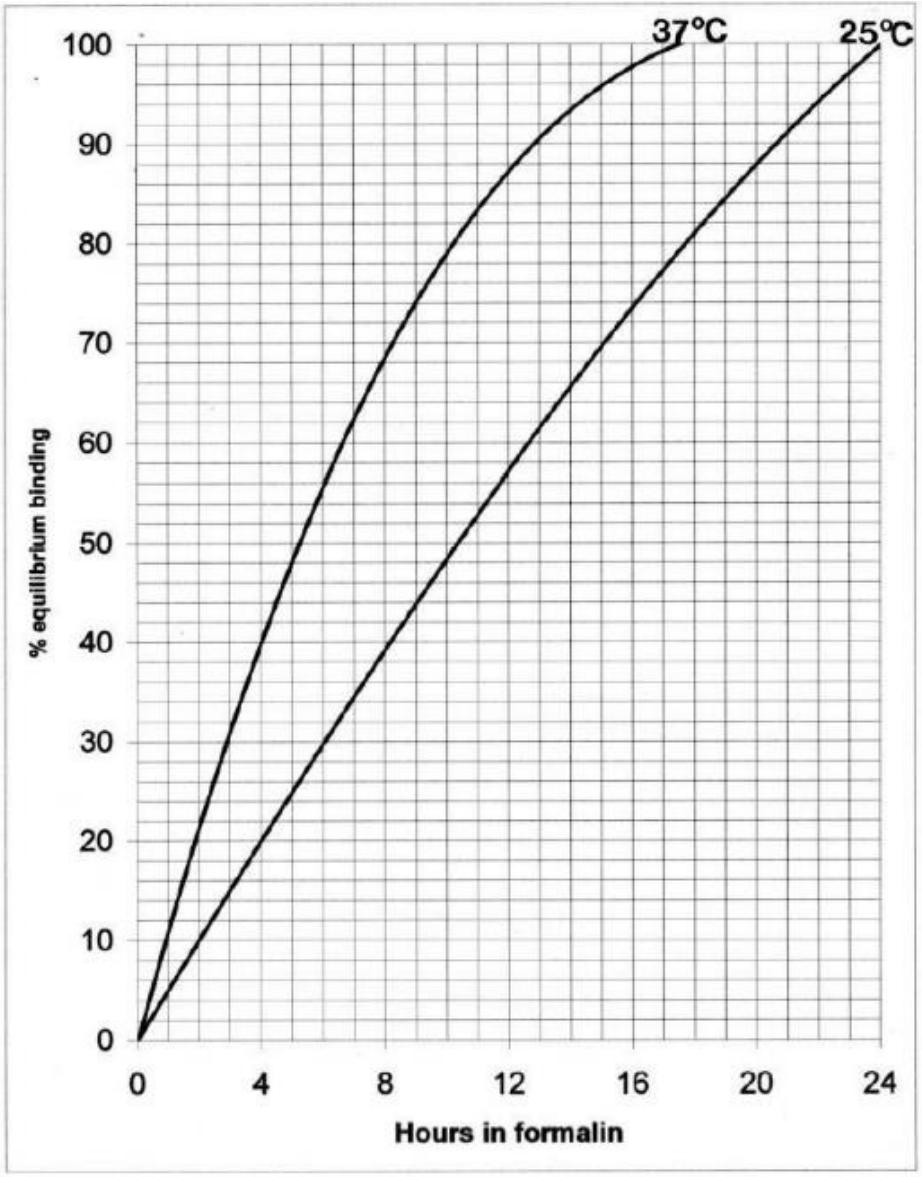

Fox CH, Johnson FB, Whiting J, Roller PP: Formaldehyde fixation. J Histochem Cytochem, 1985; 33(8):845-853

Stage 2

- **Processing in alcohols, xylene.**

Stage 3

- **Paraffin impregnation**

Stages of histological processing are carried out either in containers (jars) placed in thermostats or in histoprocessors

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Histological blocks - plastic cassettes in which biological tissue fragments are imbedded with paraffin

- paraffin blocks are installed in the microtome and thin sections with a thickness of 2 to 5 microns are made from them, which are applied to the histological glass.

Depending on the objectives of the study, slides are stained with histochemical dyes (there are hundreds of stains and their modifications).

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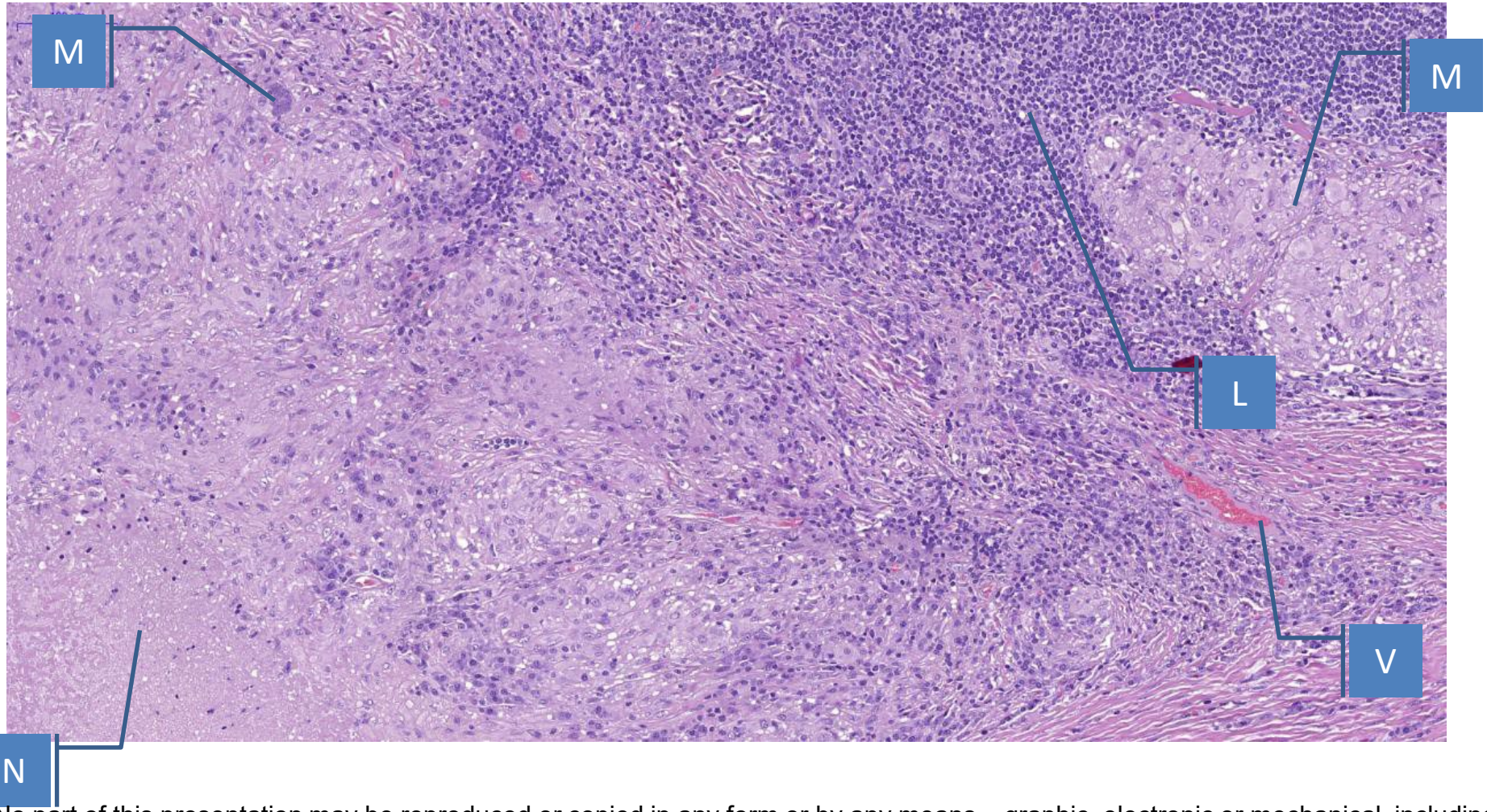
Hematoxylin-Eosin

Hematoxylin stains nuclei in shades of blue and purple (basophilic structures).

Eosin stains the cytoplasm, basement membranes, fibrous elements of the stroma in shades of pink, erythrocytes in orange-red color (eosinophylic structures).



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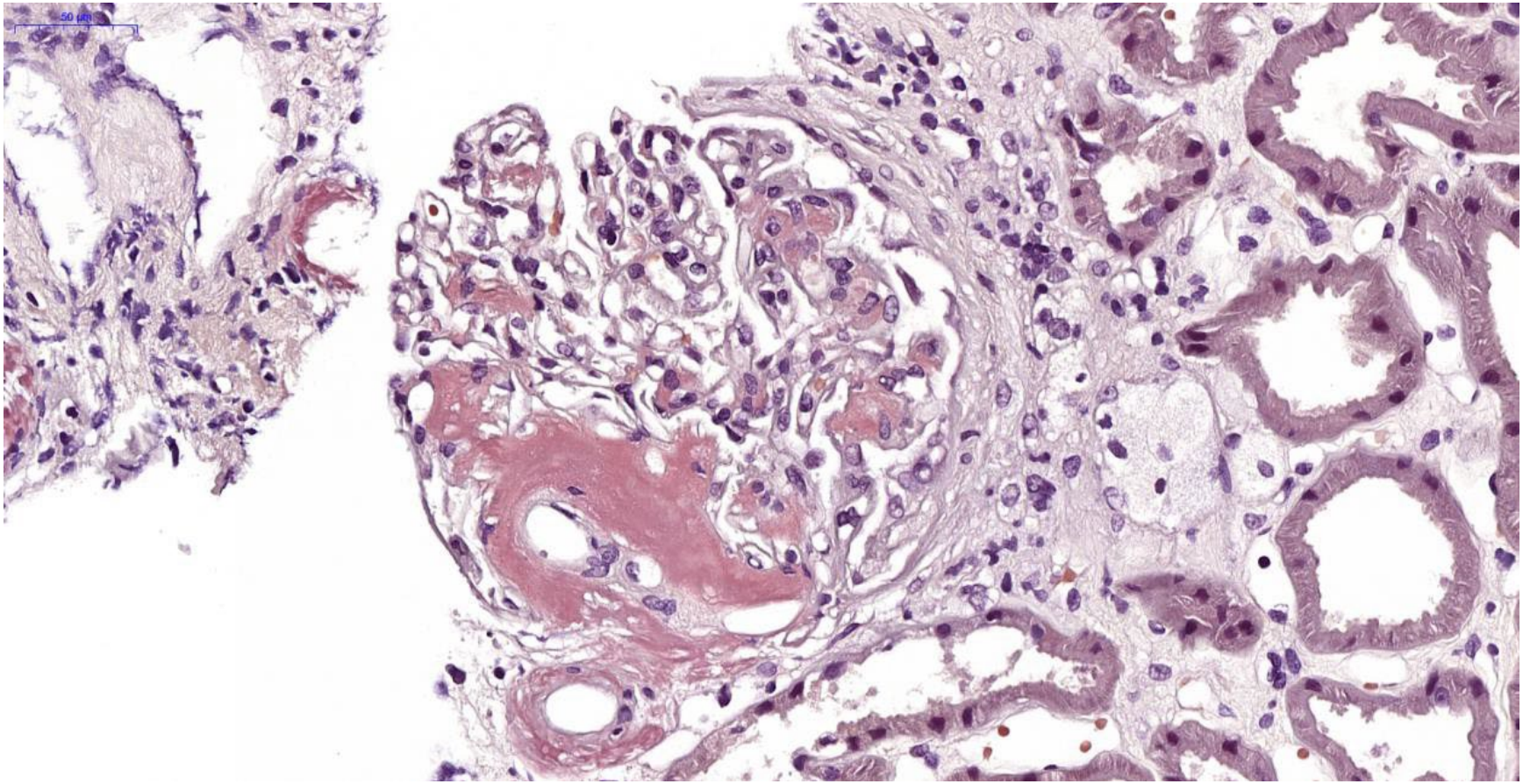
Congo-red stain

Positively colored areas, when examined in polarized light, acquire an apple-green stain.



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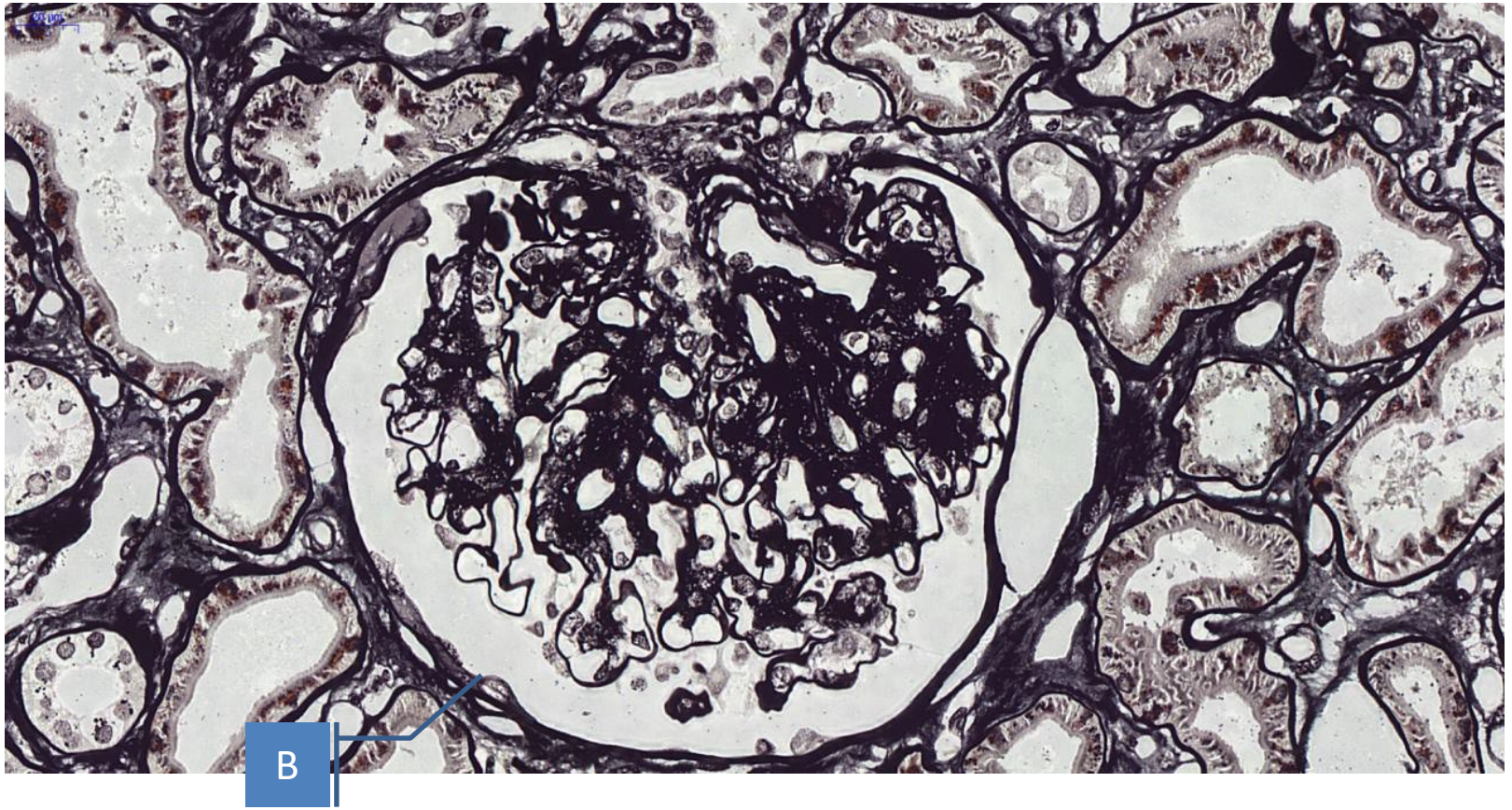
Impregnation with silver salts
(Silver stain)

- staining reticulin fibers in various localizations (basement membranes),
- staining organelles



result of methenamine-silver staining of the basement membranes of the glomerular capillaries.

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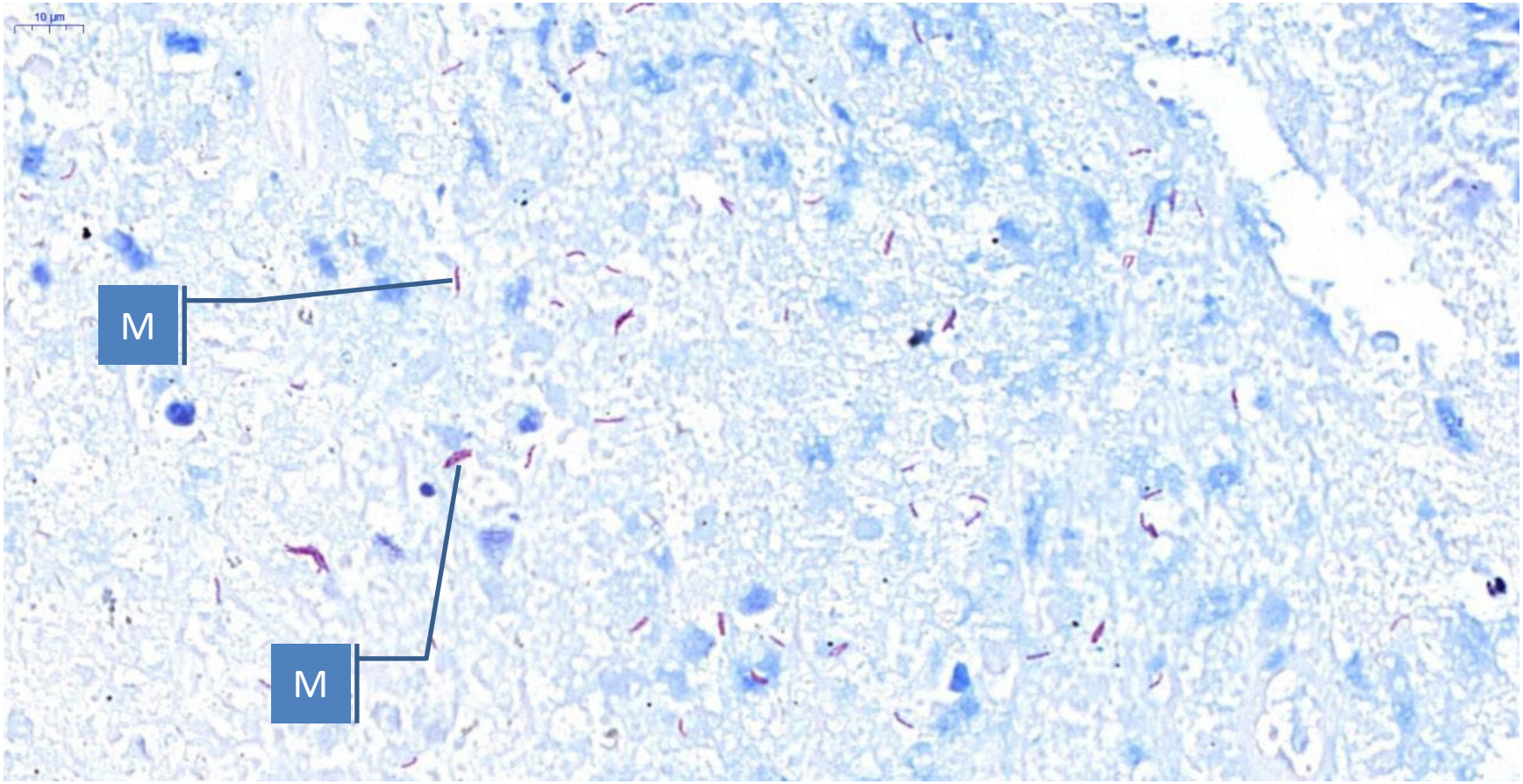
Ziehl-Nielsen stain

Bright red (fuchsin) staining of acid-fast Mycobacterium tuberculosis (Koch's sticks).



