CYTOCHEMICAL AND CYTOENCIMOLOGICAL METHODS

1. METHODS OF CYTOCHEMISTRY

We achieve the localization and identification of some chemical cellular compounds on the biological specimen destined to the photonic microscope studying with the aid of cytochemistry.

The cytochemical techniques allow to observe the substance localization at cellular level with color reaction, making simultaneously a chemical analysis and a morphological one.

1.1.The Specimen Riping

The specimen riping is acleved with the same techniques described above.

1.2.The Fixing

a) Physical agents – criofixing, criodehydration and criosubstitution.

The essential factor in the physical agents fixing methods is represented by the specimens freezing at a low are the temperature.

The physical fixing advantages are the following:

- It stops the enzymatic activities and the post-mortem cellular autolysis.
- The cellular structures conserving may complete with the best cytological chemical fixing results.
- The moving off of soluble or diffusible substances is more reduced than in arry other fixing processes.

b) Chemical agents must perform the following conditions:

- To keep the substances in their sector
- To keep the substance away from dissolving
- To keep their structure
- To allow the normal chemical reaction going on.

The most used fixing agents are:

1. Carnoy for – glycogen

- Mucopolysaccharides
- Proteins
- Nucleic acids

2. Buffered 10% formol for:
- Proteins
- Lipids
3. Potassium dichromate sublimate for phospholipids

The fixing time is more exact in cytochemistry and cytoenzymology, because it can a risc an extractive action fixing agent on the chemical compounds.

The volume of the fixing agent must be enough large to prevent its dilution with the water from specimen. It is preferred the cold fixing at 4 C, that prevents the autolysis but increases the fixing time. The fixing agents must have a pH close to physiological values (pH=7,2 – 7,4)

1.3. The including

The greatest part of cytochemica and cytoenzymologic methods are achieved by the criotome technique and those use chemical fixing agents. They use the paraffin inclusion.

1.4.The sectioning

The sectioning is made as describe for the paraffin including specimens. For thefragmesnts fixed by physical, the sectioning is made by the previous described technique.

The section are glued with albumin, glycerin, amidin, etc., on the port-object. The glue solution has to be chosen depending on the cytochemical reaction that have to be done. The cytochemical reaction have like purpose to give a signal (color one)to inform about one existing substance group.

1.5.To put in evidence of some substances by cytochemical methods

a) The evident rendering of sugars by cytochemical technique

The sugars are aldehyde-cetone of polyhydroxylic alcohols with special role in the cellular activity. They are first class energetic substance (glycogen, for example); they can combine with the proteins or lipids with structural role.

There can be making evident the polysaccharides and the mucopolisaccharides neutral and acids, glycoproteins and glycolipids.

b) The cytochemical lipids evident making

The lipids are obtained by joining an alcohol with superior fatty acids and have energetic and structural role.

They are classified as following:

- Simple lipids – TG with Sund III, IV are stained in yellow orange
- Complex lipids

To make evident cytochemically the lipids we use general staining reaction to make evident certain radicals, reaction for the sugar compounds or the proteic ones.

Results: the total lipids are stained blue.

c) The cytochemical proteins evident making

The proteins are the most important group of organic compounds from the cell. The cytochemicals methods detect only the proteic primary structure. The electropolar characters of the protein (a protein car be):

- Electronegative – when it prevails the functional carboxyl groups (COO) and the protein has affinity with the basic staining matters (anionic).
- The isoelectric point is the pH value where a protein ia electric equilibrate (the electronegative ionizable groups and the electropositive ones are in equal number) and is determined by the methylene blue extinction.

Results: the basophilic substance (with COO) are stained in blue with maximum intensity at alkaline pH.

We use the Brachet and Feulgen methods to make evident the nucleic acids.

2. CYTOENZYMOLOGIC CURRENT METHODS

2.1. General data

The enzymes are organic biomolecules that catalyse the synthesis and the degradation reactions from the living organism; these biocatalyzers have a fundamental role in metabolic processes and on all the growing and tissue regeneration ones. Some enzymes activities depend only on their protein structure, but others demand the presence of some cofactors. The cofactor can be a metallic ion (K^{+}, Mg^{++}, Mn^{++}) or an organic molecule named coenzyme (they act like intermediary transporters in all enzymatic reactions).

