HISTOCHEMISTRY

- Histochemistry is a method of staining tissue that provides information concerning the presence and location of intracellular and extracellular macromolecules.

- Histochemistry is complementary to biochemical analysis of tissue homogenates, since histochemical techniques can give simultaneously biochemical and morphological information.

The general protocol used for histological and histochemical study can be divided into the following main parts:

- Sampling
- Fixation
- Dehydration
- Clarification
- Infusion
- Embedding

- Sectioning
- Adhesion of section on a slide
- Staining
- Dehydration
- Mounting
- Observation

- The goal of the histochemistry techniques is to detect specific molecules in tissue sections, and therefore it is possible to study their distribution "in situ", that is in the tissue.

- These molecules cannot be readily distinguished by general staining techniques excepting those containing some pigments like hemoglobin in the blood or melanin in the epidermis.

- The tissue has to be treated to reveal the molecule we are interested in.

Histochemical techniques can be divided in two groups:

- Chemical reactions
- Histo-enzymology

1. Chemical Reactions

- Chemical reactions are modifications of tissue molecules that allow them to be colored.
- There are histochemical procedures for staining carbohydrates, proteins and nucleotides.
- PAS (Periodic Acid Schiff) is the most popular histochemical technique for detecting free or conjugated carbohydrates that can be visualized when they are relatively abundant in the tissue (Figure).

1. Chemical Reactions

- The chemical modification consists in the oxidation by periodic acid of close carbon links that have hydroxyl groups.
- This reaction forms aldehyde groups that are recognized by the Schiff reactive, providing a brilliant red color.
- PAS technique is able to discriminate different types of carbohydrates by adjusting the procedure.

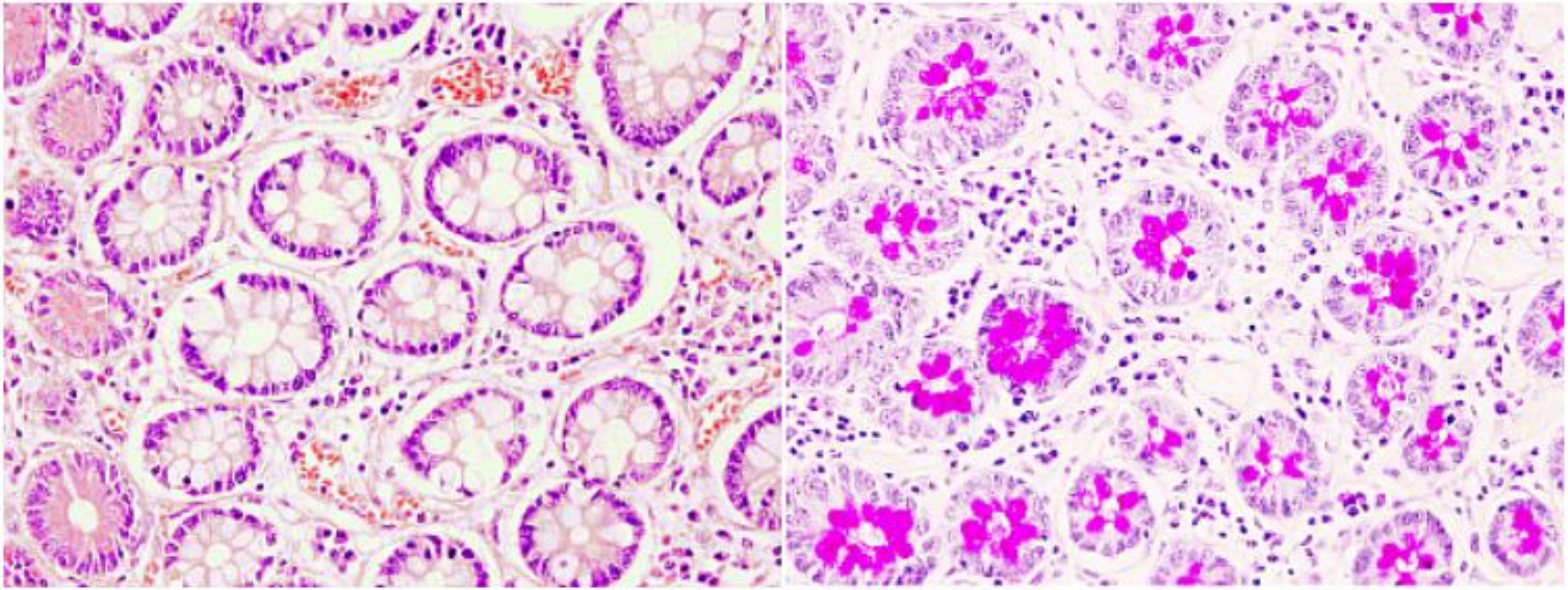


Figure 1. Haematoxylin-eosin staining (on the left) and PAS-haematoxylin (on the right) staining of human intestinal crypts in transverse view. Goblet cells are stained in pink with PAS histochemistry because of the high content of mucopolysaccharides, whereas in the general staining they are not stained, cells look clear. Nuclei are stained with haematoxylin.

2. Enzymatic Reactions

- Histo-enzymology, or enzyme histochemistry, is based on the capacity of some enzymes to keep their activity after the tissue fixation.

- These enzymes, and the cells they are located in, can be visualized after the conversion of soluble and colorless substrates to insoluble and colored products by the enzyme activity.

2. Enzymatic Reactions

- Substrates are specific for the enzyme and the products precipitate in the place where the enzyme is.

- There is a diversity of enzymes that can be detected with this method, such as peroxidases, phosphatases, dehydrogenases, acetylcholinesterase, and some others.

2. Enzymatic Reactions

- It should keep in mind that fixation and embedding of the tissue may affect the enzyme activity.
- Embedding should be avoided because dehydration and high temperature may damage the enzyme and therefore its activity.
- That is why histo-enzymology is mostly done on freezing sections where no embedding is needed.