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Cryostat microtomy

•A method used for express diagnostics.

•tissue immediately after cutting by a pathologist is placed in special cryostat microtomes.

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•This technique allows a preliminary diagnosis to be obtained within 20 minutes.

•Also, this method is used to perform some additional stains (for example, staining for fats - Sudan-III, since fats are dissolved in alcohols during routine histoprocessing).

Technician with cryostatic microtome (“C”)

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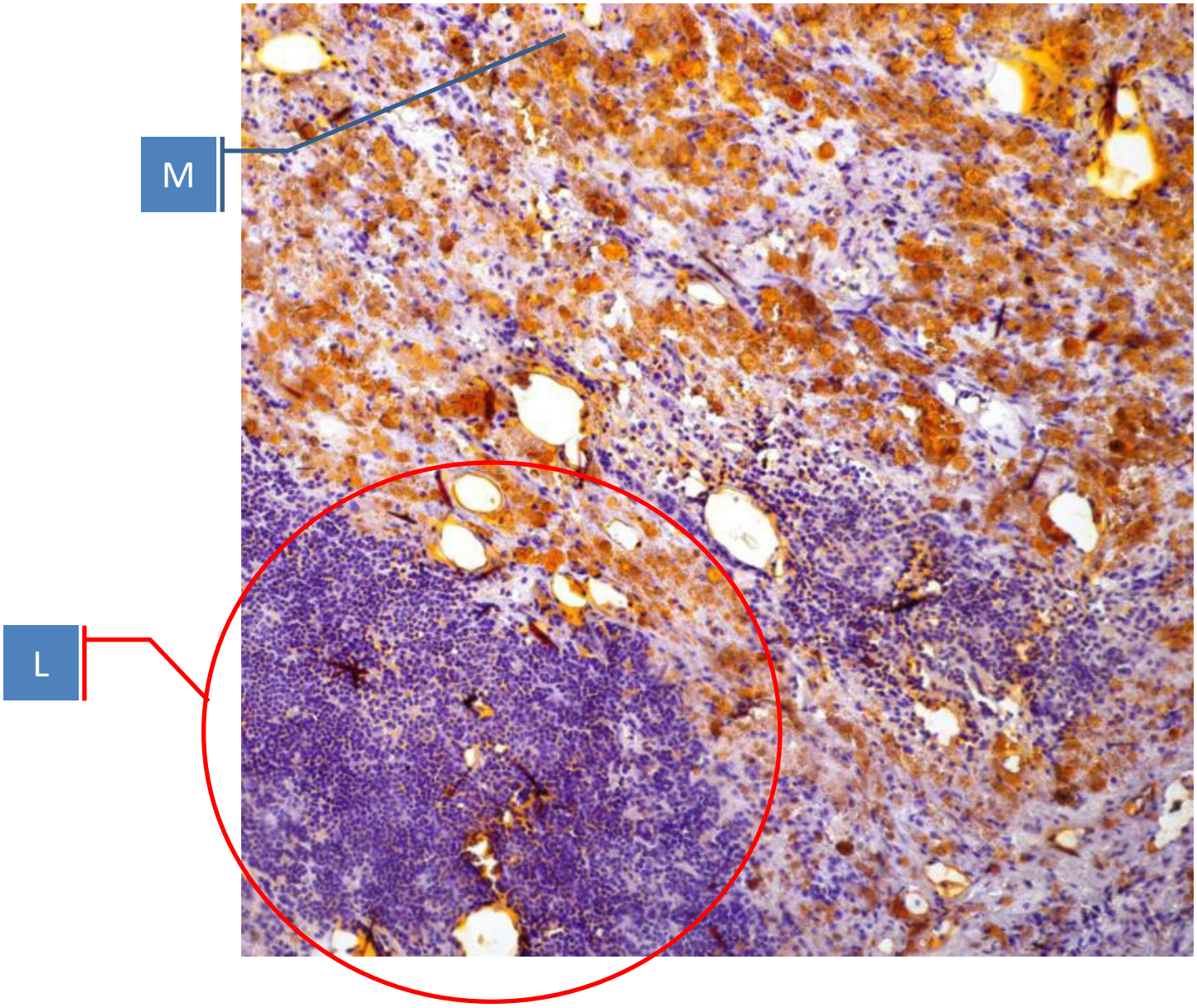

Fields of macrophages with lipid-containing cytoplasmic inclusions of golden-orange color (Sudan-III stained cryostat section).

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Additional morphological research methods.

Currently, the most common in practice are:

- Immunohistochemical study (IHC);
- In situ hybridization (CISH, SISH or FISH);
- Immunofluorescence analysis (ELISA);
- Electron microscopic examination.

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IHC
(**I**mmuno**h**isto**c**hemistry)

- Immunohistochemistry is the use of antibody-based reagents for localization of specific epitopes in tissue sections.

- This method is based on the principle of "antigen-antibody" interaction.

- A positive reaction in this case is denoted by the term "expression", indicating the identified antigen. For example, "cells express CD20".

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The range of indications for IHC investigation is extremely diverse and, among other includes:

- Differential diagnosis of cancer.
- Making an accurate diagnosis in the case of a tumor of unknown origin, including cases with metastasis.
- Assessment of the tumor's malignant potential.
- Determination of target molecules in tumor cells for personalized therapy (especially important for tumors of the breast, lung, brain, cervix, intestines, stomach ...
- Diagnosis of endometrial pathology in infertility…

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A basic principle in diagnostic immunohistochemistry

- the utilization of panels of antibodies, rather than single antibodies directed against markers of the suspected correct diagnosis.

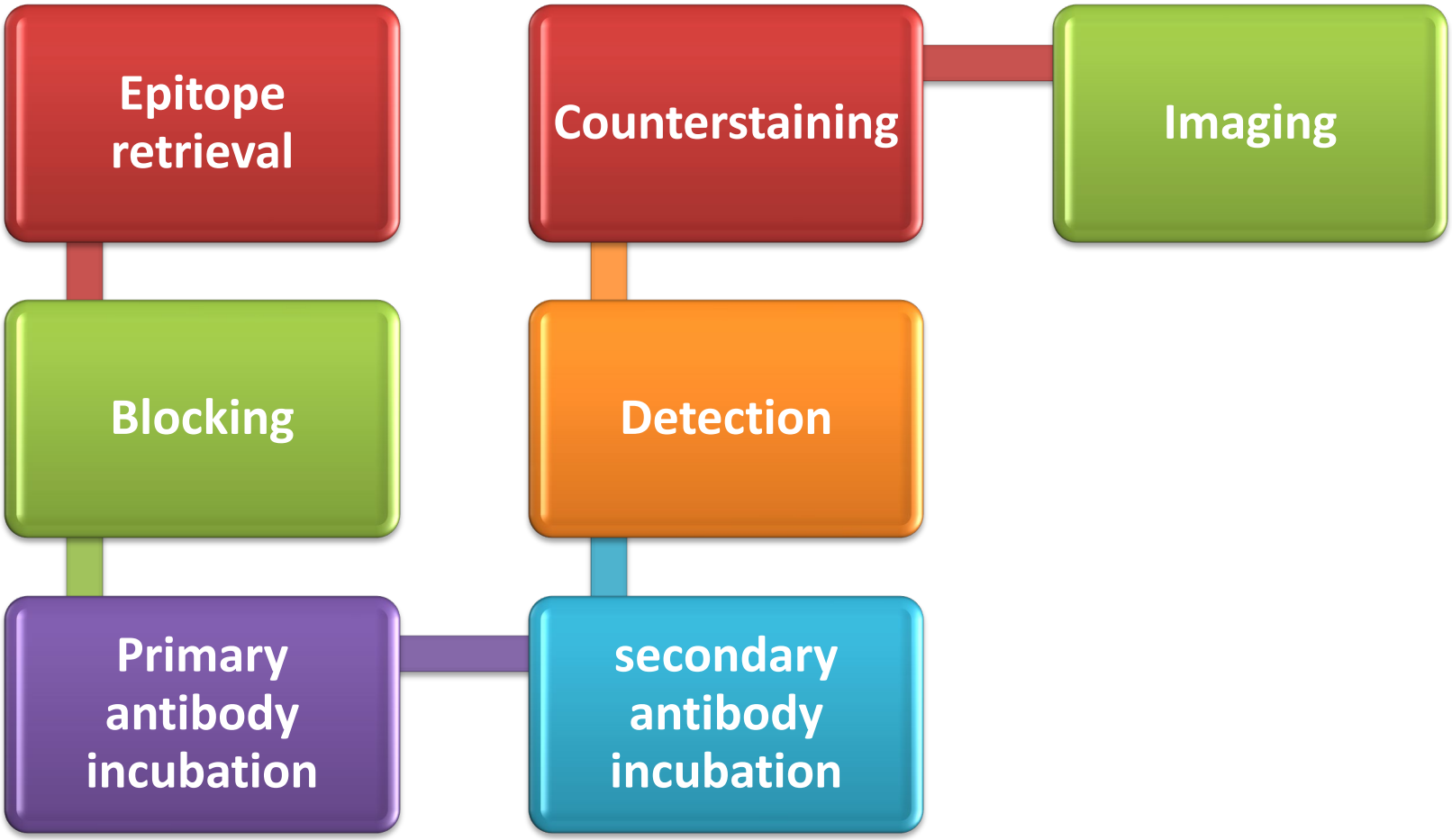
*

ANTIBODY TO	SMALL CELL CARCINOMA	MELANOMA	LYMPHOMA	EFT	RMS	PDSS	DSRCT
Cytokeratins	Positive	Variable	Negative	Variable	Rare	Positive	Positive
Melanocytic markers	Negative	Positive	Negative	Negative	Negative	Negative	Negative
CD45	Negative	Negative	Positive*	Negative	Negative	Negative	Negative
Desmin	Negative	Variable	Negative	Rare	Positive	Negative	Positive
FLI1/ERG, NKX2.2	Negative	Negative	Negative	Positive	Negative	Negative	Negative
Synaptophysin	Positive	Negative	Negative	Variable	Rare	Negative	Negative

EFT, Ewing family of tumors; RMS, rhabdomyosarcoma; DSRCT, desmoplastic small round cell tumor; PDSS, poorly differentiated synovial sarcoma.
*Lymphoblastic lymphomas may be CD45 negative. In children, screen with TdT and CD43 instead of CD45.

* Enzinger and Weiss's soft tissue tumors / John R. Goldblum, Andrew L. Folpe, Sharon W. Weiss.—6th ed.

IHC steps



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automated systems

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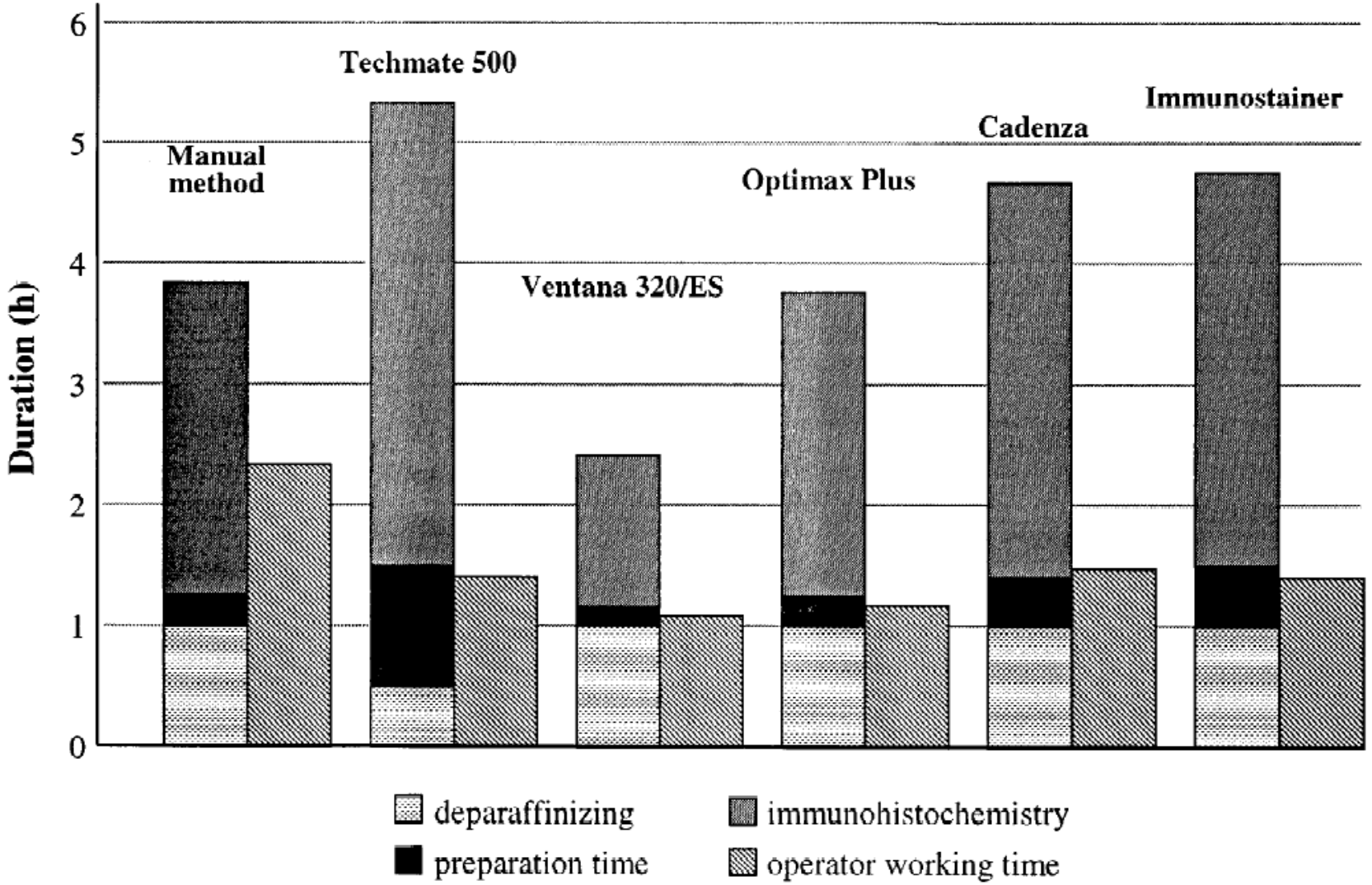
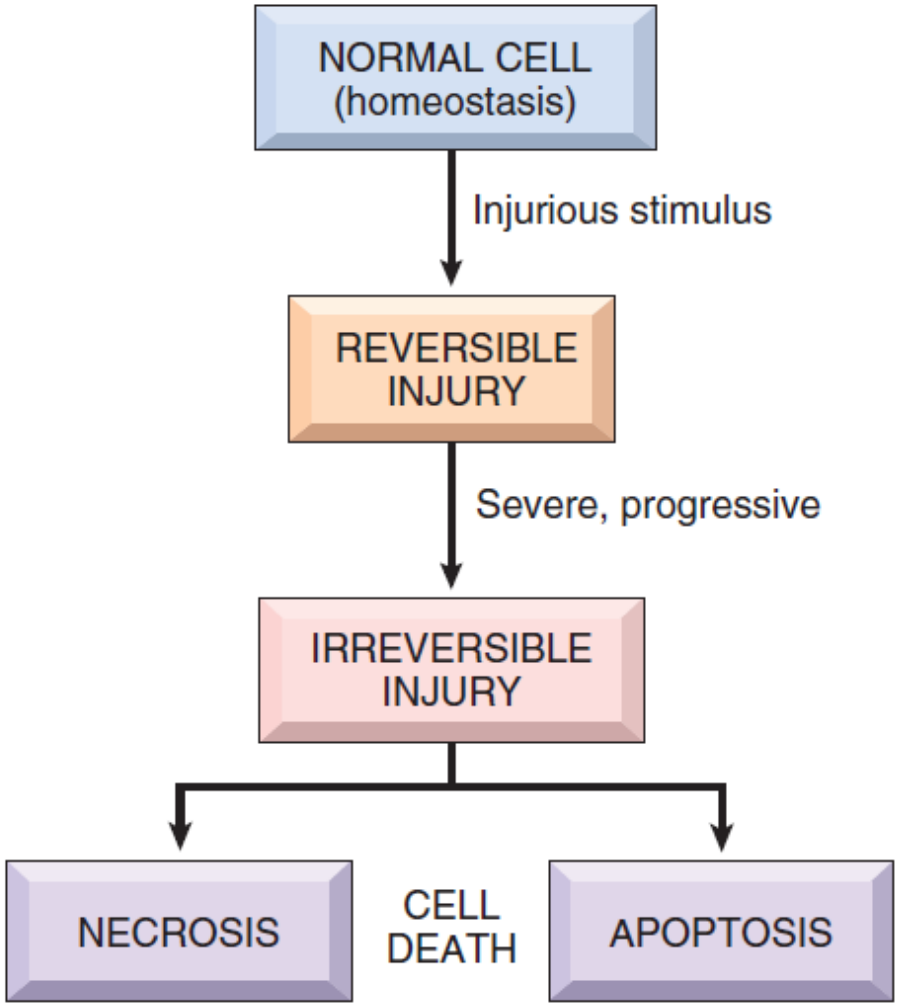


Le Neel, T., Moreau, A., Laboisse, C., & Truchaud, A. (1998). Comparative evaluation of automated systems in immunohistochemistry. Clinica Chimica Acta, 278(2), 185–192. doi:10.1016/S0009-8981(98)00146-6 (https://doi.org/10.1016/S0009-8981(98)00146-6)

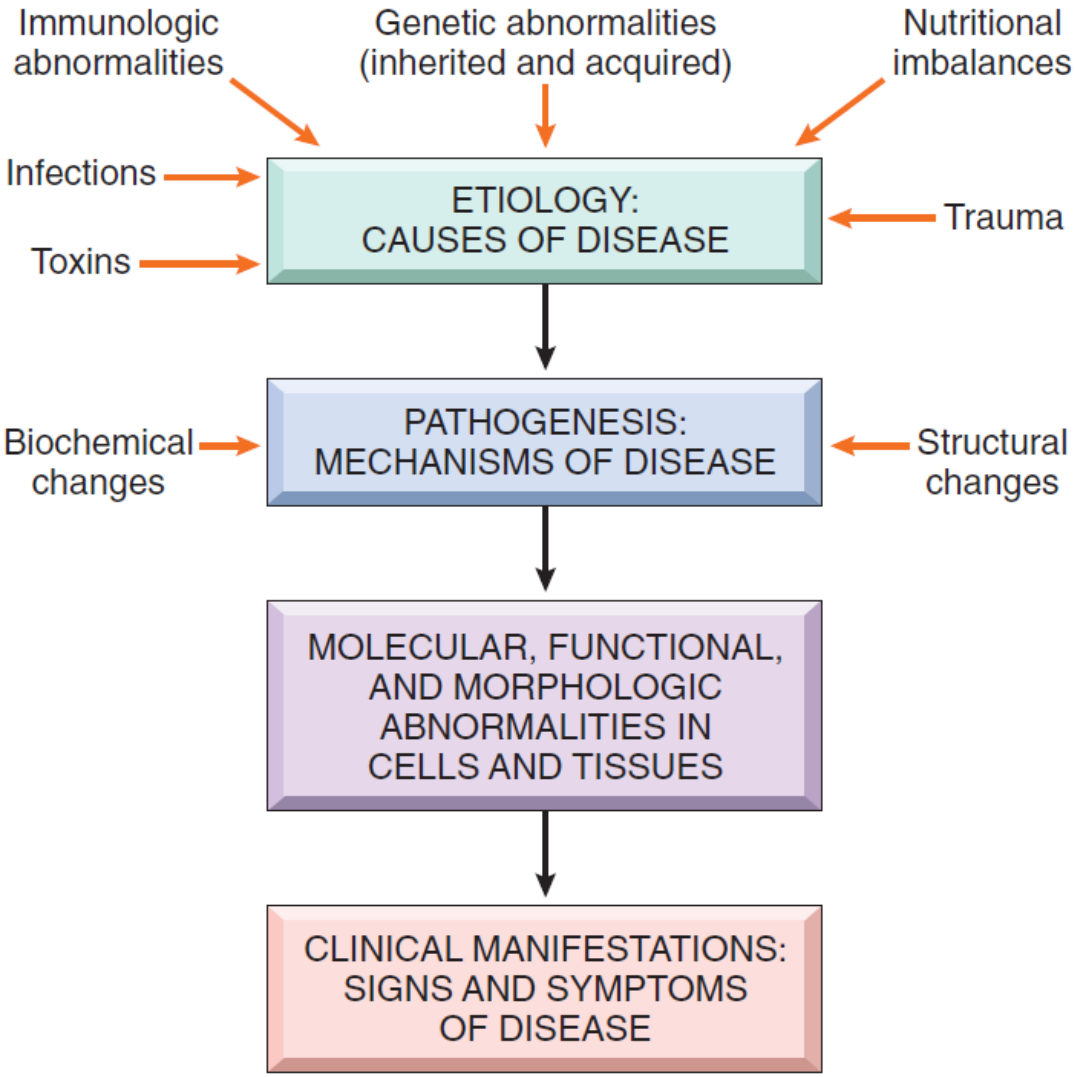

CELL INJURY

Sequence of reversible cell injury and cell death. Necrosis and apoptosis are the two major pathways



From: Kumar, V., Abbas, A. K., & Aster, J. C. (2017). Robbins Basic Pathology (10th ed.). Elsevier - Health Sciences Division.

