What is histochemistry?

- It is a histological technique used for studying chemistry of tissues and cells
- Histochemistry
- Enzyme histochemistry
- Immunocytochemistry
- In situ hybridization

Examples of Histochemical Methods:

Iron (ferric ions – Fe\(^{3+}\))
- Perls’s reaction:
  - sections of tissues are incubated in a mixture of potassium ferrocyanide and hydrochloric acid
  - result: insoluble dark blue precipitate of ferric ferrocyanide
- Diagnostic application:
  - patients with diseases that store iron (e.g., hemochromatosis)

Examples of Histochemical Methods:

Lipids
- stained with dyes soluble in the lipids
- e.g., Sudan IV, Sudan black, Oil red, Nile blue
- fresh frozen (cryostat) sections are used for the most authentic picture of tissue lipids

Examples of Histochemical Methods:

Nucleic Acids – DNA
- Feulgen’s reaction:
  - hydrolysis of DNA by hydrochloric acid
  - this process leads to the formation of aldehyde groups
  - free aldehyde groups react with the Schiff reagent
  - result: insoluble red substance

Examples of Histochemical Methods:

Nucleic Acids – RNA
- RNA-rich organelles are stained with basic dyes
  - i.e., toluidine blue, methylene blue
  - Since the RNA is not the only basophilic substance in the tissue it is necessary to incubate a control slide with ribonuclease
Histochemistry
Examples of Histochemical Methods: Saccharides

- Saccharides can be detected by **PAS reaction** (periodic acid-Schiff)
- It is based on oxidative action of periodic acid (HIO₄) → aldehyde groups
- These aldehyde groups react with Schiff’s reagent (as in Feulgen’s reaction)
- → a new compound with a purple color (PAS-positive substances)

Histochemistry
Examples of Histochemical Methods: Saccharides

- PAS-positive substances are:
  - polysaccharides (glycogen)
  - glycosaminoglycans/mucopolysaccharides (hyaluronic acid, chondroitin sulfate)
  - proteoglycans
  - glycoproteins (thyreoglobulin, collagen)
  - glycolipids (lipofuscin)
- Clinical application:
  - biopsies of tissues from patients with diseases that store glycogen (glycogenosis), glycosaminoglycans (mucopolysaccharidoses)...

Enzyme Histochemistry

- Enzymes are the catalysts of most of biochemical reactions
- Frozen tissues are used
- Principle:
  - 1st reaction (histochemical):
    - enzyme + substrate → product
  - 2nd reaction:
    - demonstration of the product

Enzyme Histochemistry – Examples

- Peroxidase
  - sections are incubated in a solution containing hydrogen peroxide and DAB (diaminoazobenzidine)
  - DAB is oxidized in the presence of peroxidase
  - result: insoluble, black, electron-dense precipitate
- Clinical application:
  - diagnosis of leukemias (detection of the peroxidase activity in blood cells)
- Peroxidase is used also as a label in immunocytochemistry and in ISH

Immunocytochemistry

- Is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions
- Antigens:
  - proteins, glycoproteins, proteoglycans
- Antibodies:
  - serum proteins known as immunoglobulins
  - formed in the humoral immune system by plasma cells
  - there are five types of antibody found in the blood IgA, IgD, IgE, IgG and IgM.
  - IgG is the commonest and the most frequently used antibody for immunohistochemistry

Antibodies

An antibody molecule is composed of four polypeptide chains, two identical light chains and two identical heavy chains
Antigenic Determinants (Epitopes)

- The specific site of an antigen that binds to an antibody is called an **antigenic determinant** or **epitope**
- Most antigens have a variety of epitopes that generate a number of different antibodies that are called **polyclonal**
- A single immune response to an antigen is termed **monoclonal**

Monoclonal antibodies

- Monoclonal antibodies are "cloned" antibodies that all respond to exactly the same epitope of the same antigen – they are exceptionally specific
- Activated B cells (plasma cells) contain a mixture of antibodies directed against different epitopes
- Activated B cells have a limited lifetime when grown in culture
- How can we select a single B cell clone (monoclonal) and propagate the cell line?

Monoclonal antibodies

- Antibodies can be raised in mice by injection of an antigen.
- Repeated injections create a pool of activated B cells.

![Monoclonal antibodies](image)

Methods of Labelling Antibodies

- Enzyme labels (peroxidase)
- Colloidal metal labels (EM)
- Fluorescent labels
- Avidin-biotin techniques
Fluorescent Compounds

- Fluochromes are substances which can absorb radiation in the form of ultraviolet or visible light
- This absorbed radiation causes an "excited state" followed by emission of radiation of a different wavelength
- The fluorescent substances then appear as shiny particles on a dark background
- Fluorochromes: FITC (fluorescein isothiocyanate), rhodamine, DAPI, etc.

Avidin-biotin Techniques

- These methods rely on the affinity of the glycoprotein avidin for biotin
- Biotin, a low molecular weight vitamin, is easily conjugated to antibodies and enzyme markers
- Up to 150 biotin molecules can be attached to one antibody molecule → increased sensitivity

Methods of Localizing Antigens

- Direct method
  - primary antibody is conjugated directly to the label
- Indirect method (more sensitive)
  - first step: nonlabeled anti-x antibody is bound to the antigen
  - second: the labeled antiantibody binds to the anti-x antibody

In Situ Hybridization Techniques

- Allow the demonstration of specific nucleic acid sequences in their cellular environment
- Are based on hybridization of a nucleic acid probe (small lengths of nucleic acid of known base sequence) to the target
- Applications:
  - detection of abnormal genes
  - identification of viral infection, etc.

In Situ Hybridization Techniques

- Probes labelling:
  - radioisotopes
  - coupling of the nucleotides with biotin
  - fluorescent labels (FISH)