- The NADPH diaphorase activity is performed in the nervous system by the endothelial and neuronal nitric oxide synthase enzymes.

- Nitric oxide has been involved in the control of the blood flow and neuronal activity.

- By using histo-enzymology, neurons (Figure) and endothelial cells expressing these enzymes can be quickly and easily identified in tissue sections.

- The enzymatic reaction is: NADPH + nitroblue tetrazolium = $NADP^+$ + formazan.

- Formazan is the colored and insoluble product of the reaction that can be observed at light microscopy.

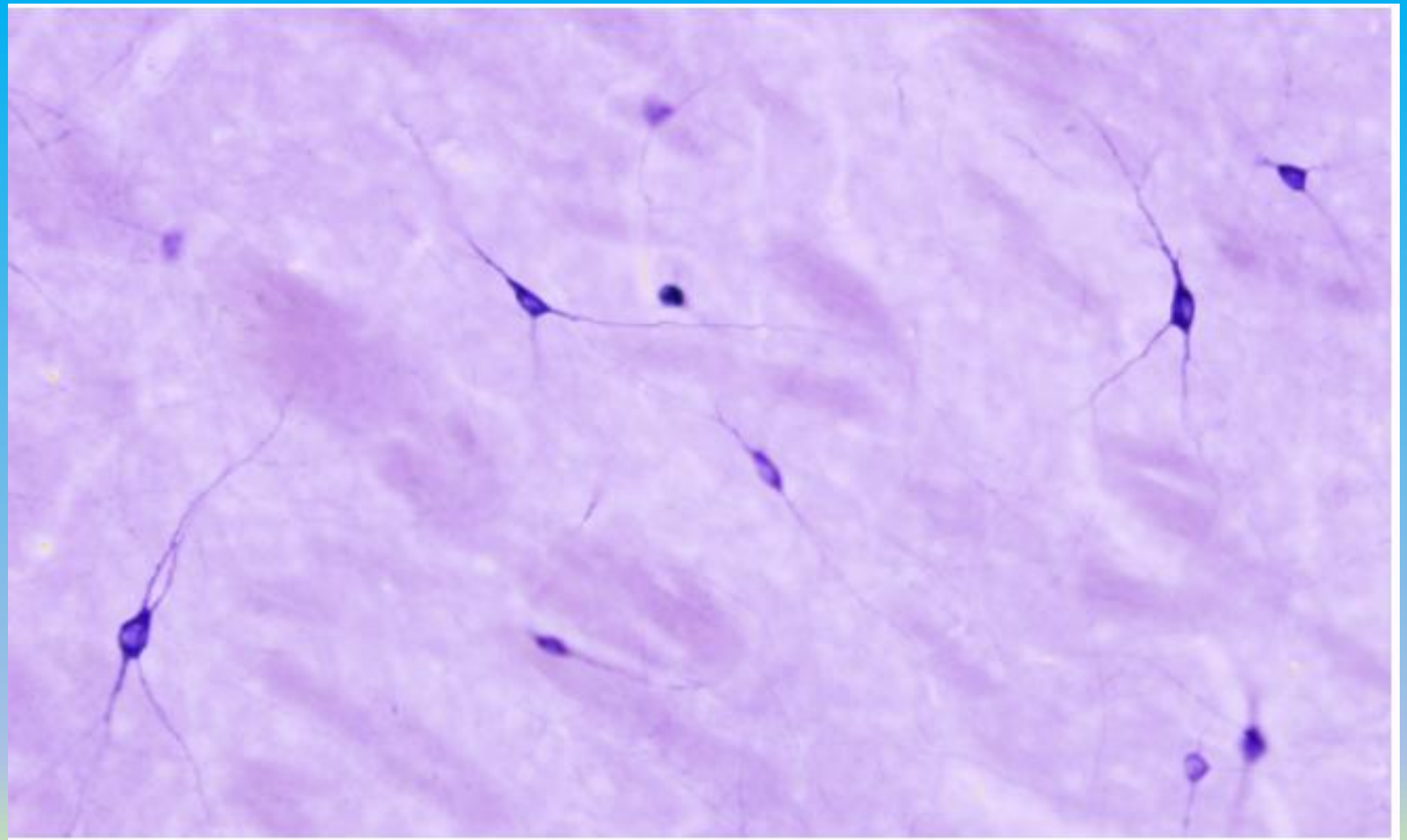


Figure NADPH diaphorase histoenzymology. NADPH diaphorase activity in a 60 µm section of a rat brain. Neurons expressing nitric oxide enzyme activity are colored in dark blue.

3. Lectins

- Lectins, such as selectins, are proteins bearing molecular domains that are able to recognize carbohydrates and carbohydrate bonds.

- The recognition is so specific that lectins are used to identify carbohydrates present in glycoproteins of cell membranes and extracellular matrix, as well as mucopolysaccharides.

- Lectins recognize some cell types and tissues, and are used as tools to study those tissular components.

Stain	Target
Pigments and Minerals	
Alizarin red S	Calcium
Bile–Hall's bilirubin stain	Bilirubin
Copper stain	Copper; commonly used to diagnosis Wilson's disease
Fontana–Masson silver method	Argentaffin granules (present in carcinoid tumors) and melanin
Prussian blue	Iron
Turnbull's blue (iron stain)	Ferrous iron in tissue
Sudan black B stain (for lipochrome)	Lipofuscins
Gomori's methenamine silver	Urate crystals
Von Kossa stain for calcium	Calcium
Carbohydrates	
Alcian blue	Acid mucopolysaccharides, acetic mucins
Alcian blue/periodic acid–Schiff (PAS) stain	Differentiates neutral and acetic mucosubstances
Colloidal iron	Mucopolysaccharides
Congo red	Amyloid
Mucicarmine stain	Mucin
PAS stain	Glycogen
Periodic acid–Schiff, digested stain with diastase (PAS-D)	Detects glycogen by digesting out sugars, followed by PAS staining
Thioflavin S stain	Amyloid deposits in tissue
Lipids	
Oil red O stain	Lipids in frozen sections
Sudan black B stain (for fat)	Lipids
Connective Tissue	
Verhoeff–Van Gieson stain	Elastin fibers
Weigert's resorcin–fuchsin	Elastin fibers
Fraser–Lendrum method	Fibrin
Jones' silver stain	Basement membrane of the glomerulus in kidney
Phosphotungstic acid–hematoxylin, Mallory's (PTAH stain)	Muscle cross-striations and fibrin
Reticulin stain	Reticular fibers
Trichrome stain, Masson's method	Differentiates between collagen and smooth muscle in tissue
Methyl green pyronin (MGP) stain	Stains DNA green and RNA red