CAUSES OF CELL INJURY



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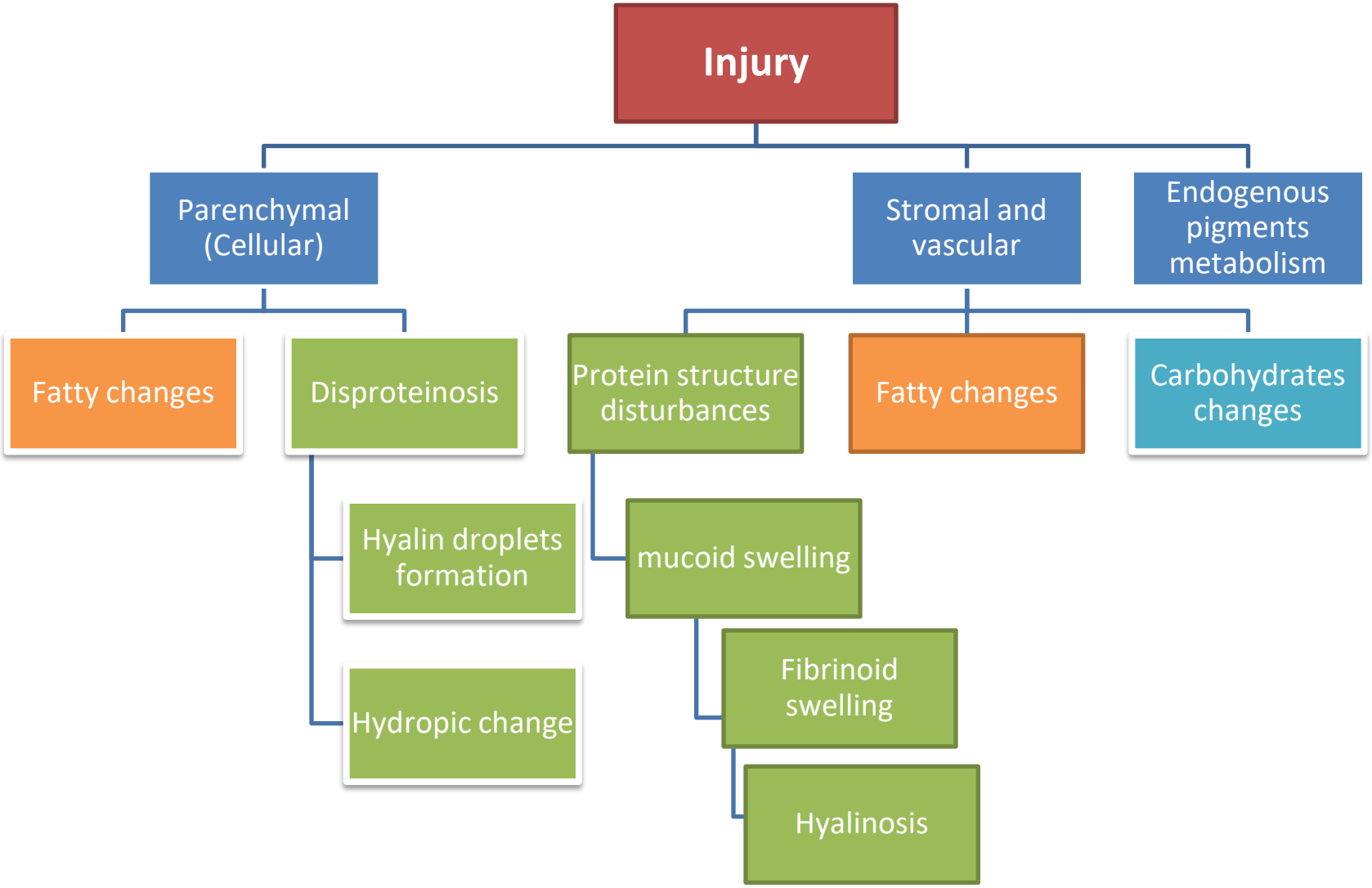
Mechanisms and Morphology of Cell Injury

3 type of events



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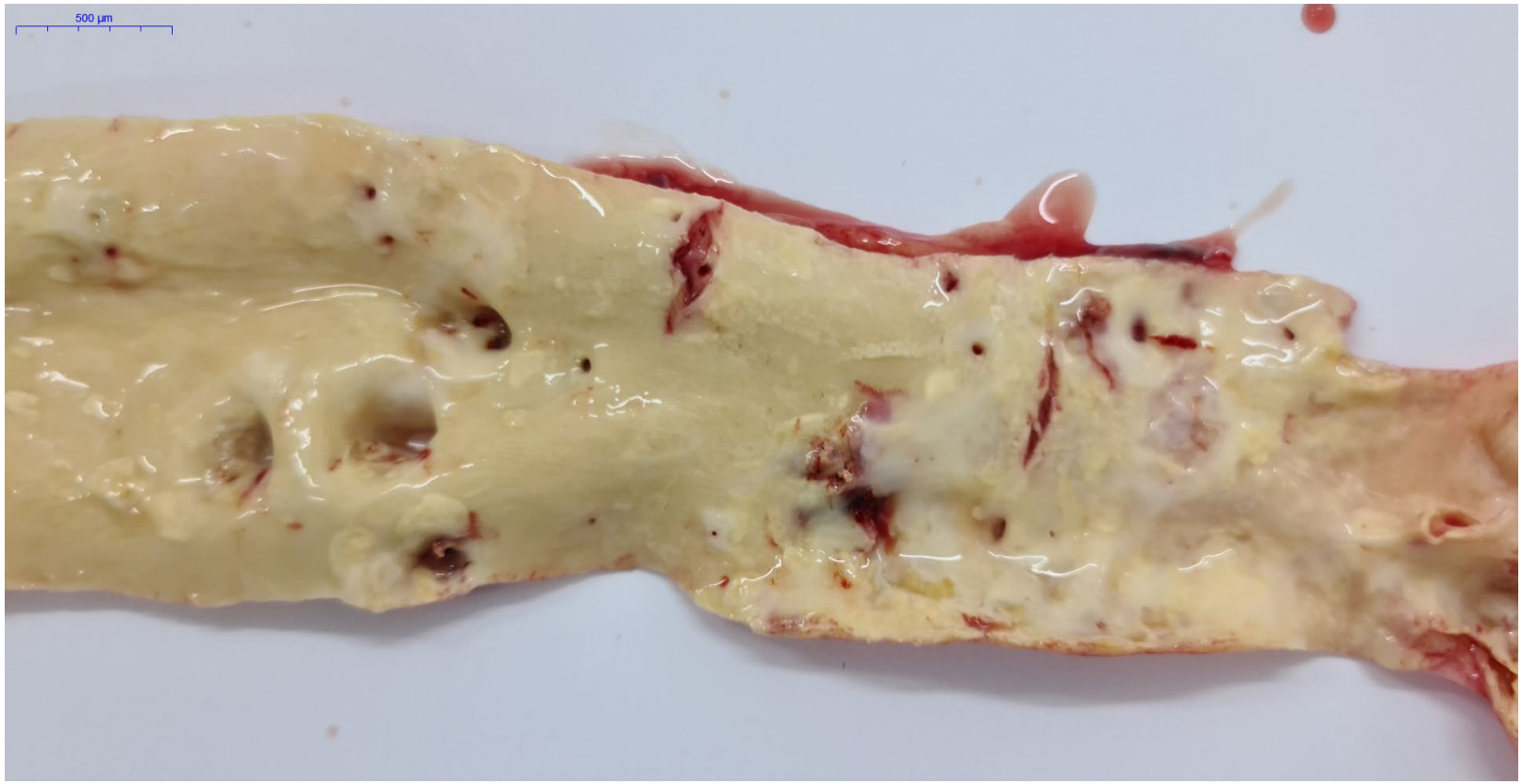
Morphogenesis of injury

- **Infiltration** - excessive penetration of metabolic products into cells, vascular walls, intercellular substance with subsequent accumulation.



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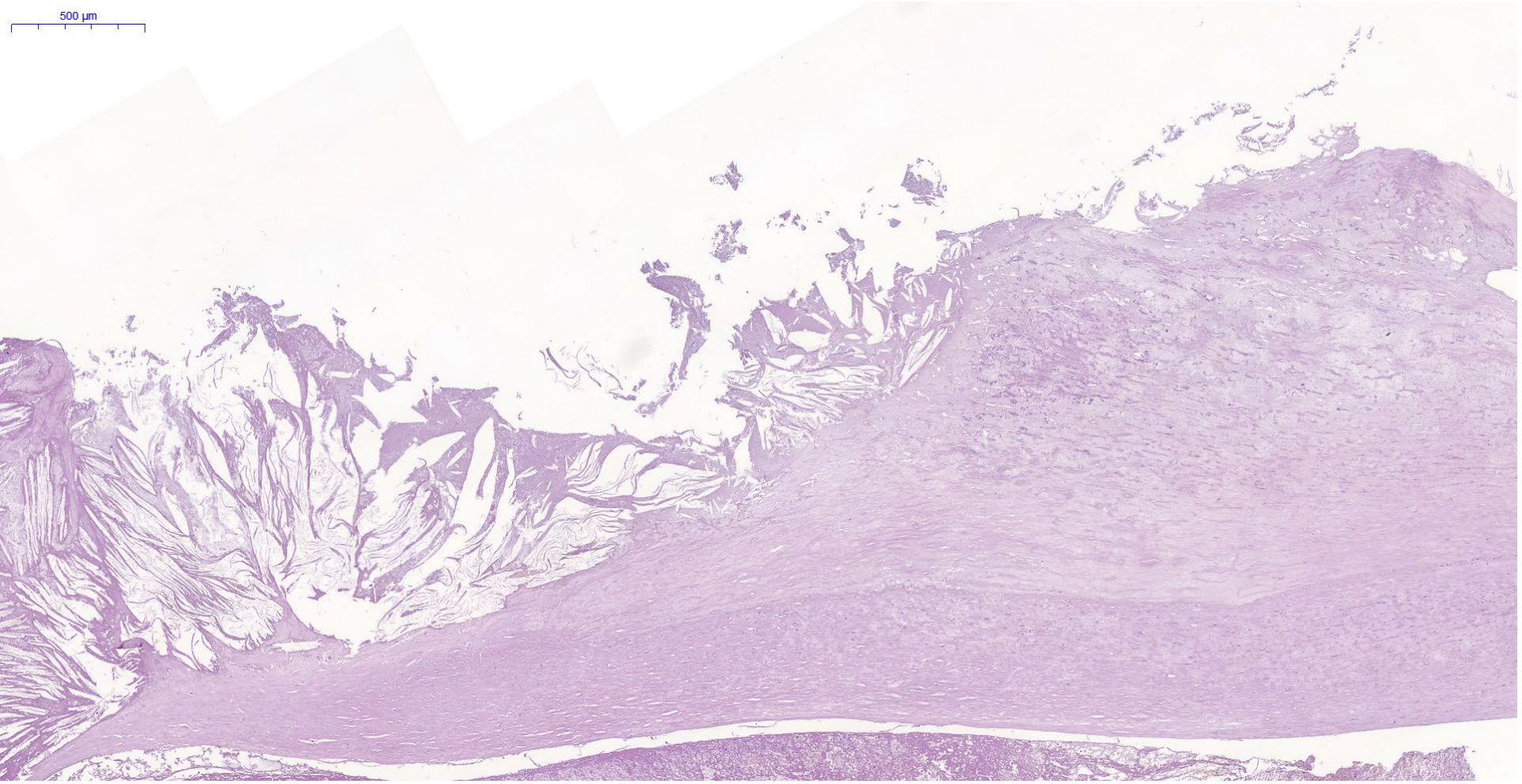
Morphogenesis of injury



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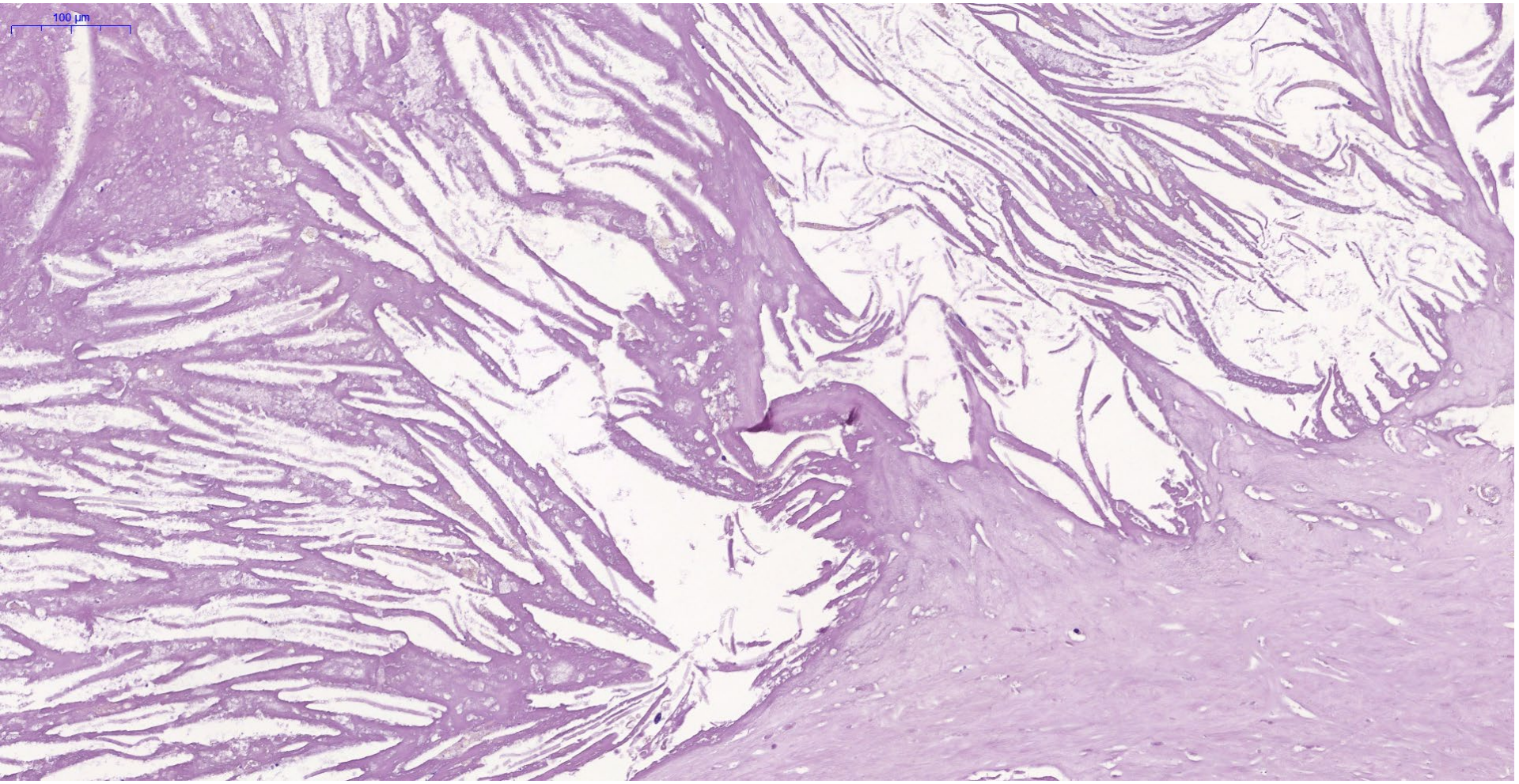
Morphogenesis of injury



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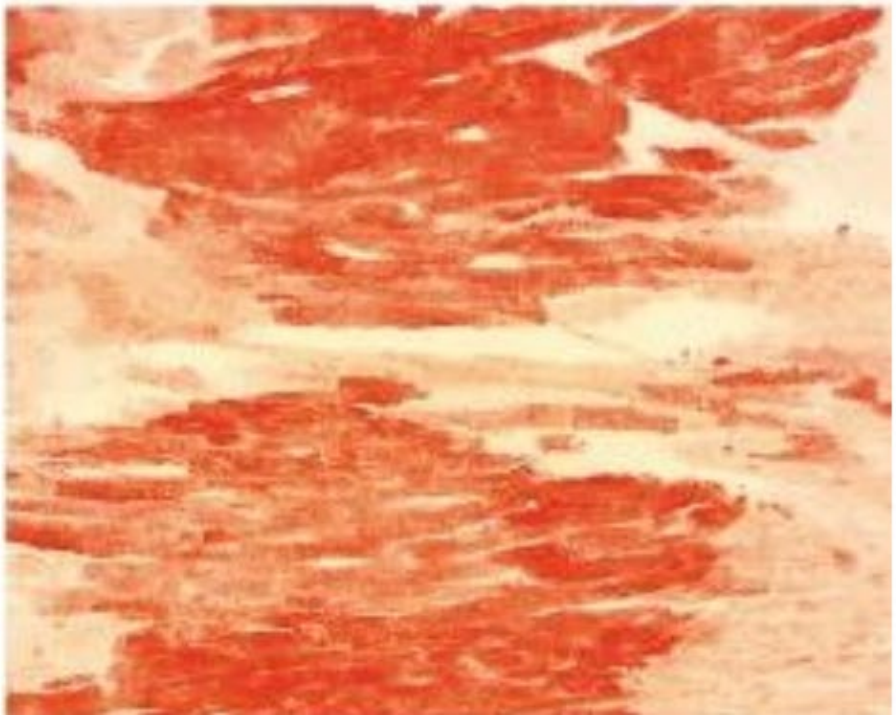
Morphogenesis of injury

- **Decomposition** - disintegration of cellular ultrastructures, intercellular substance or chemicals with the accumulation of decay products.



