Lectures of
Histological Microtechniques

(Master Stage)
Second Semester 2023-2024

Course period 15 weeks

By
Professor
Dr. Khulood Naji Rasheed

Anatomy and Histology Department

Histochemistry: Visualizing Cellular Chemistry

Branch of science concerned with the identification and distribution of the chemical constituents of tissues. it is one of the most widely used techniques to help scientists localize and visualize cellular components, tissues, and other living structures.

This technique uses different stains and indicators, which reacts with the cellular components, to develop tiny colored structures that could be easily observed under a microscope. Histochemistry involves the aspect of both Chemistry and Histology.

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 inside the tissue. These molecules cannot be readily distinguished by general staining techniques. Histochemical techniques can be divided in two groups: chemical reactions and histoenzymology.

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 (Fig. 1).

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. Schiff reactive contains pararosaniline (a component of the basic fuchsin), which has been previously treated with sulfuric acid. PAS technique can discriminate different types of carbohydrates by adjusting the procedure.

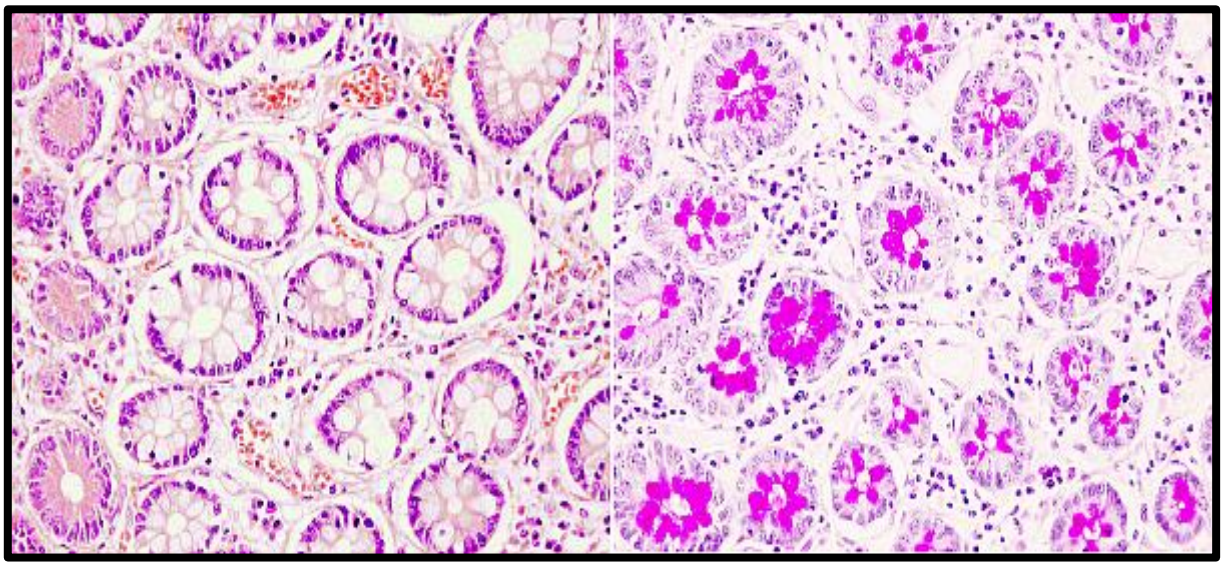

Fig. 1: H&E staining (on the left) and PAS-hematoxylin (on the right) staining of human intestinal crypts in transverse view. Goblet cells are stained in pink with PAS histochemistry because of the high content of mucopolysaccharides, whereas in the general staining they are not stained, cells look clear. Nuclei are stained with hematoxylin.

2. Enzymatic reactions

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

Enzymes that can be detected with this method include peroxidases, phosphatases, dehydrogenases, diaphorases, acetylcholinesterase, and some others. 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 histoenzymology is mostly done on freezing sections, where no embedding is needed.

Special Stains

It provides with an increased level of detail, enabling the easy visualization and identification of specific cells, tissue types, cellular products, and morphology of microorganisms needed for a precise diagnosis. Based on classical dye staining methods, special stains technique provides valuable information in the evaluation of numerous abnormal or disease conditions.

Examples include:

- Hematoxylin, Eosin, Alcian blue (cartilage, polysaccharides)
- Masson trichrome (skin: identification of collagenous connective tissue).
- Van Gieson trichrome (connective tissue)
- GMS silver stain (lung: identification of Pneumocystis or Aspergillus spp.).
- Perl's Prussian blue iron (liver: identification of ferric (Fe3+) iron in tissue preparations or blood and bone marrow smears).
- Ziehl-Neelsen (acid-fast bacillus) (lung: identification of acid-fast bacilli).
- Alcian blue (intestine: identification of acid mucopolysaccharide and acidic mucins).
- Periodic acid-Schiff (PAS) (kidney: identification of high proportion of carbohydrates, such as glycogen, glycoproteins, and proteoglycans).
- PAS-Alcian blue (intestine; combination of staining properties of both Alcian blue and Periodic acid-Schiff for identification of similar tissue components).
- PAS - Alcian blue (polysaccharides)
- PAS - hematoxylin (polysaccharides)
- Nissl staining (nervous tissue)
- Woelcke staining (nervous tissue, myelin)
- Hall's method (bilirubin or bile pigment in tissue section).
- Von Kossa method (the sections for calcium).
- Gomori trichrome (blue or green, e.g., submucosa, identification of muscle fibers, collagen, and nuclei).
- Gomori's method (reticulum fibers by silver nitrate method).
- Gomori's method (for hemosiderin a tissue pigment).
- Mayer's Mucicarmine method (demonstrate mucin in a tumor or epithelium).