CARBOHYDRATE DEMONSTRATION

- Mucins and glycogen are the two main entities to be considered in tissue carbohydrate demonstration, of which mucins are the largest group comprising conjugates.

- The role played by glycogen in body metabolism as a glucose precursor is well established, but the precise function of mucin in all its forms has yet to be fully understood.

- Most surfaces are coated with mucin having an obvious lubricatory function, but it has been suggested that this coating also forms a favorable environment for ionic and molecular diffusion.

GLYCOGEN

- This is a simple polysaccharide which consists of branched or straight chaind D glucose units and ban be seen with the electron microscope to occur in the following three main forms:

- Alpha

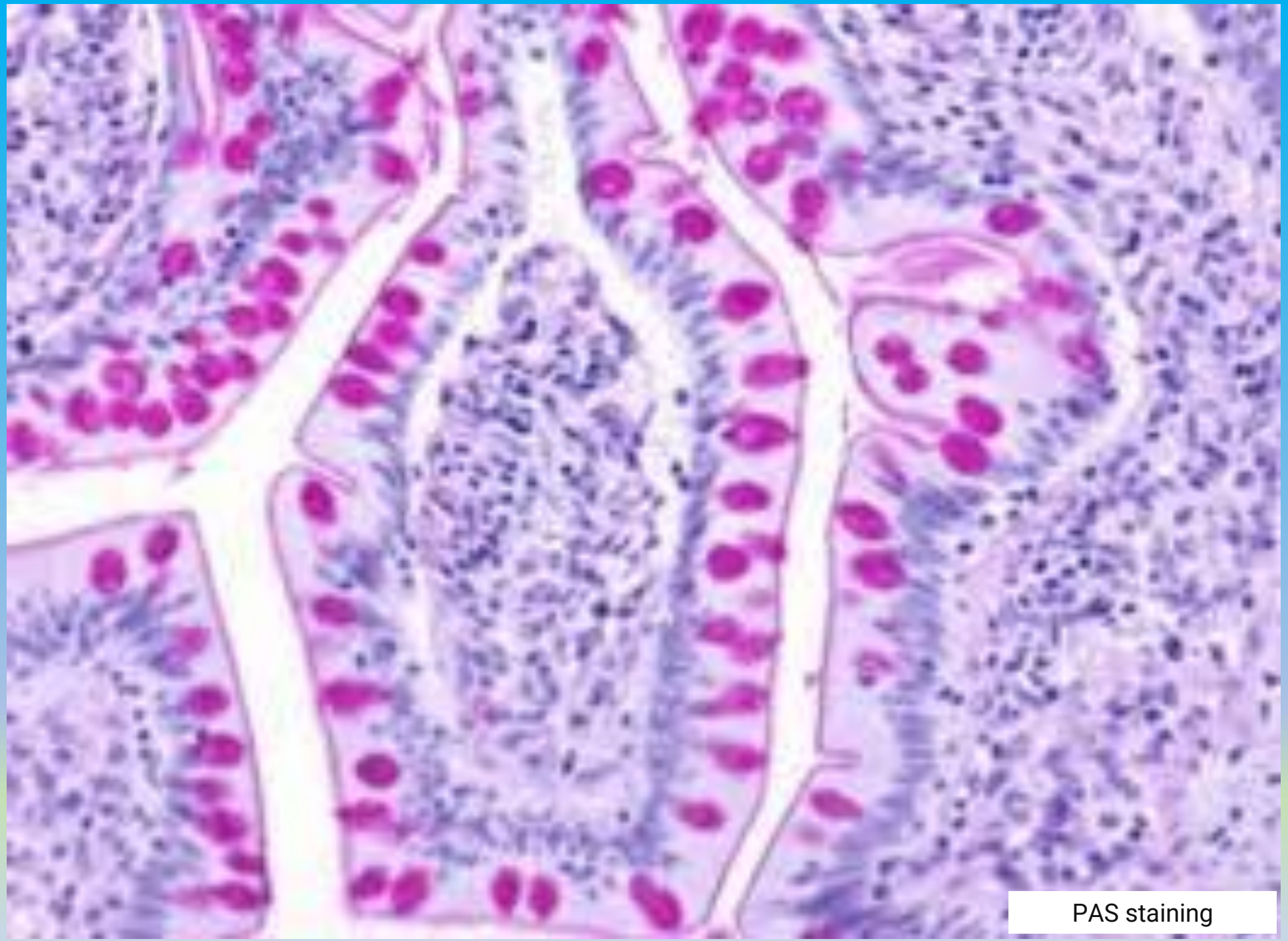
- Beta

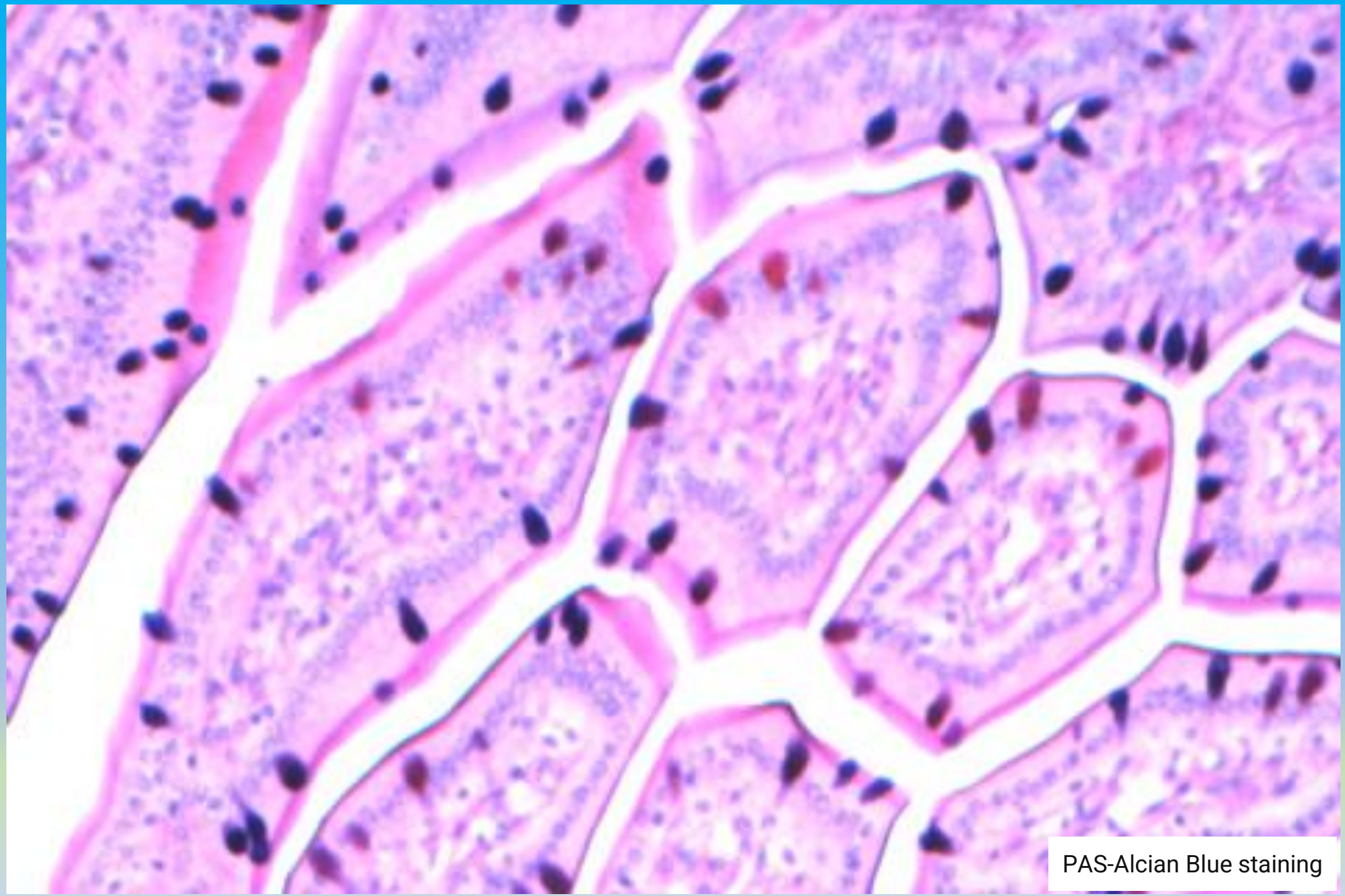
- Gamma

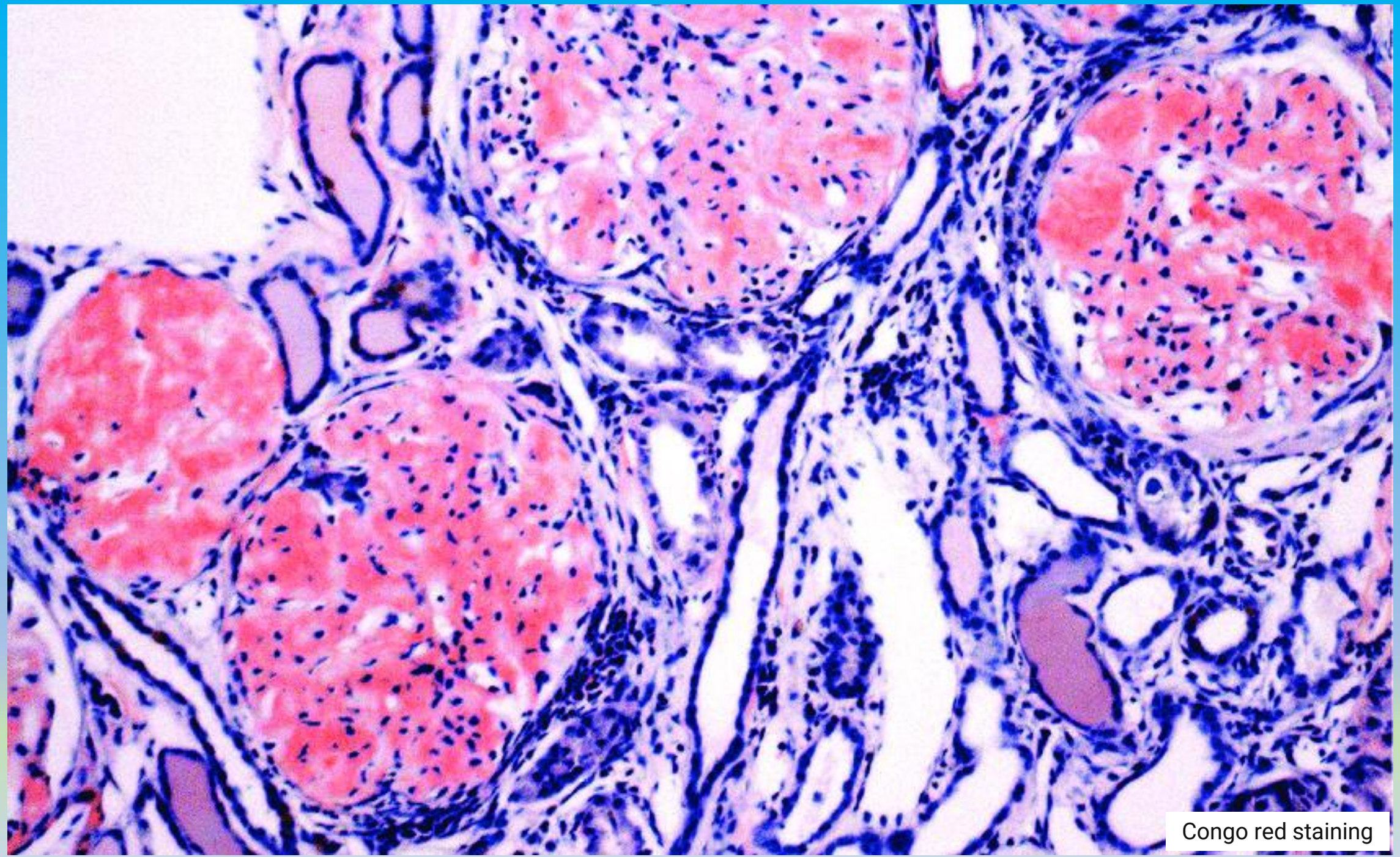
- Under normal conditions, glycogen is intracytoplasmic and found in greatest amonts in liver, cardiac muscle and skeletal muscle with significant quantities also present in hair follicles endometrial glands vaginal and ectocervical epithelium.