Morphogenesis of injury

- **Transformation** - the formation of products of one type of metabolism from products that are used to build proteins, fats and carbohydrates.

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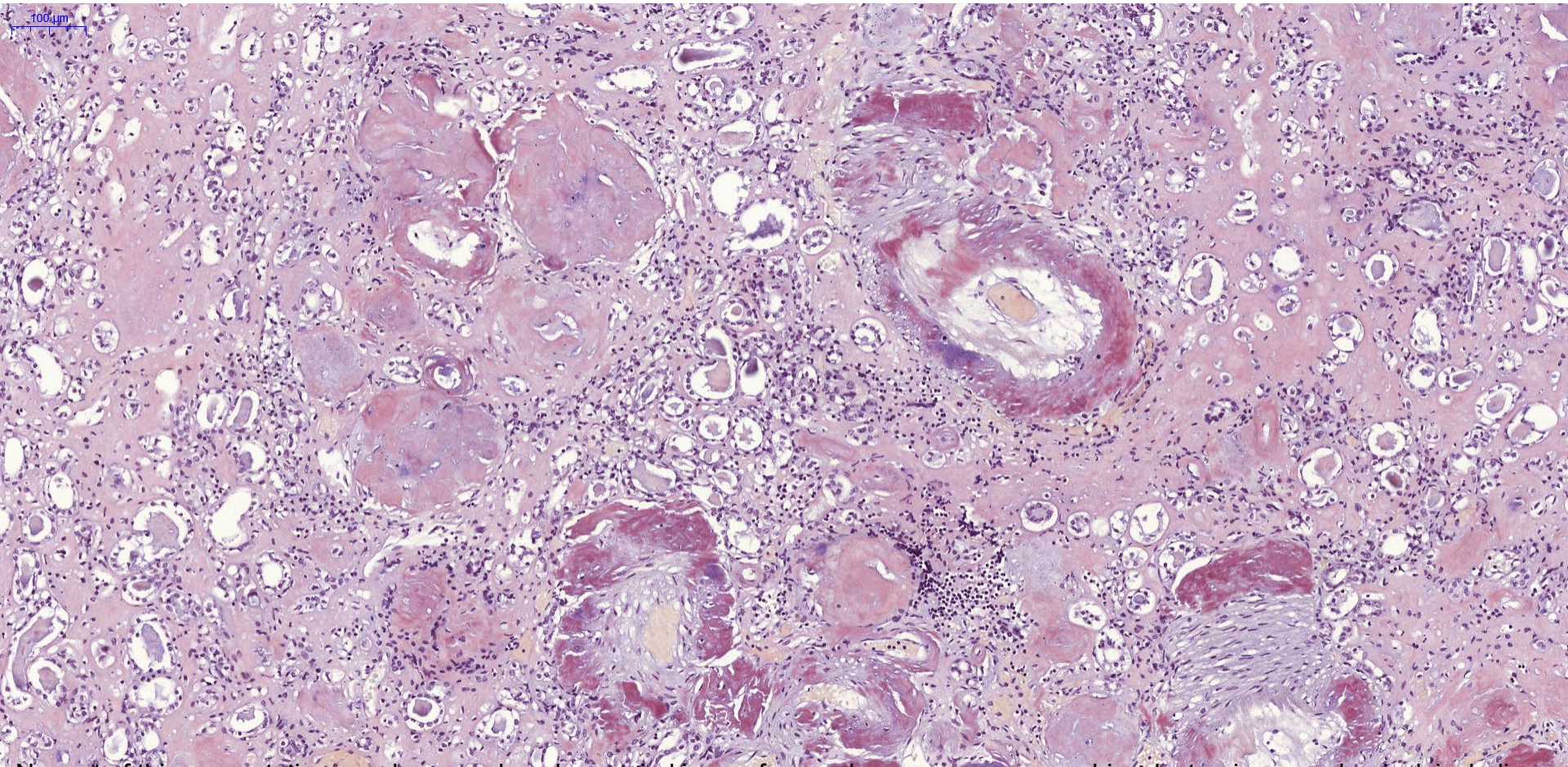
Morphogenesis of injury

Perverted synthesis is the formation of substances in cells or tissues that are not normally found.



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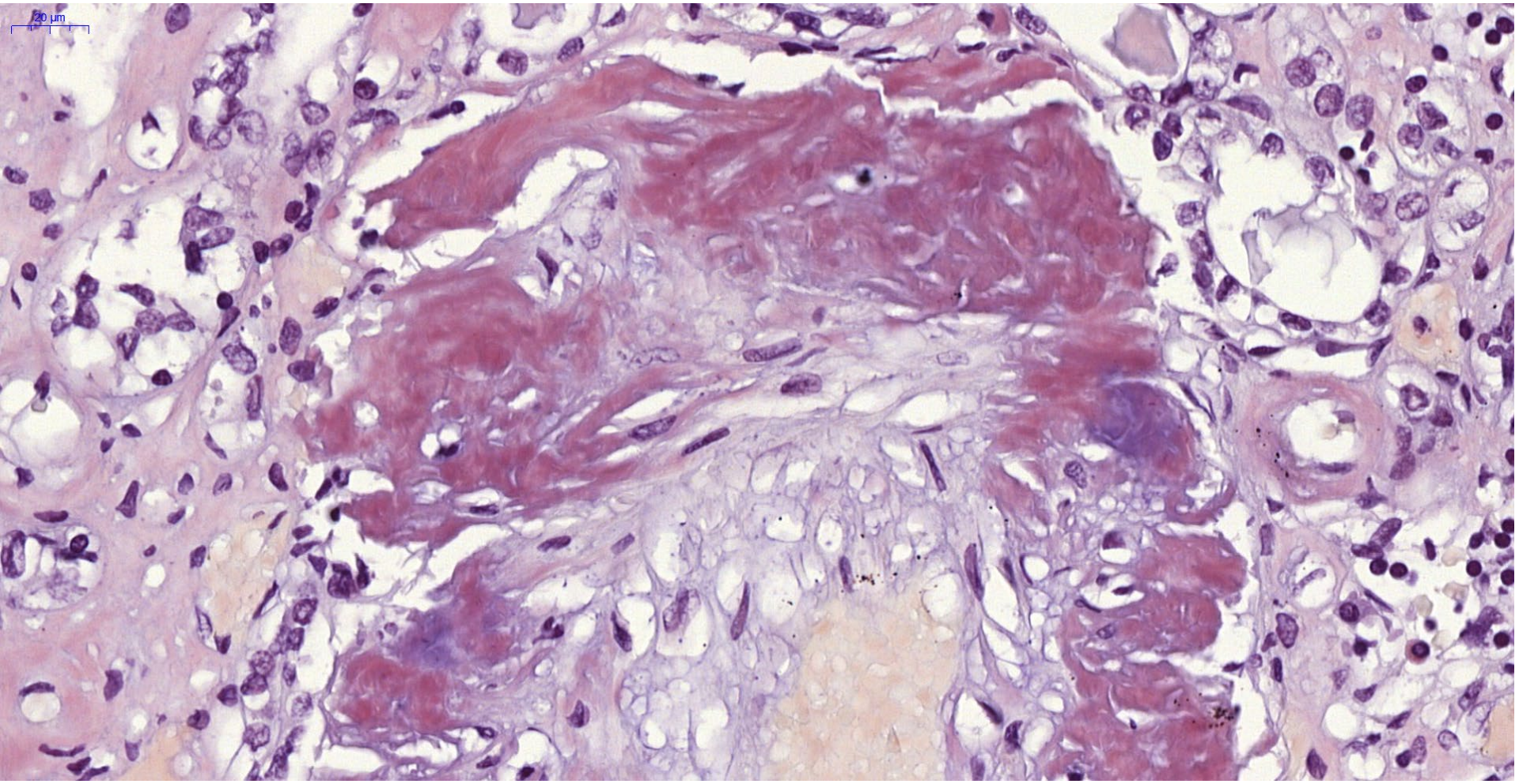

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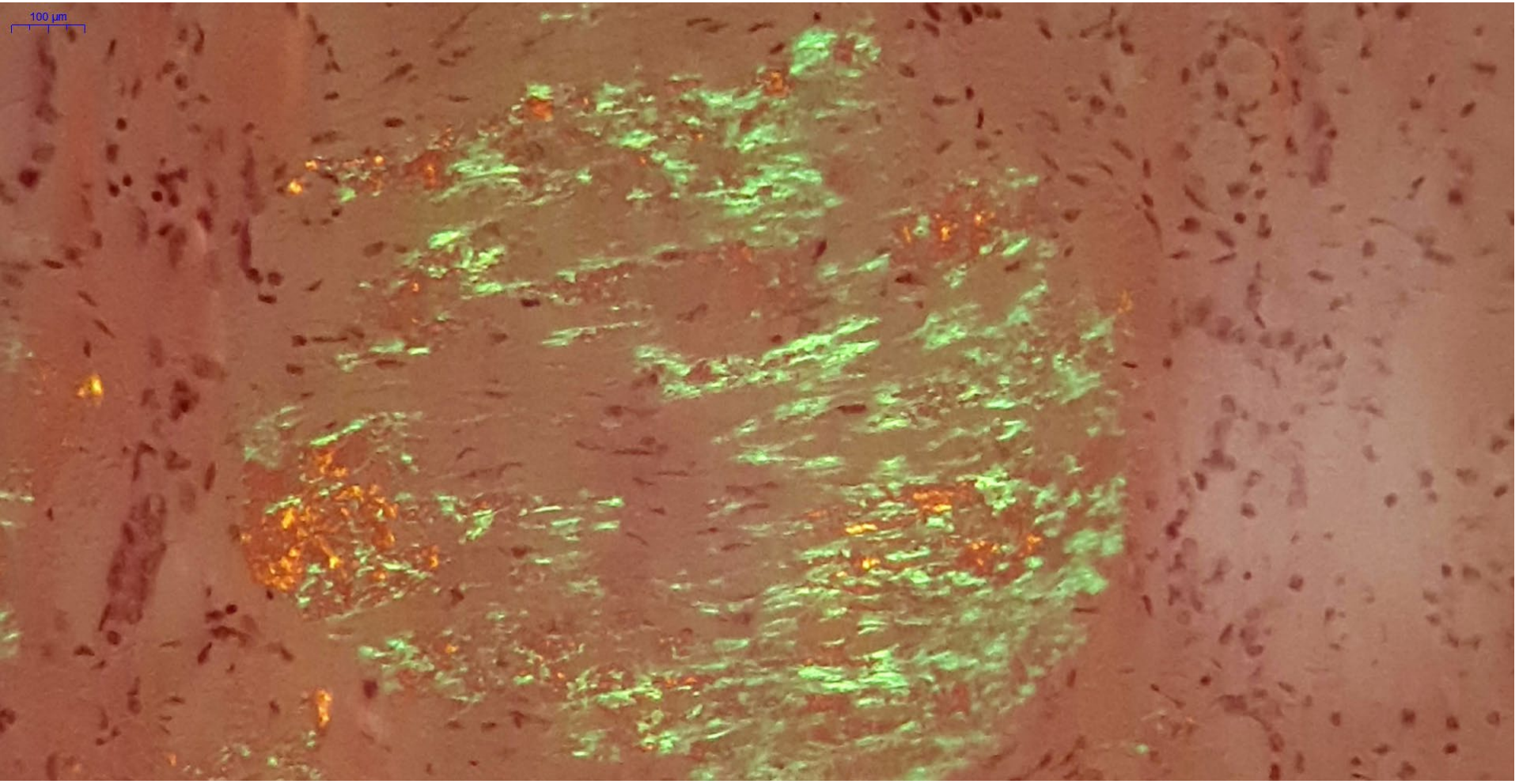
Polarized microscopy

«apple-green»



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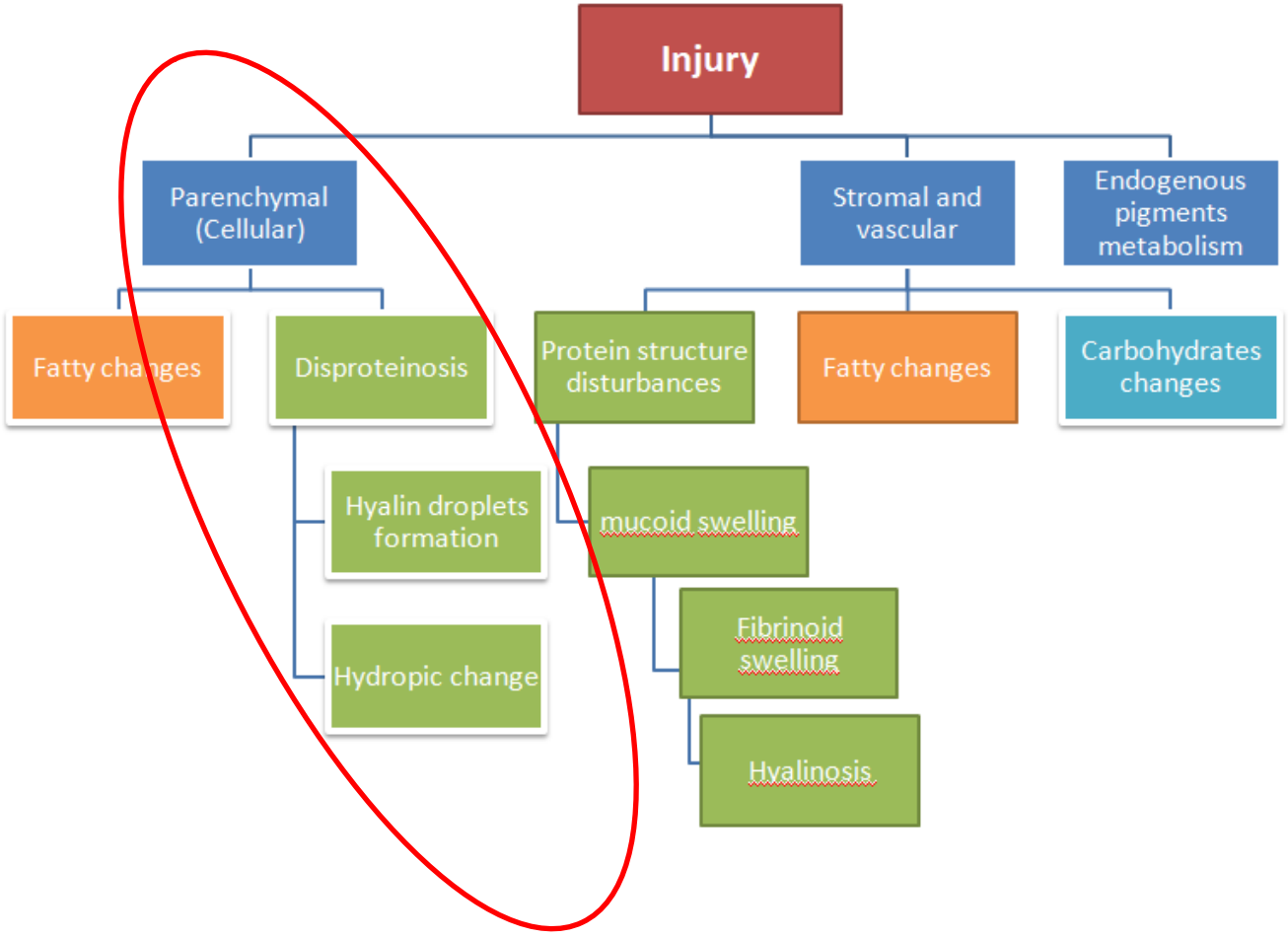

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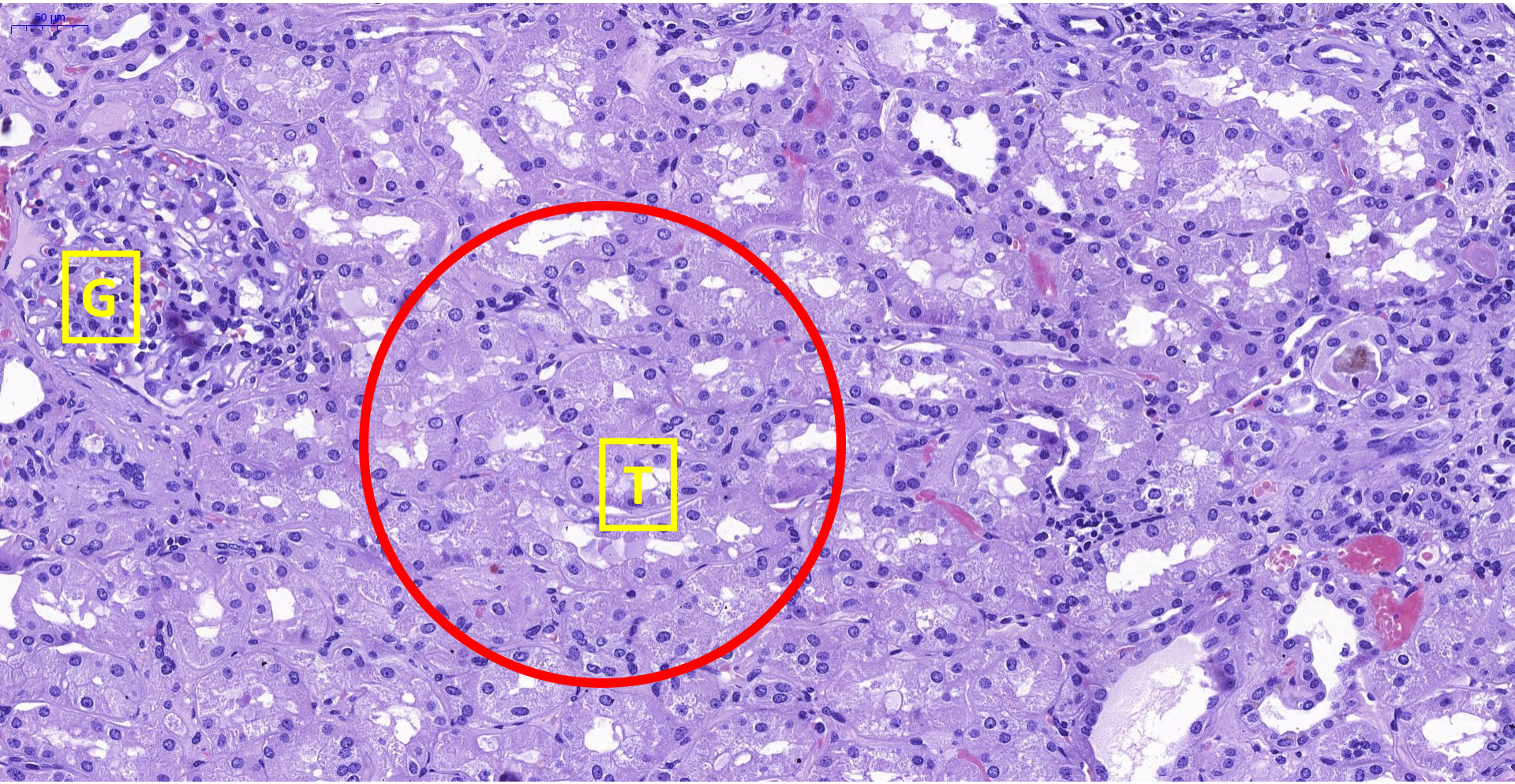
Hyalin droplets formation