There are 6 kinds of enzymes:

1. Oxido-reductases (for oxido-reducing reactions)
2. Transferases (they transfer functional groups)
3. Hydrolases (hydrolysis reactions)
4. Lisses (addition at the double link)

5. Isomerases (for isomerizations reactions)
6. Ligases (links making with ATP degradation)

The cytoenzymology's purpose in the enzymatic activity detection into the cell and the achievement of a metabolic status. The enzyme placement into the cell is made with the aid of chemical reaction between the enzymes and substratum that have like final product a stained and insoluble precipitate; when it is soluble, it is made insoluble by a "capture reaction".

The kinetics of the enzymatic reaction depends on the kinetics of the chemical reactions catalyzed by enzymes. It is made upon Michaelis-Menten equation; Enzymes concentration the quantitative analysis of enzymatic activity is easy achieved when the substratum or the product is stained pH value - most enzymes have a characteristic pH at which their activity is maximal; the optimal pH for an enzyme is not all the time the same with the pH of the normal medium.

The working temperature - it is known that the speed of the reactions catalyzed by enzymes increases with temperature, on the temperature interval in which the enzyme is stable and keeps its whole activity; usually, the working temperature is 37 C.

The activators of the reactions of the enzymatic catalysis are substances that increases the reaction speed through the unblocking of a proteic part that mask the active part of the enzyme or through the protection of the enzymes by inhibitors.

The inhibitors of the enzymatic catalytic reactions – the inhibition of some enzymes by specified metabolites is an important element in the intermediary metabolism regulation.

Incubation time – through biochemical researches it was established that 20-30 minutes represent the optimal time.

2.2. The Cytoenzymologic Technique

The main stages are similar to those of classic cytology:

1. **The riping**. In comparison with classic cytology, the cytoenzymologic reaction are made only on tissue or organ fragments harvested "in vivo" or immediately after the sacrifice of the experience animal.

2. the fixing conserves the enzymatic activity. There may be used:

- **Physical fixing agents** – the quick freezing of the fragments in liquid nitrogen at 180 C
- **Chemical fixing agents** – glutaraldehyde, alcohol, acetone

3. **The incubation** represents the most important step. It offers the optimal conditions for enzyme's activity.

To use of incubation medium has two major aims:

- To allow the forming of the enzyme-substratum complex that represents, in fact, the enzymatic activity.
- To make the reaction product to become insoluble (enzyme + substratum).

The enzymes have two parts:

- A inydrosoluble part or lysoenzyme
- A liposoluble part or desmoenzyme

The lysoenzymes are lost during different manual labour to obtain the specimen, making evident the desmoenzymes. When the reaction product is soluble, as for hydrolases, it has to be made insoluble by a proceediag named “capture”.

To achieve those purposes, the incubation medium has tocontain:

- The specific substratum of the enzyme that have to be evident
- The substance that assures the product insolubilisation
- The substance that allows the visualization
- Buffer substances that give a certain pH to the incubation medium

The incubation time has a real importance, showing that the enzyme saturation with substratum is made in 20 minutes.

4. **The Section Mounting** it is achieved with hidrosoluble medium- the glycerine and Apathy syrup
5. **The Visualisation –** it is achieved the detecting and localization of a certain colour with rough or diffuse aspect. The visualisation shows: the enzymatic activity place and the tinctorial intensity that shows the enzymatic activity intensity.

5.1. The Cytoenzymologic Detection Of The Oxido-reductases

The enzymes from the group of oxido-reductases catalyze the oxidation and reduction reaction from the living cells.

The oxido-reduction reaction are those reactions where occurs the transfer of an electron from the electron- giver (the reducing agent) to an electron acceptor (oxyding agent). The electrons transport is fuel of the cellular activities because it gives a great amount of free energy, kept under the macroenergic phosphate of ATP, after the oxidative phosphorylation process.

Succinate dehydrogenase

The enzyme activity is making evident by Niclas technique. There are used section obtained at criotome from liver or rough fresh muscle. The principle is the following: the SDH moves

off the hydrogen from the succinic acid salts that are transformed in fumarate. The hydrogen is accepted by a tetrazolium salt. The positive result represents a blue-pale precipitate.

2.4. **The Cytoenzymologic Detection of Hydrolases**

The hydrolases are enzymes that catalyze the hydrolytic reaction of complex molecules; The proteins in amino acids, polysaccharides in monosaccharides, lipids in fat acids and glycerine.

The Acid Phosphatase

It is the marker enzyme on lysosomes. Its activity is detected with the Gomori technique. The principle of this reaction consists in the fact that the acid phosphatase act over the β-glycerol phosphate that is hydrolysed in glycerol and sodium orthophosphate.

Results: the places with acid phosphatase activity appear over the shape of black granules.