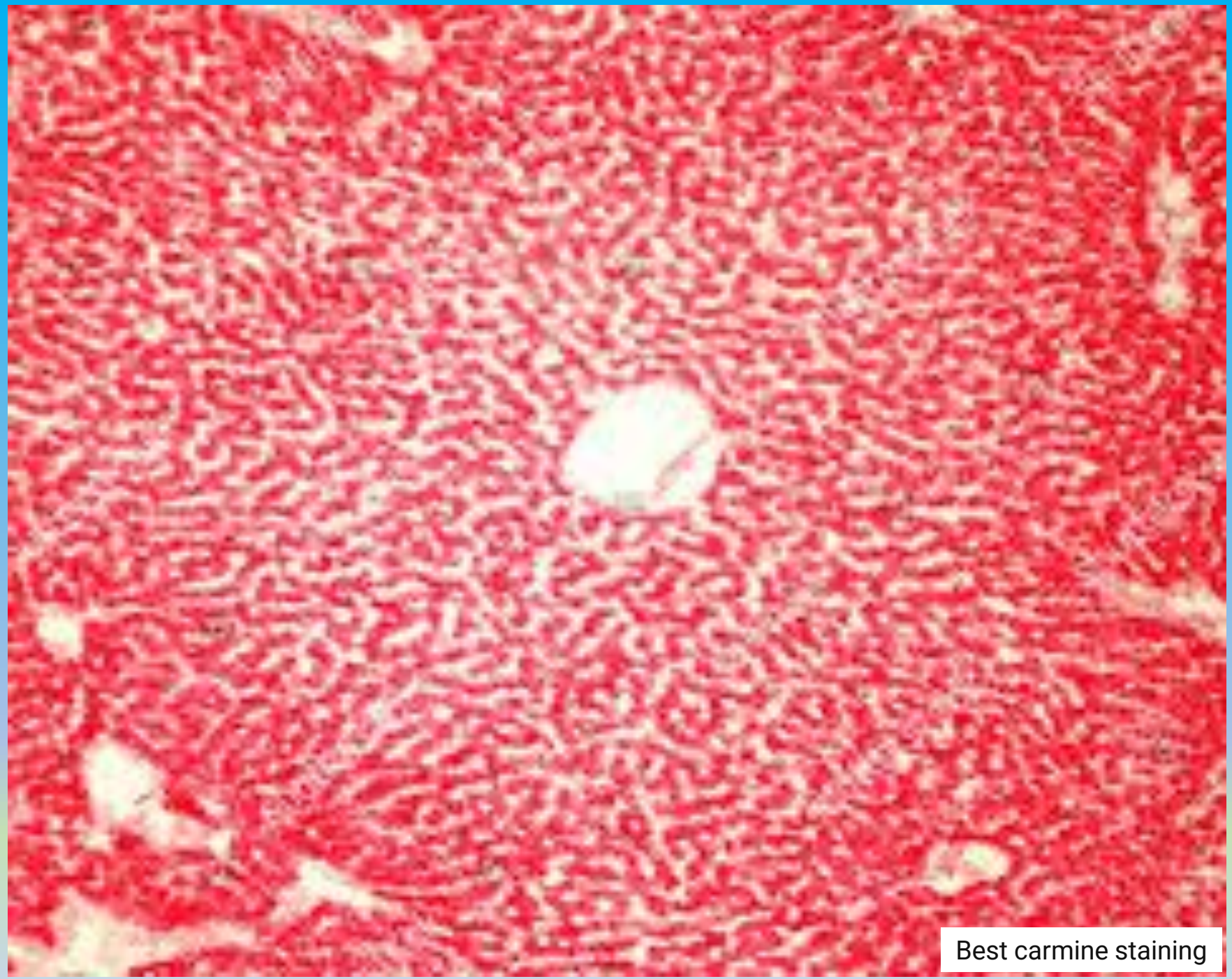
- Umbilical cord, mesothelial cells, neutrophil leukocytes and megakaryocytes also contain glycogen.

- It is considered that glycogen exists in tissue within a protein environment, rather than chemically bound to protein.