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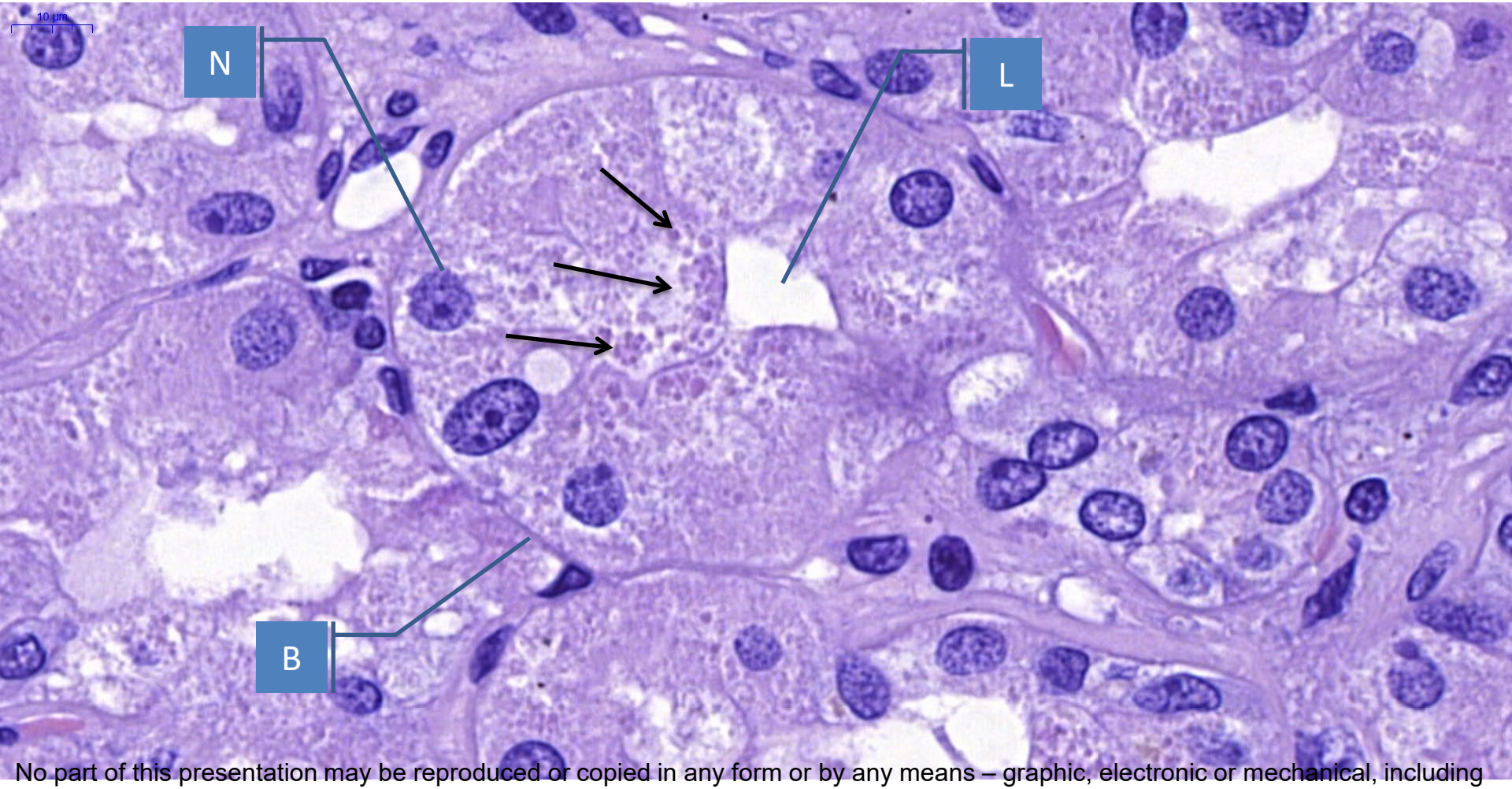
Hyalin droplets formation

- Eosinophilic (pink) hyaline-like protein clumps and drops appear in the cytoplasm, merging with each other.



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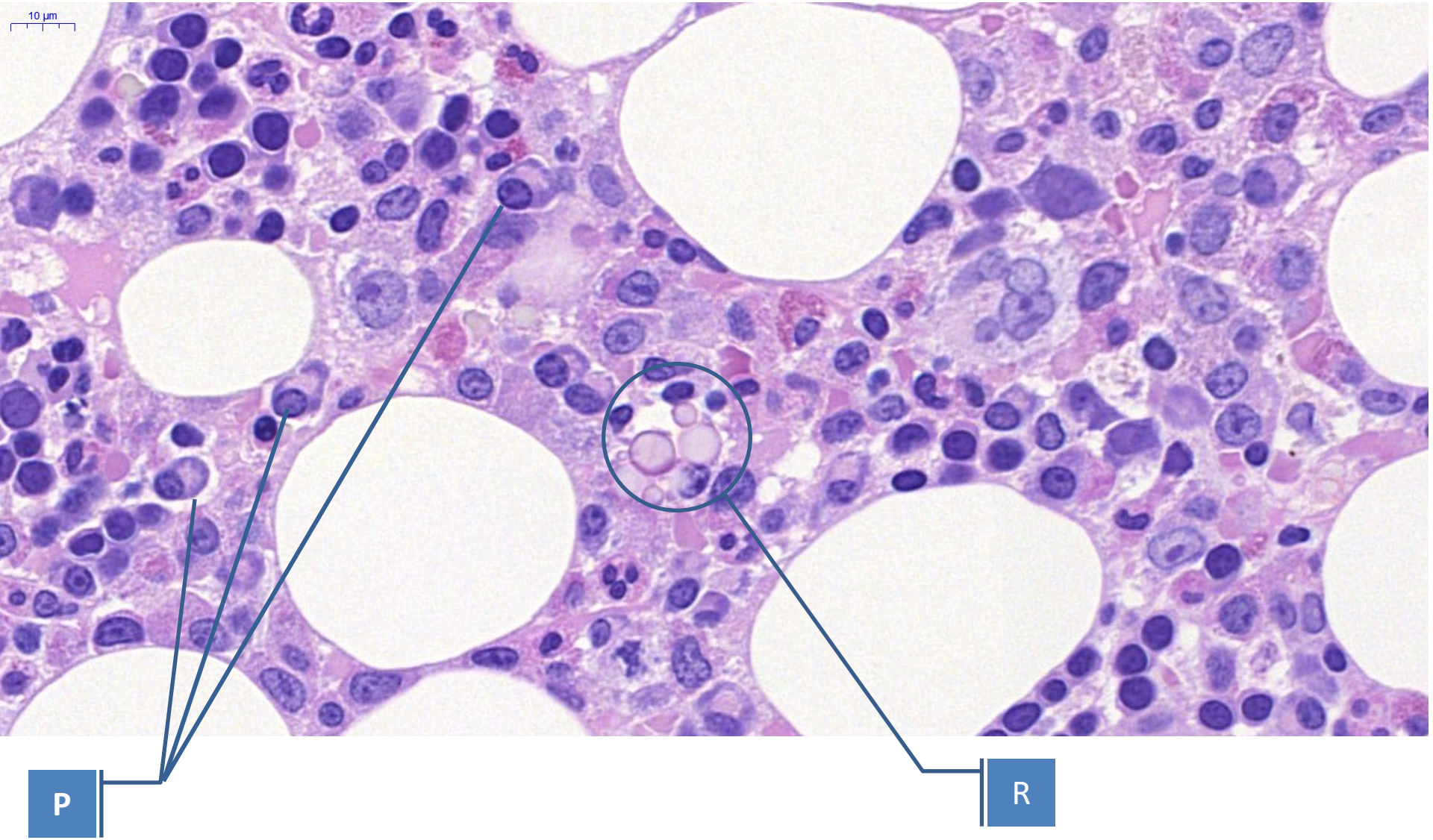
Roussel's bodies



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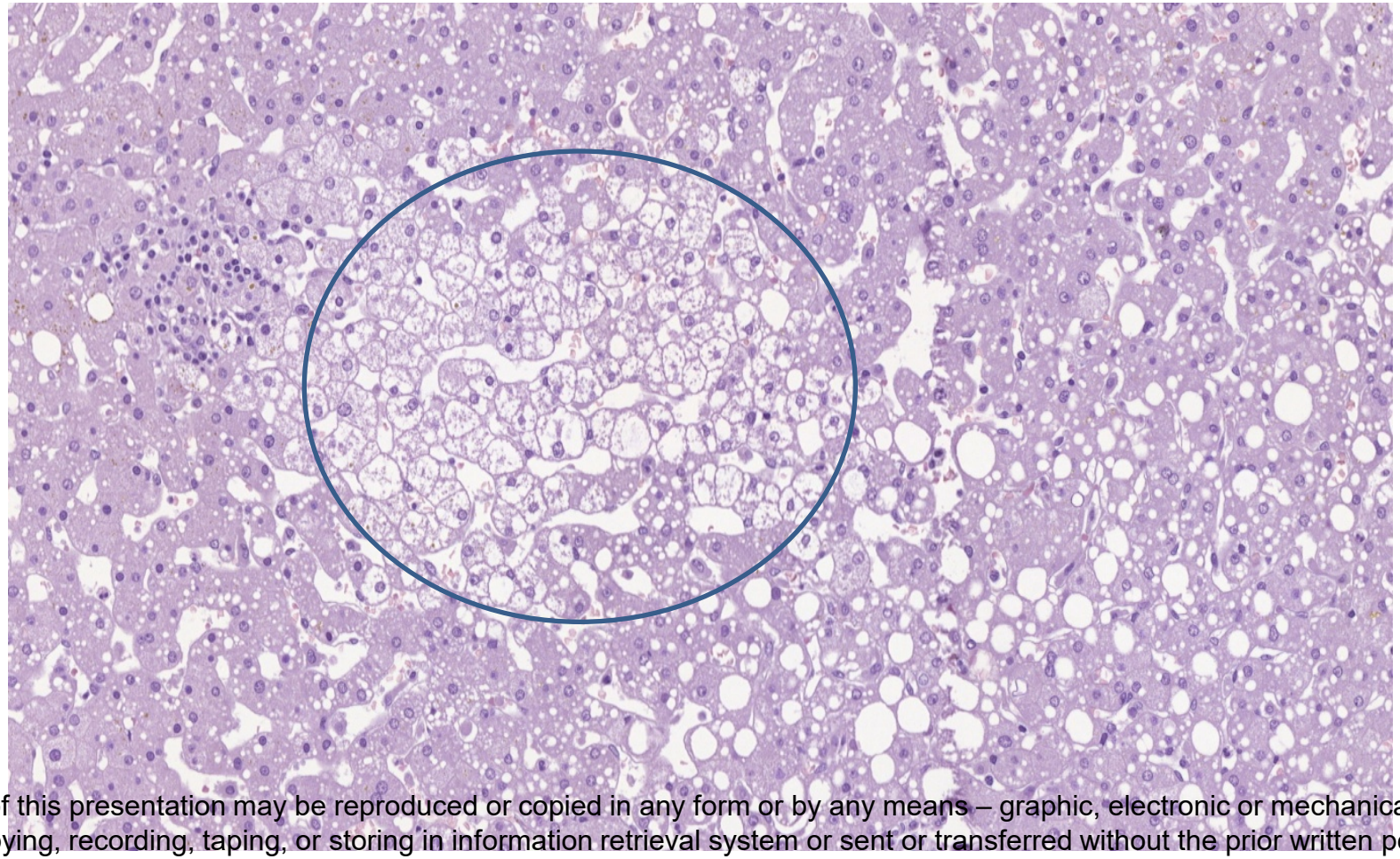
Hydropic changes (Cellular swelling)

- associated with increased permeability of the plasma membrane.



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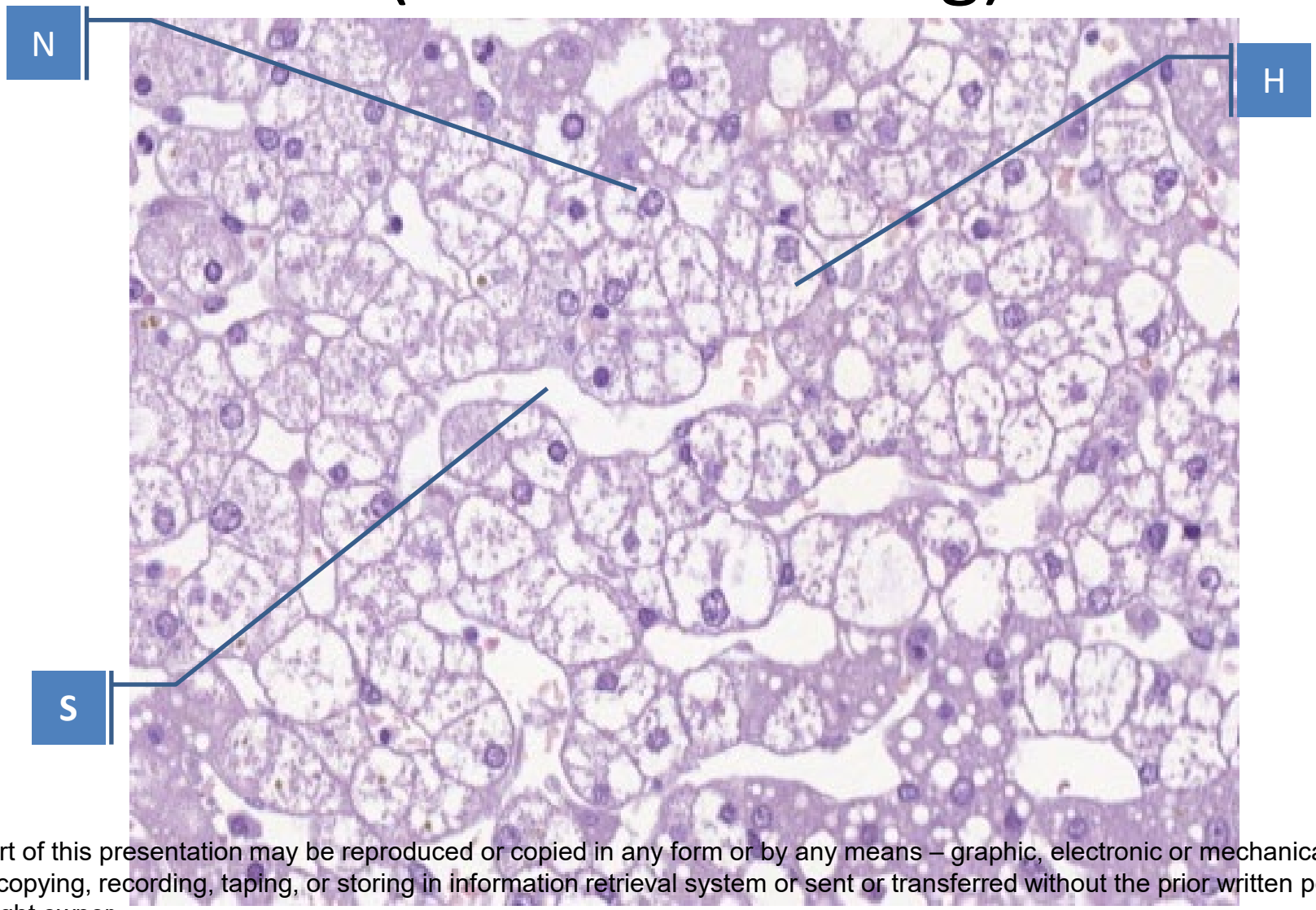
Hydropic changes (Cellular swelling)



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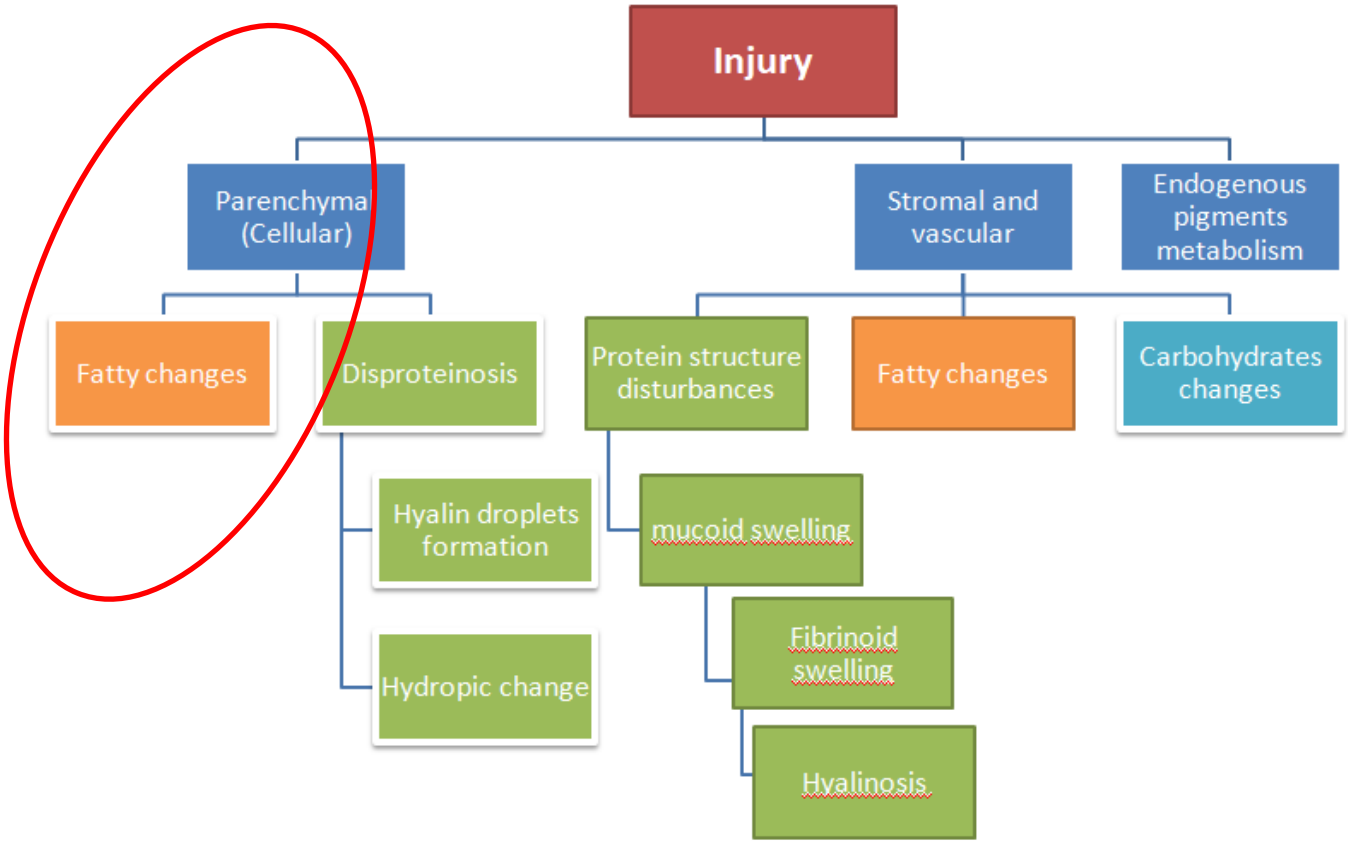

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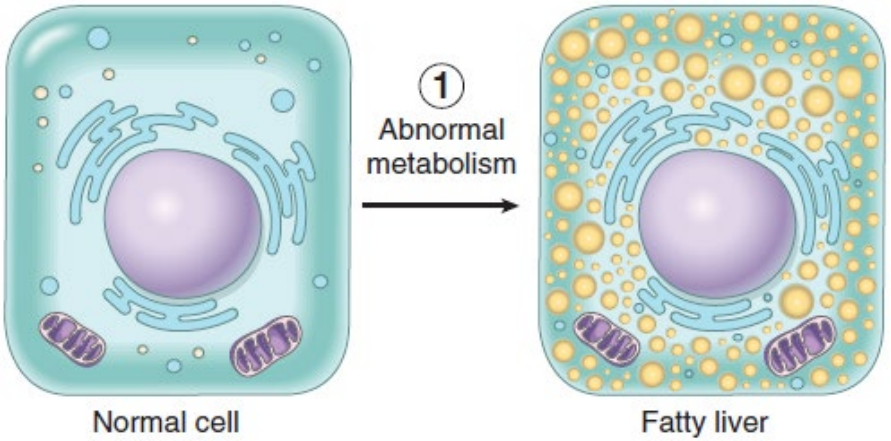
Fatty changes

- s manifested by the appearance of triglyceride containing lipid vacuoles in the cytoplasm. It is principally encountered in organs that are involved in lipid metabolism, such as the liver.