PAS staining


PAS-Alcian Blue staining

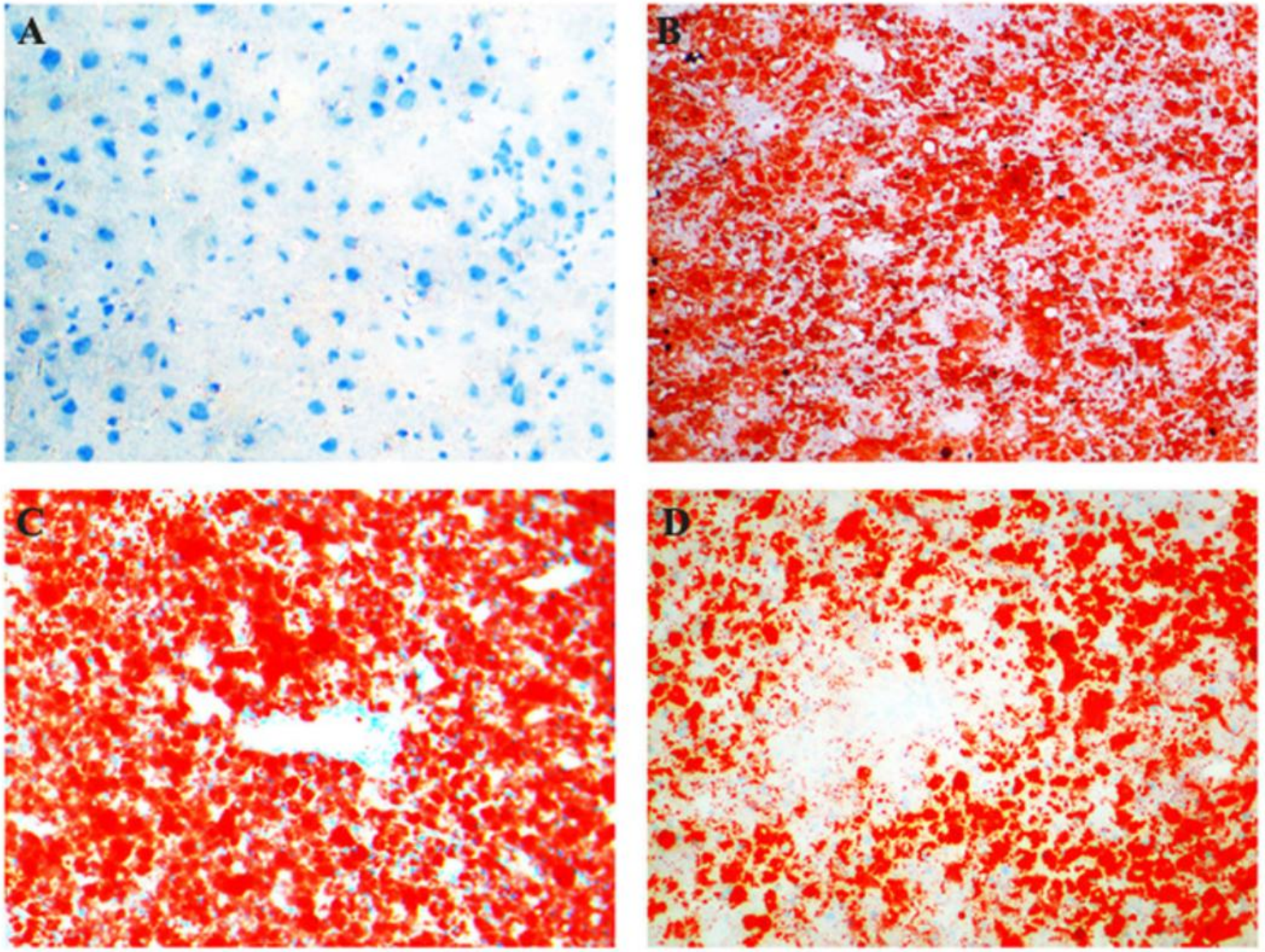

Congo red staining



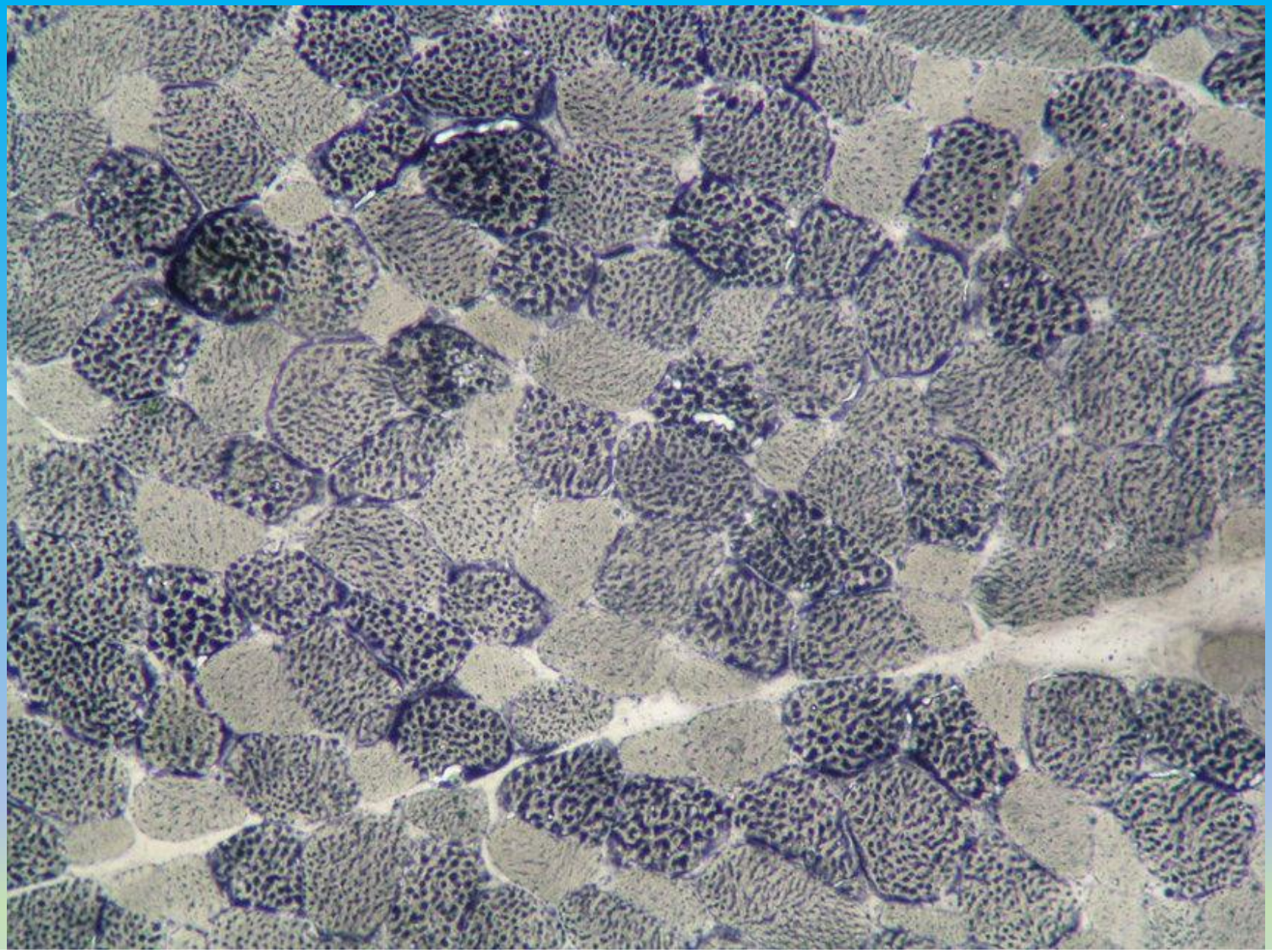
Best carmine staining

LIPIDS

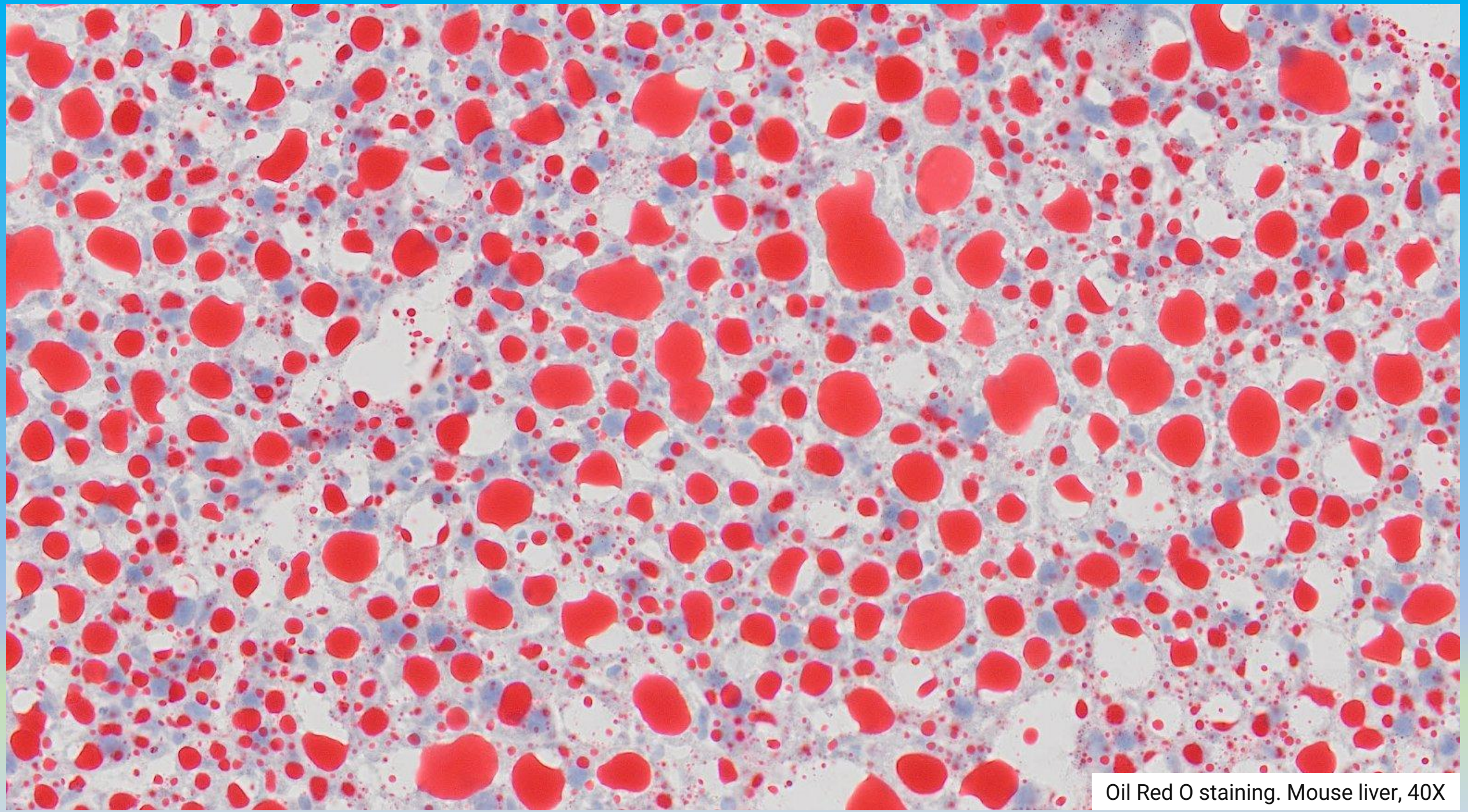
- Stained with dyes soluble in the lipids
- Sudan IV, Sudan black, Oil red o for light microscopy
- For electron microscopy, osmium tetraoxide
- Such methods are used to show normal lipid distribution and disease related lipid accumulation (fatty change in the liver)