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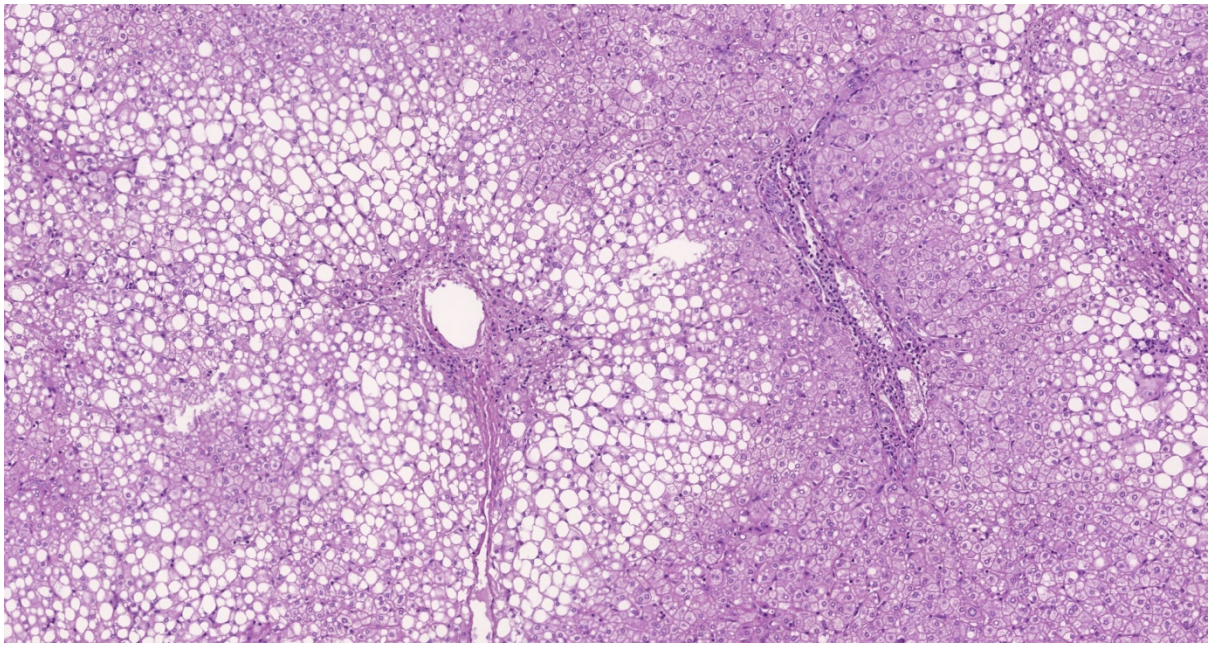

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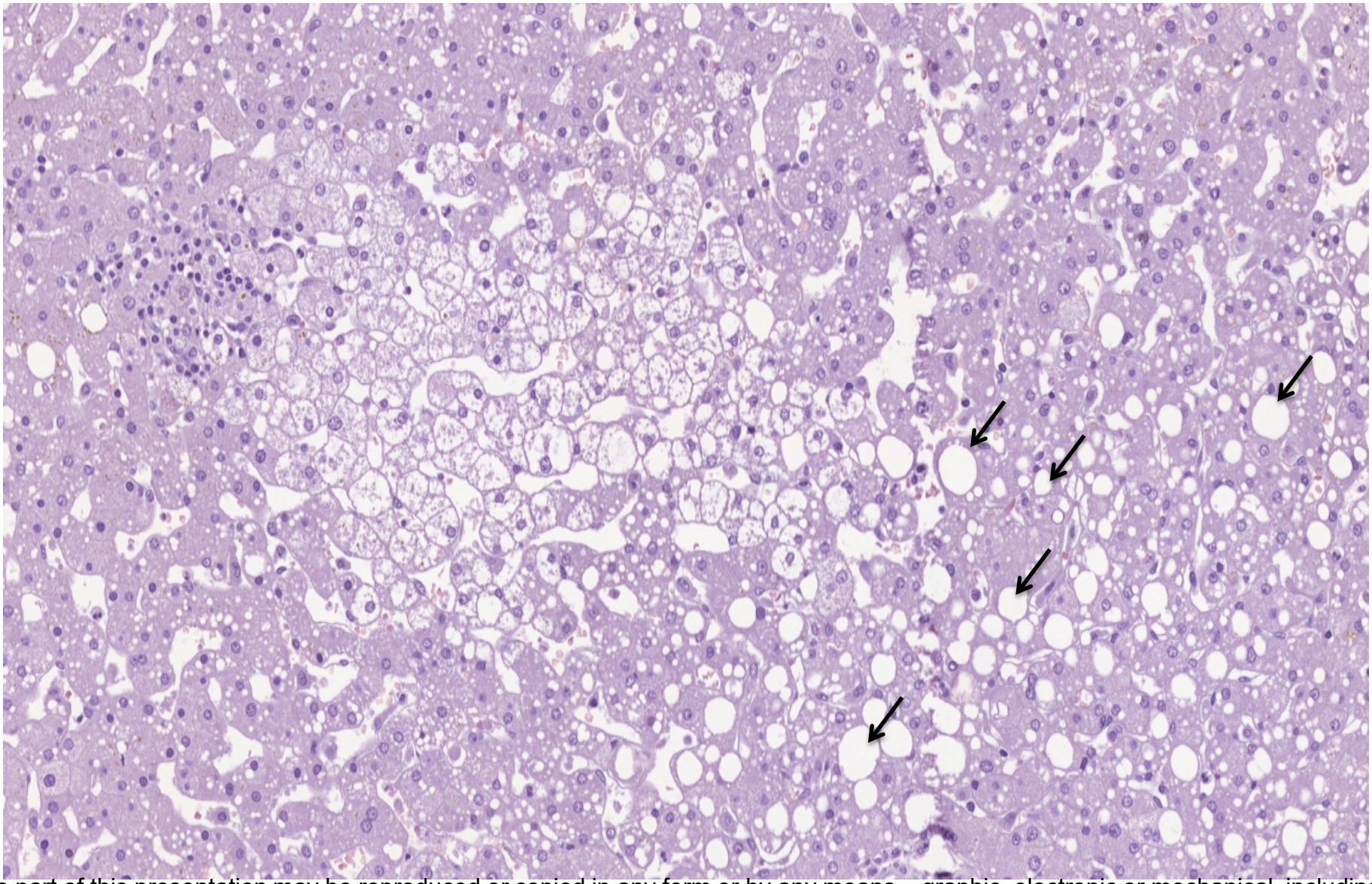
*From: Kumar, V., Abbas, A. K., & Aster, J. C. (2017). Robbins Basic Pathology (10th ed.). Elsevier - Health Sciences Division.
** Own data

Fatty changes – “goose liver”



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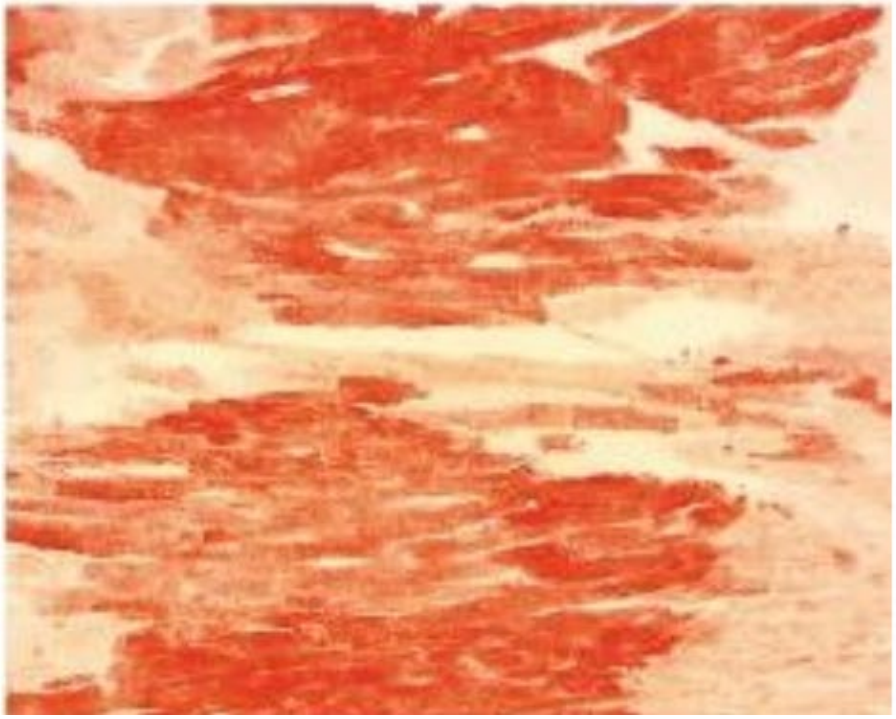
Fatty injury of the myocardium

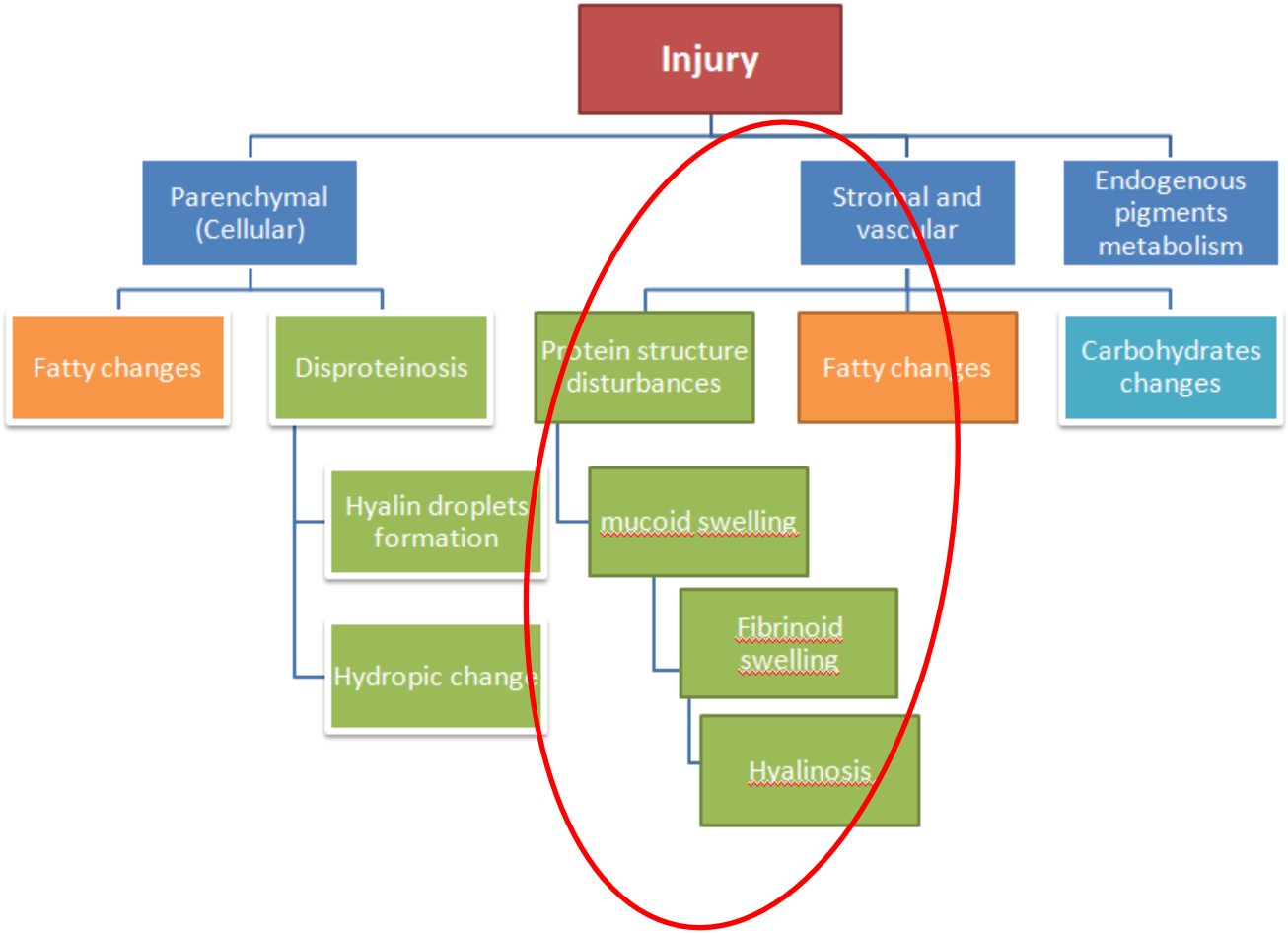
("tiger heart")



Lipid accumulation inside of cardiomiocytes (Sudan-III stain)

The clinical significance of myocardial fatty degeneration is a decrease in myocardial contractility (heart failure).

Stromal and vascular injury



Stromal and vascular injuries develops in the stroma of organs and vascular walls.

Stromal and vascular protein injury

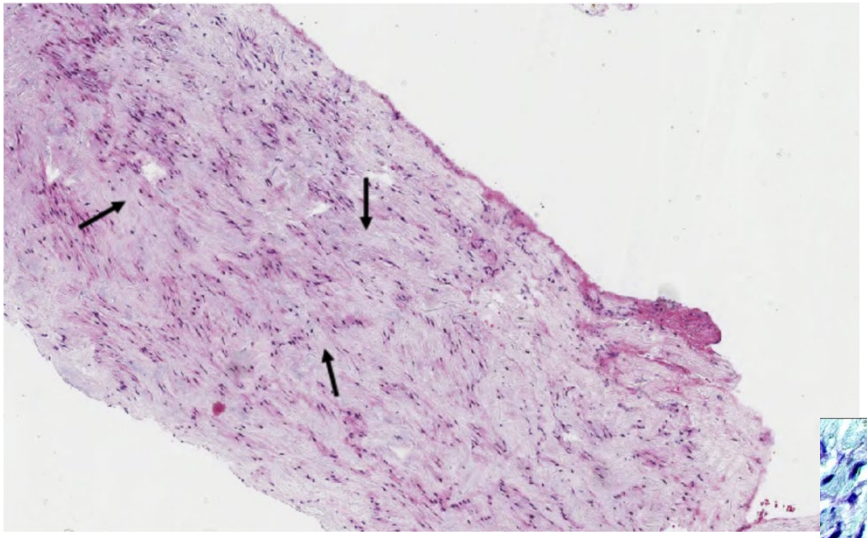
Step 1: Mucoid swelling

- superficial and reversible disorganization of connective tissue with the accumulation and redistribution of glycosaminoglycans.

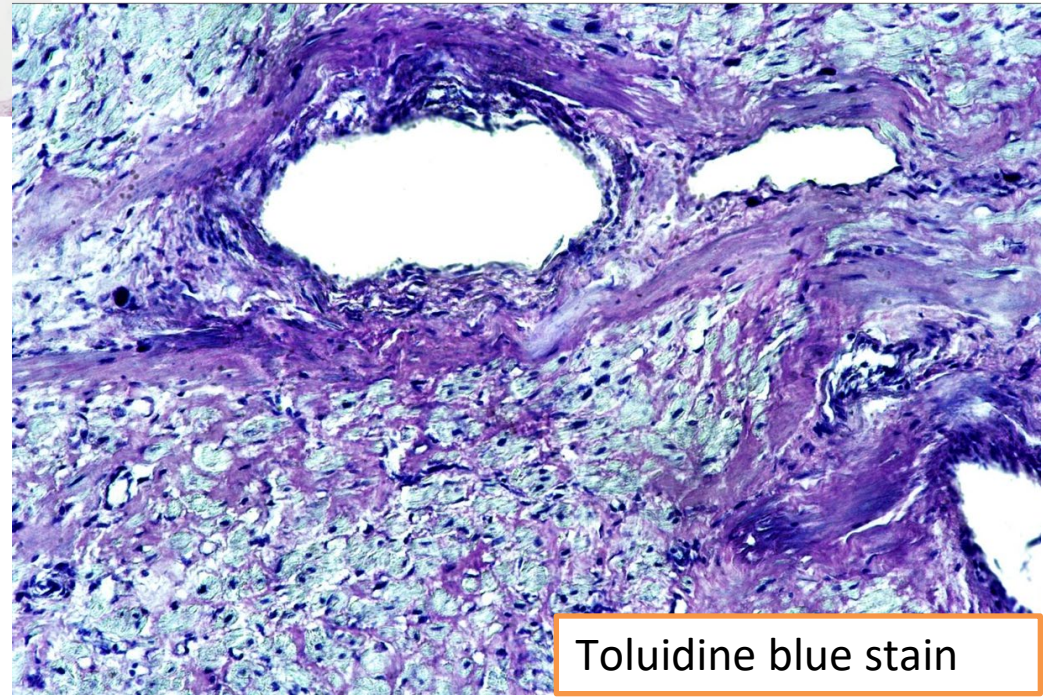
Glycosaminoglycans have hydrophilic properties, which leads to hydration and swelling of the main substance of the connective tissue.

Causes:
Rheumatic diseases;
Atherosclerosis;
Hypertonic disease;
Hypoxia.

metachromasia



H&E stain



Toluidine blue stain

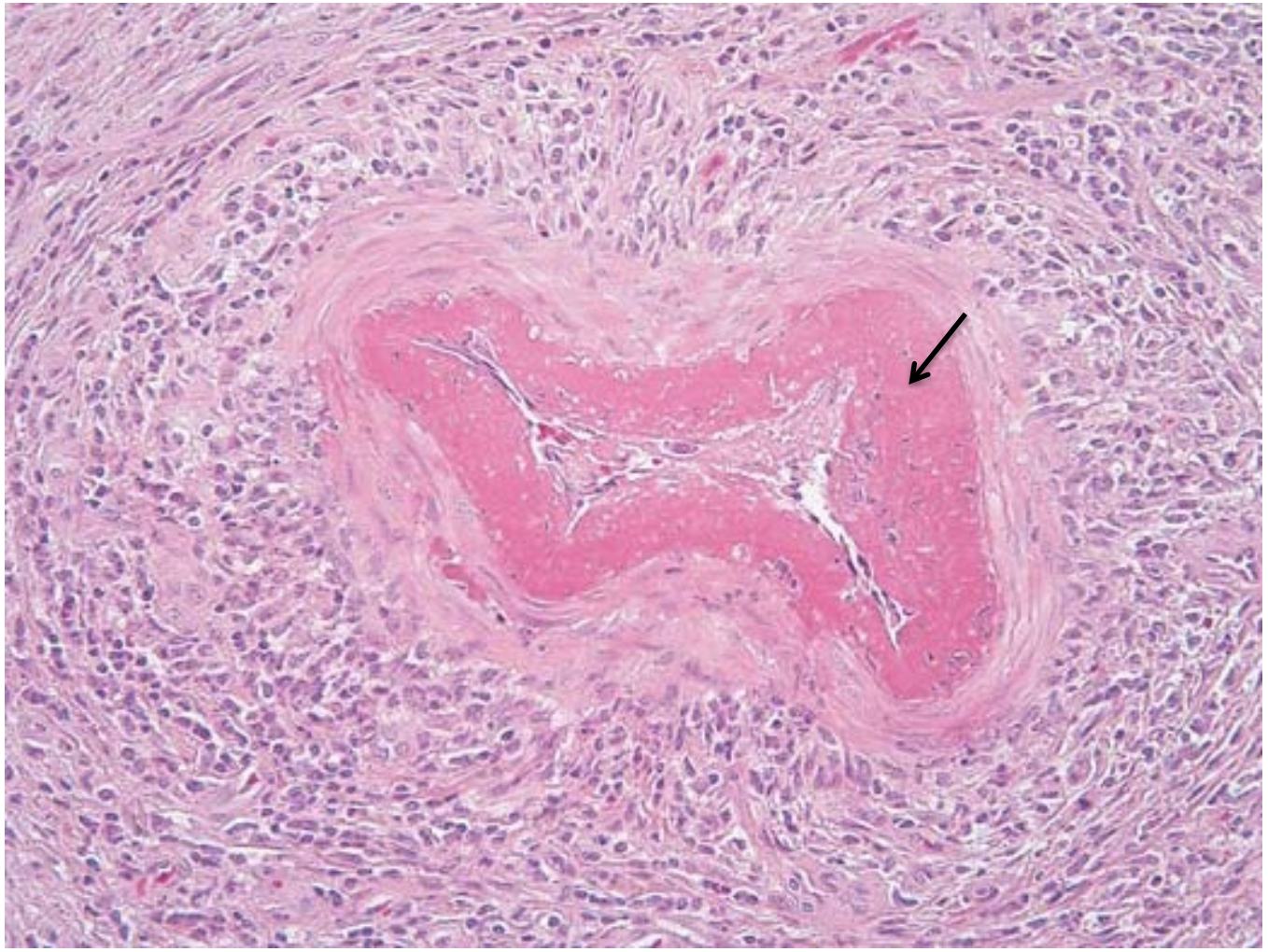
From: Tzavellas G, Skripochnik E, Landau D, Wain RA, Tassiopoulos AK. Intimomedial mucoid degeneration of the peripheral arteries. J Vasc Surg Cases Innov Tech. 2019 Oct 10;5(4):452-455. doi: 10.1016/j.jvscit.2019.04.005. PMID: 31660471; PMCID: PMC6806643.

Stromal and vascular protein injury

Step 2: Fibrinoid swelling (*disorganization*)

-**deep and irreversible disorganization of the connective tissue with the destruction of its basic substance and fibers and the formation of fibrinoid.**

“Fibrinoid” is a complex substance, an essential component of which is **fibrin**.



Microscopically, homogenization and eosinophilia of fibrous structures and the main substance of connective tissue is observed.

Macrophages may appear around the fibrinoid focus. Macroscopically, organs and tissues are little changed.

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Stromal and vascular protein injury

Step 3: **Hyalinosis**

“Hyaline Refers to Any Reddish, gomogeneous Material That Stains with Eosin” *

Alcoholic hyaline is composed of cytoskeletal filaments;
Hyaline found in arterioles of the kidney is derived from basement membranes;
Hyaline membranes in the lung consist of plasma proteins deposited in alveoli.

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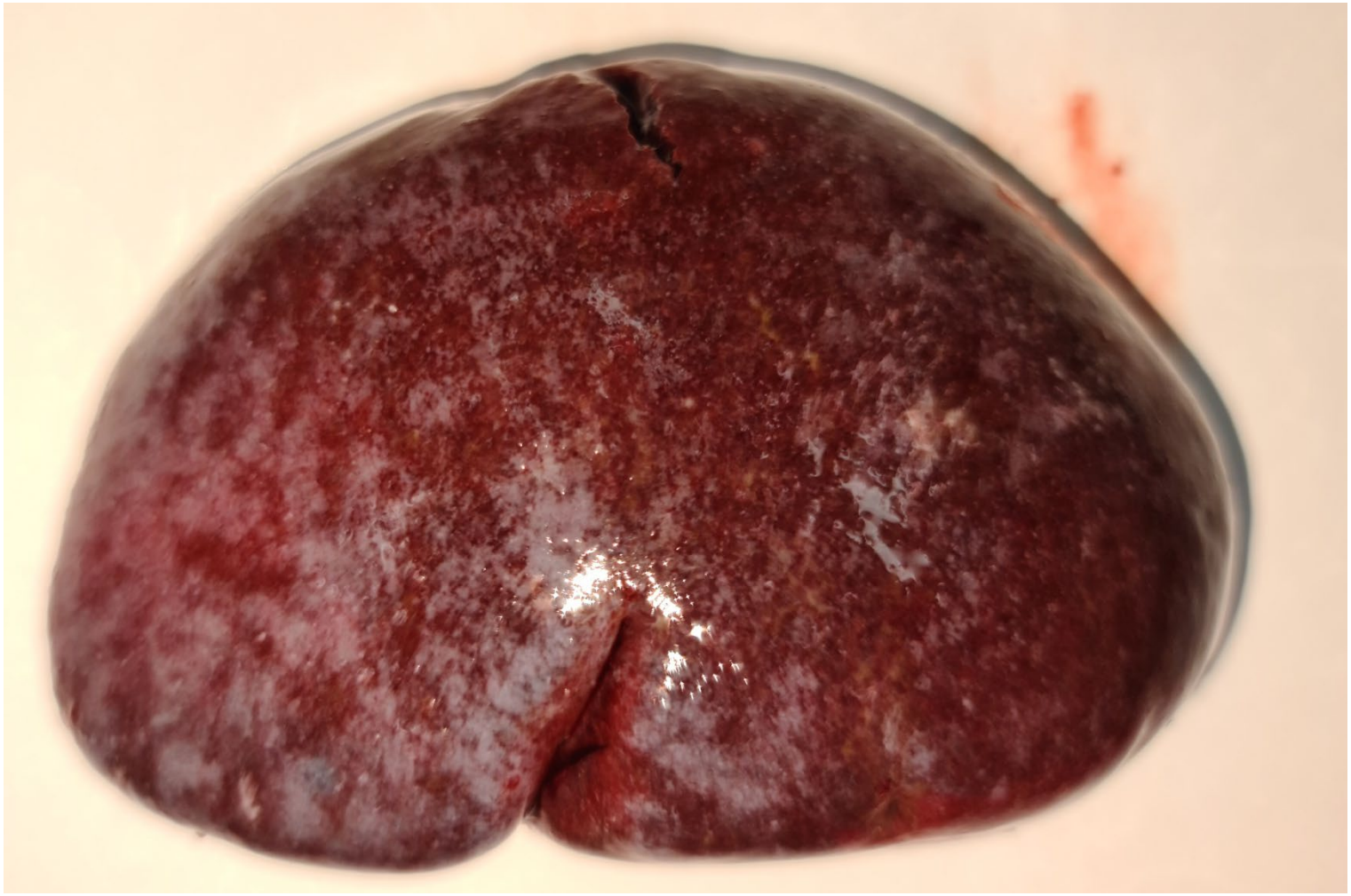
Examples of hyalinosis

«glazed spleen»



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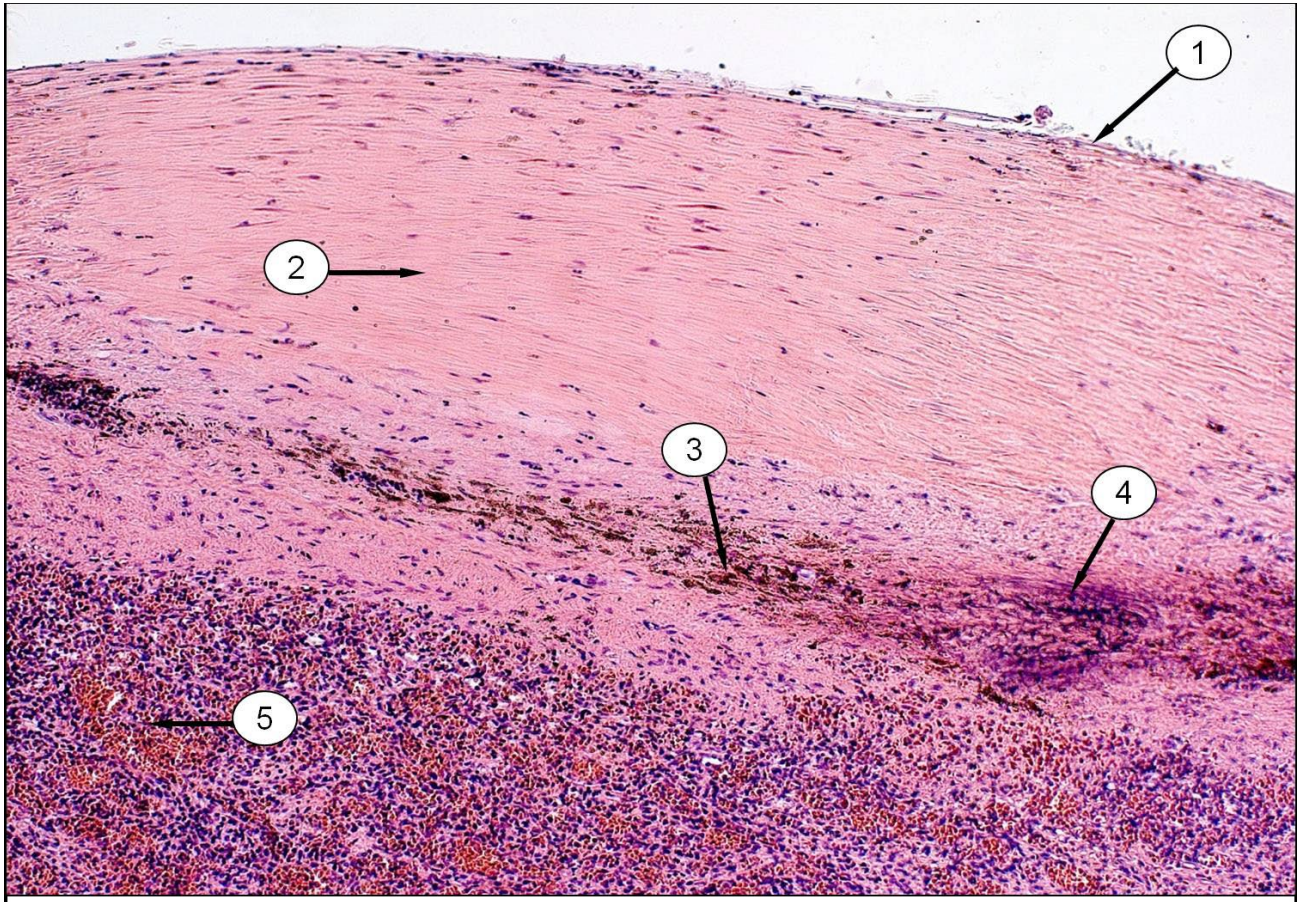
Examples of hyalinosis «glazed spleen»



1 - наружный край капсулы селезёнки; 2 - гиалиноз капсулы «глазурная селезёнка»; 3 - очаговый гемосидероз; 4 - кальциноз капсулы селезёнки; 5 - полнокровие синусоидов красной пульпы.

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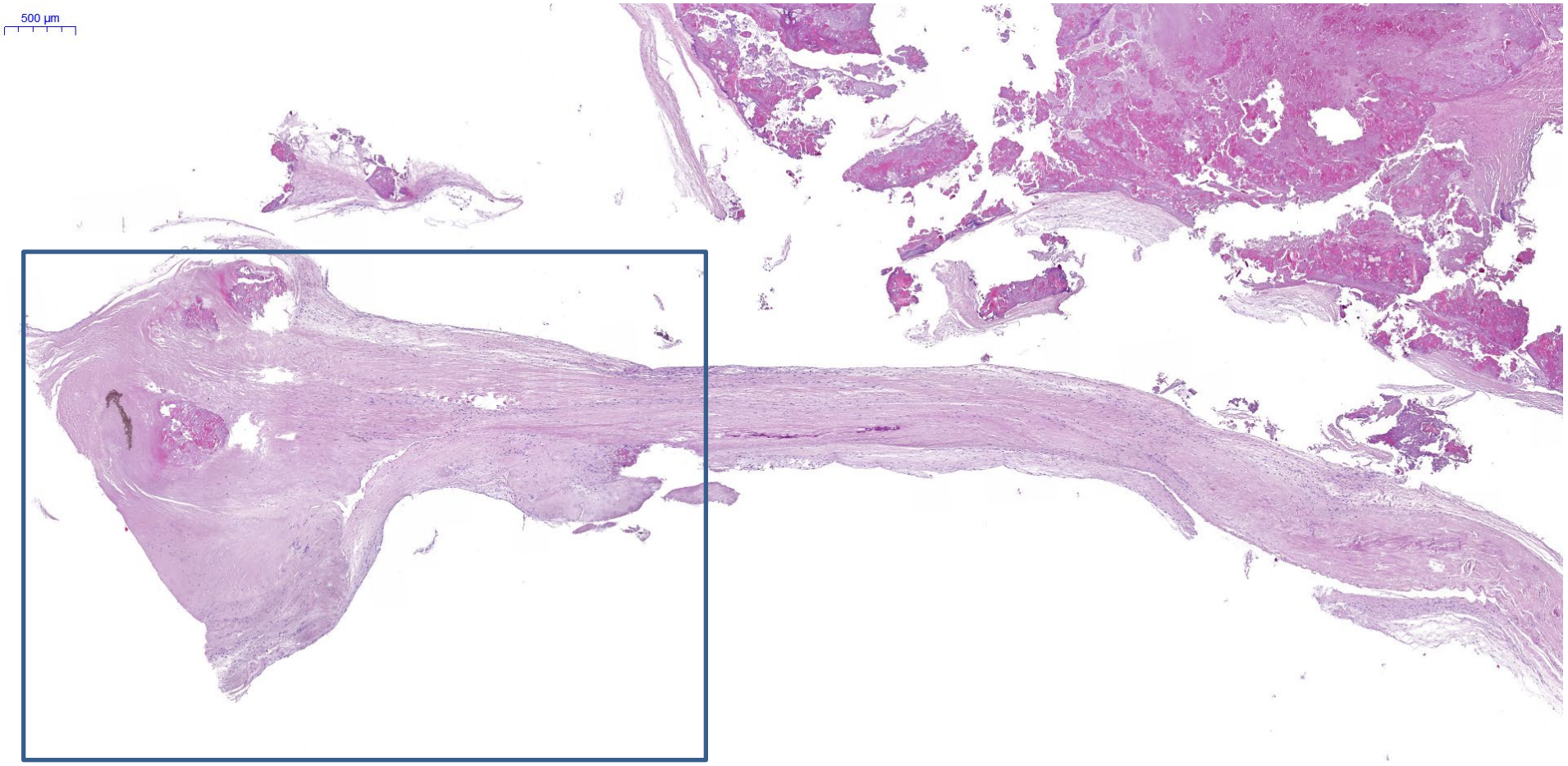
3 steps in 1



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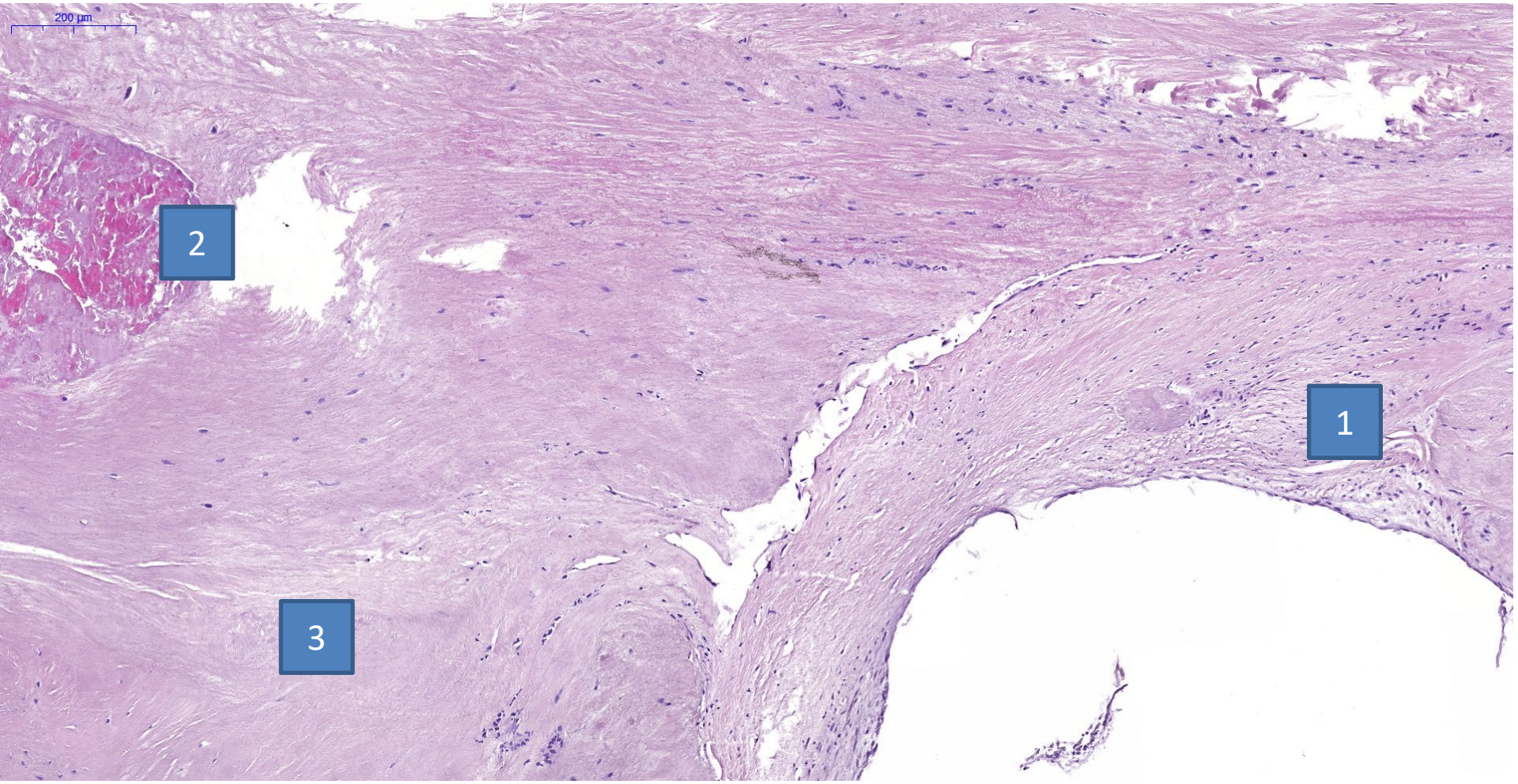