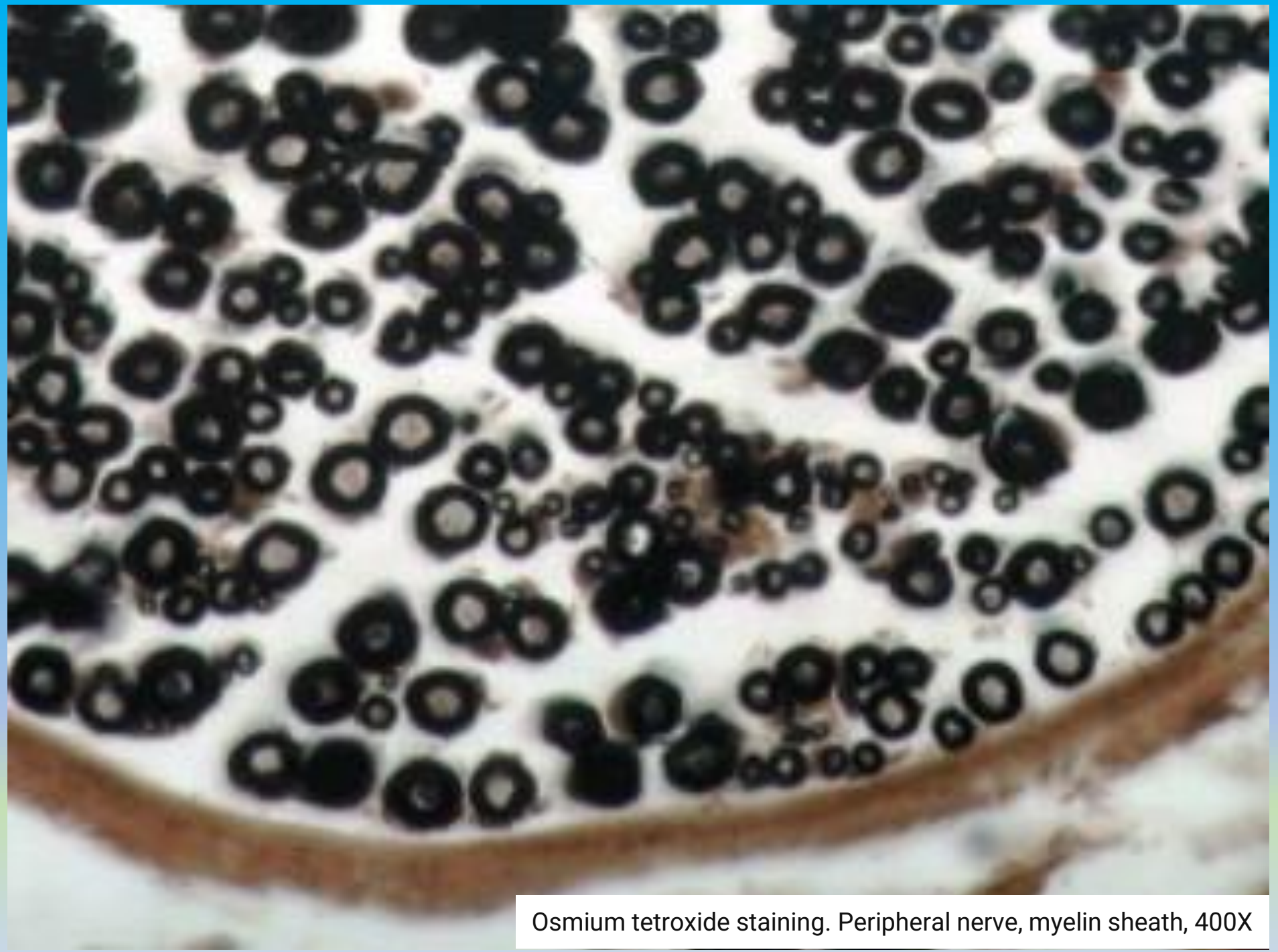
Sudan IV staining of hepatic tissues in each group of rats. Hepatic steatosis emerged until 8 th week and aggravated gradually with the consumption of high-fat diet (B). Panacinar steatosis was observed at the end of 24 th week (C). These changes were improved in the liver of simvastatin-treated rats (D).



The lipid storage can be well demonstrated by Sudan Black stain which revealed small lipid droplets in type I muscle fibers (ob. ×40).



Oil Red O staining. Mouse liver, 40X



Osmium tetroxide staining. Peripheral nerve, myelin sheath, 400X

PROTEINS AND AMINO ACIDS

- Older methods of portein detection are nonspecific for proteins but specific for some amino acids.
- Million reaction for tyrosine
- Sakaguchi reaction for arginine
- Tetrazotized benzidine reaction for tryptophan

PROTEINS AND AMINO ACIDS

- Specific class of enzymes can be detectable by the techniques for enyzme histochemistry
- Specific proteins can now be localized by using immunohistochemistry.

NUCLEIC ACIDS

- Nucleic acids can be localized by specific or nonspecific methods.

- DNA is found mainly in nuclei and the same amount, while RNA is present both in nuclei and in cytoplasm and widely variable.

- Feulgen reaction determines the amounts of Dna
- Methyl green pyronine stain to detect not only Dna but also rna
- Acridine orange thefluorescence is yellow green if the complex contains dna and red orange if the complex contains rna.

NUCLEIC ACIDS

- Feulgen reaction determines the amounts of DNA
- Methyl green pyronine stain to detect not only DNA but also RNA
- Acridine orange: The fluorescence is yellow green if the complex contains DNA and red orange if the complex contains RNA.

DISADVANTAGE OF HISTOCHEMISTRY

1. Cannot be used for real time in vivo analysis of any tissue (requires the removal and killing of the tissue).

2. For looking at changes in tissue over time, each point in time requires a new tissue sample from a new animal.

3. Tissue preparation and histochemical analysis may alter specimen morphology or chemistry depending on the methods.

THANK YOU FOR YOUR GRAND ATTENTION