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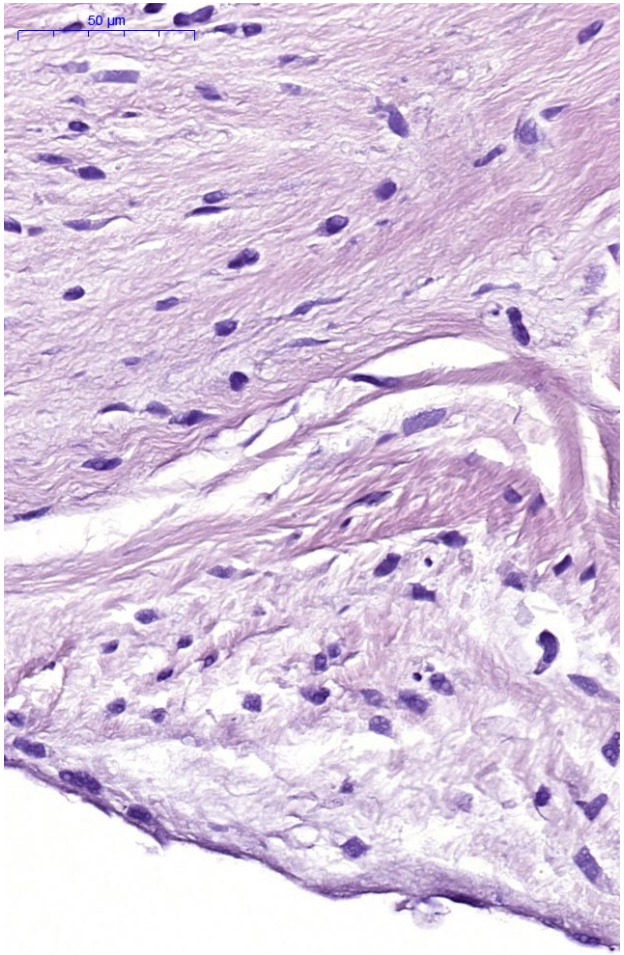
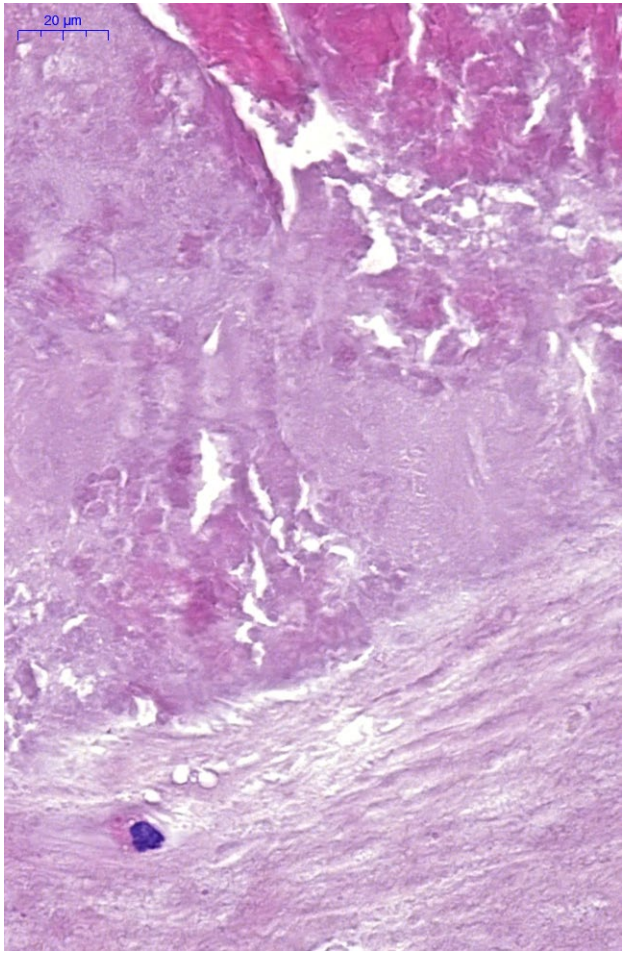
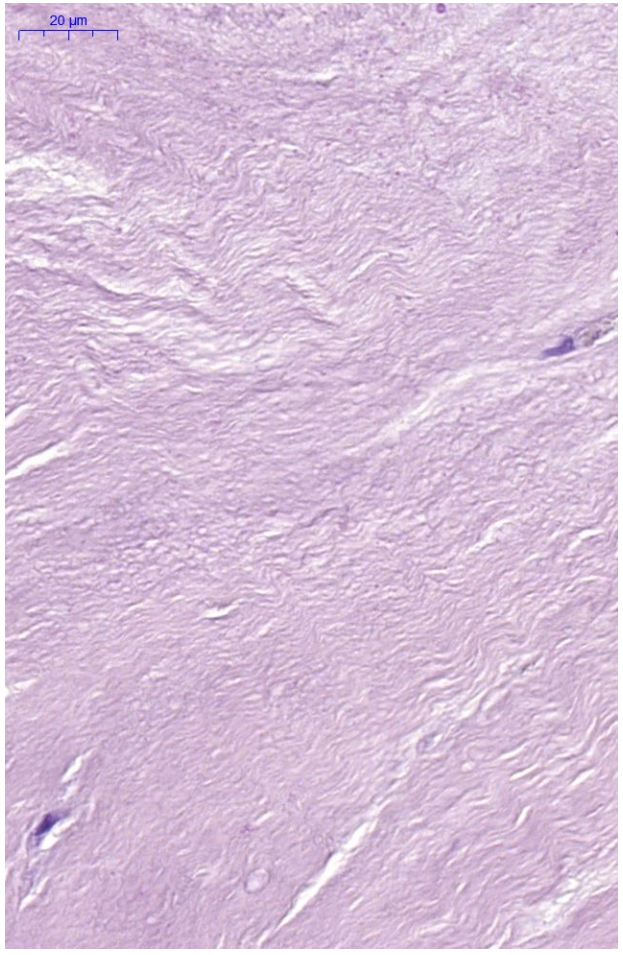


Mucoid swelling



Fibrinoid swelling
(*disorganization*)



Hyalinosis

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The clinical significance of hyalinosis

-is determined by its localization:

- Widespread hyalinosis of arterioles can lead to renal failure.

- Vascular hyalinosis is accompanied by a narrowing of their lumens, which in turn leads to hypoxia, atrophy and sclerosis of the organs.

- Hyalinosis in scars often does not cause any particular disorders.

- Hyalin is hardly absorbed, therefore, in most cases, the outcome of hyalinosis is unfavorable.

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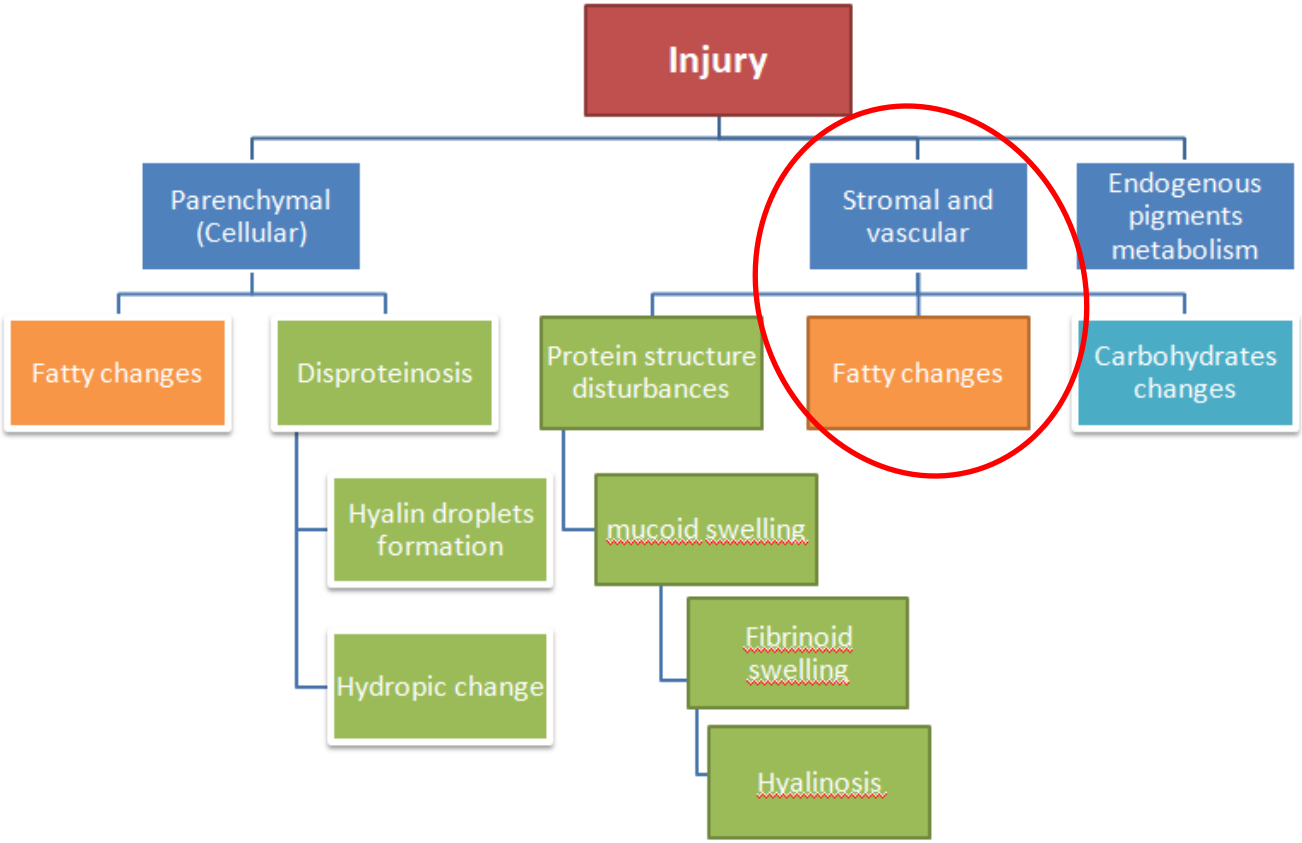
Stromal and vascular injury



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Stromal and vascular fatty injury

develops in case of metabolic disorders of neutral fats or cholesterol and its esters

- Disorders of the metabolism of neutral fats are manifested in obesity and cachexia.

- Obesity can be primary, the cause of which is unknown, and secondary.

- Types of secondary obesity:
- Alimentary
- Cerebral
- Endocrine
- Hereditary.

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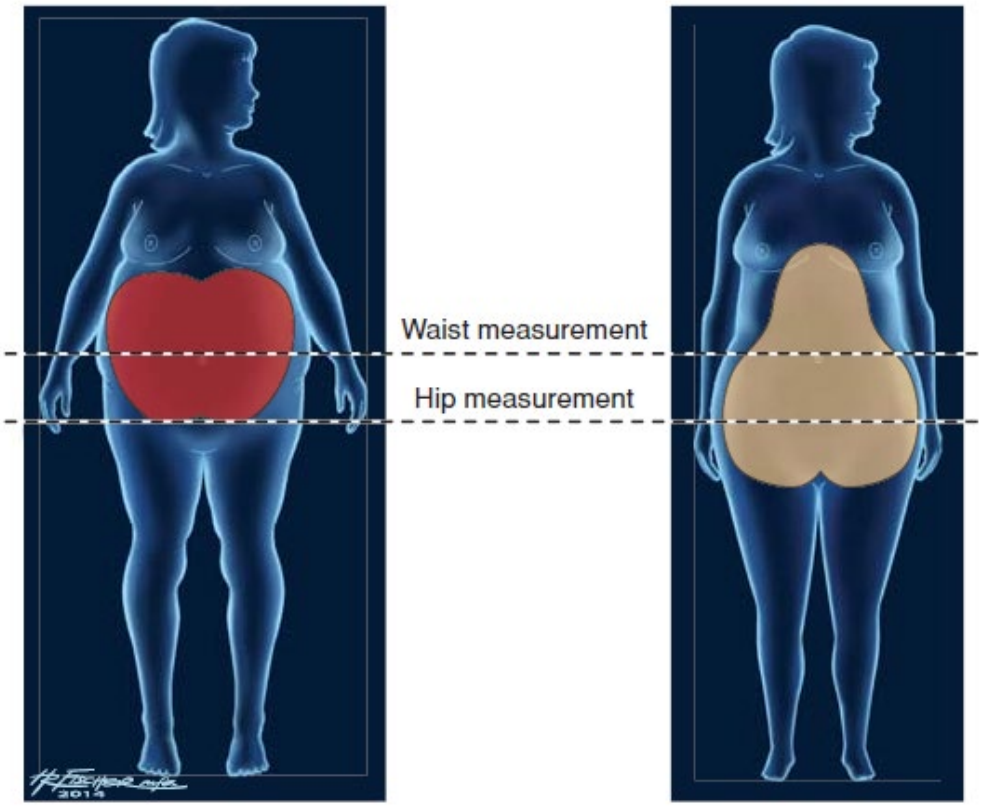
Types by fat distribution:

- Symmetrical
- Upper
- Middle
- Lower



Individuals who accumulate adipose tissue in the abdomen (“apple shaped”) exhibit increased risk of insulin resistance for glucose, type 2 diabetes mellitus and cardiovascular disease, compared to those with fat accumulations around the hips, buttocks and thighs (“pear shaped”). Standard methods to assess abdominal obesity include waist circumference and the waist:hip ratio.

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- Depending on the percentage of excess body weight of the patient, there are:

I degree - overweight is 20-29% obesity
II degree - 30-49%;
III degree -50-99%;
IV degree - more than 100%

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Cachexia

- is the opposite of general obesity.
- In case of cachexia, general atrophy of organs and tissues of the body develops.

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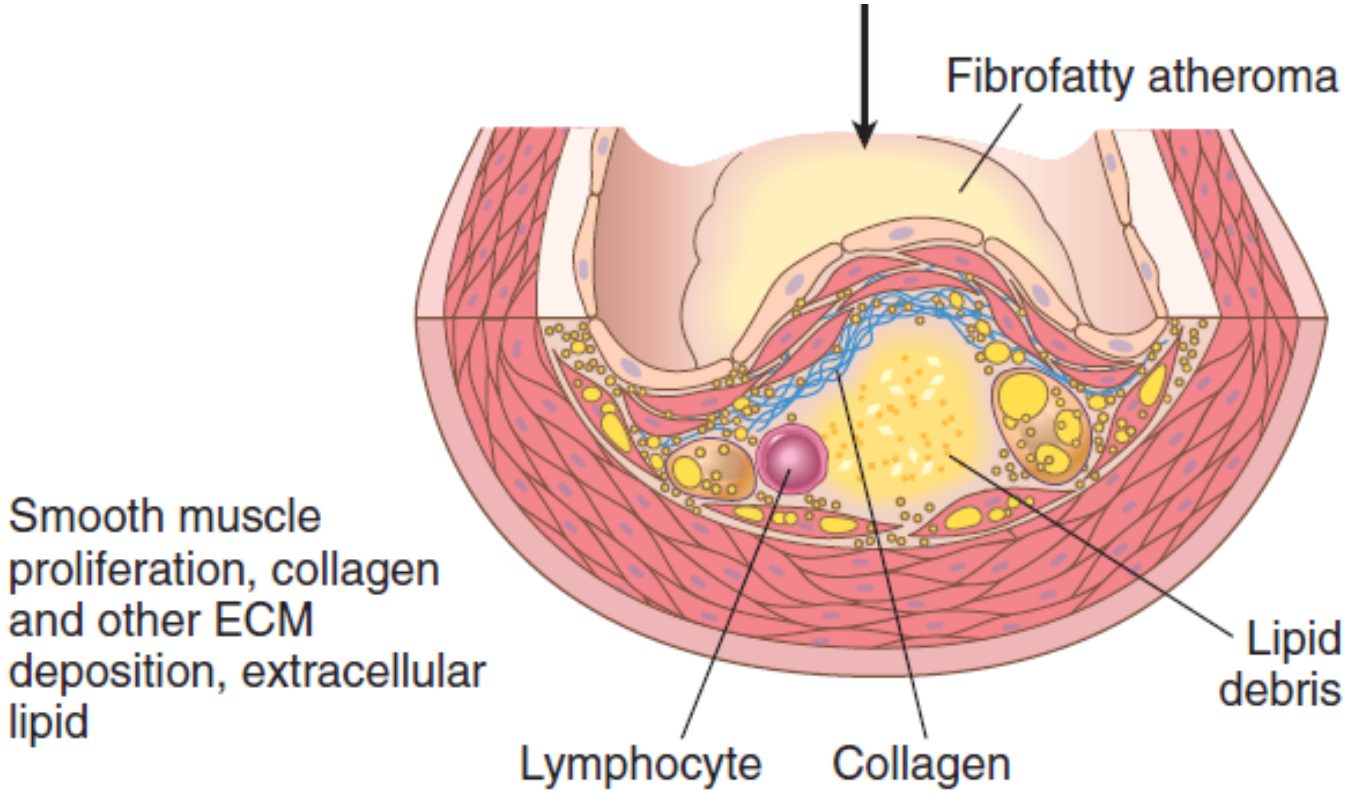
Disorders of the metabolism of cholesterol and its esters

mainly occur in atherosclerosis.

-In this disease, cholesterol and its esters accumulate in atherosclerotic plaques in the intima of large arteries.

atherosclerosis



- Lipids are localized in smooth muscle cells and macrophages as well as in extracellular area.
- The cytoplasm of these cells, due to the fatty vacuoles, has a foamy appearance (foamy, xanthoma cells).

* From: Kumar, V., Abbas, A. K., & Aster, J. C. (2017). Robbins Basic Pathology (10th ed.). Elsevier - Health Sciences Division.

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Thanks for attention!

Rudolf Ludwig Carl Virchow
«Father of the modern pathology»

Stanislav V. Gappoev.
Modern pathologist

Welcome to Pathology ☺

If you have a desire to take part in the work of the student scientific society on pathological anatomy - ask your teacher or lecturer.