Methods for cytological examination in the photonic microscope

- Tissue preparation –

A variety of technique are used today for studying cells. Because cells and organelles are too small to be examined by the naked eye, the backbone of cell biology is the microscope. However, the boundaries between the fields of cell biology and biochemistry, physiology, immunology, microbiology and molecular biology are no longer clear cut and distinct, and a modern cell biology laboratory often incorporates methods from each of these disciplines into the experimental analyses of eukaryotic cellular phenomena. We will describe not only the various forms of microscopy, but also many of the other techniques that are contributing to the explosion of knowledge conncerning cell structure and function.

The morpho-functional examination of the cell is done on microscopic specimens.

The microscopic specimen are:

1. EXTEMPORANEOUS SPECIMENS

- The glass lamina
- The lamella
- The biological material
- The conservatory fluid
- The vital dye

c) The Embedding with Paraffin

For light microscope we often use paraffin, which hardens the specimen as it cools, as an embedding medium.??

Is made in a thermostat, at 50^0C. Inside it the specimens are passed there times with a fluid paraffin which replaces the clearing agents. The impregnation paraffin fluid contains 10% beeswax to allow a better section of the included tissue.

The optimum time for a correct impregnation depends on the specimens size (3-6 hours generally) and of the structures to be seen. The vacuum inclusions degrease the impregnation time.

d) The realization at the paraffin block

Named also the black casting is represented by the specimen inclusion in a solidified paraffin block, with homogenous consistence. We cast the liquid in a shape realized with the Leuckhardt bar (like L) or made by paper on tinfoil; with a heating clip we take off the specimen from the last liquid paraffin bath and we introduce it in the caste paraffin, with the surface to be sectioned at the bottom of the shape. To identify different specimens in the blocks, we use numberd cardboard. When the block is cool and harden, the inclusion is finished.

5. THE SECTIONING

Light microscopic studies typically use 5-10 μm thick paraffin-embedded sections.

Sections are cut with a rotary microtome a machine that moves the specimen across a sharp metal blade, advancing the specimen the desived thickness after each pass.

Successive sections come off the microtome in a ribbon.

Sections are cut from the is ribbon and mounted on glass slides.

We have some different kinds microtomes:

- Rotating ones (Minot, Spencer 820) that are formed by a fixation mechanism for the port-object, the knife, the device for the specimen advance and the micrometric knob. When we section the fixed specimen, it advances through the fixed knife so we obtain series of sections. The sections are taken from the knife, with a brush and we put them on a black paper.

5.1. The Sections Application On The Port-Object

We put a drop of Mayer albumin (50g glair, 50g glycerin, 1g sodium salicylate dissolved in distilled water) on a glass lamina and we stretch with the auricular.

From the paraffin band, we take out fragments with 3-4 sections that are applied to the Mayer albumin on a degreased lamina face (with the shiny face down)

We drop of distilled water at one of the band extremities and we tilled the lamina for the sections floats, we put it horizontally on heated platen, where in few seconds the paraffin and the sections smooth out are complete. After that, the exceed water is separated and lamina is put in a wood stand and after that in a thermostat, at 37C, where in 24 hours the drying is finished and the section glue together with the lamina.

The specimen can be kept unstained a lot of time if we are keeping them away from dust.

6. THE STAINING PROCEDURE

The coloring matters classification:

a) The origin

- Natural: - at vegetable origin – hematoxylin, safranin
    - At animal origin - carmine
- Synthetics – methyl – blue

b) The behavior in the solution

1. Acid dye carries a net negative change that is attached on positively charged subcellular structures.

Some acid dyes:

- Acid fuchsin – in red
- Aniline blue – blue
- Eosin – red
- Orange G – orange

- These dyes selectively stain collagen ordinary cytoplasm and red blood cells, respectively
- Tissue components that stain with acid dyes are called acidophilic.
- Staining with acid dyes is less specific, but more substances within cells and the extracellular matrix exhibit acidophilia

These include:

- Most cytoplasmatic filaments, especially those of muscle cells
- Most intracellular membranous components and much of the otherwise unspecialized cytoplasm
- Most extracellular fibers (primarily due to ionized amino groups)

2. Basic dye carries a net positive change the are attached on negative charged subcellular structures

Some basic dyes: Methyl green – in green

Methylene blue – blue

Toluidine blue – blue

Hematoxylin – violet-blue

Tissue components that react with basic dyes are called basophilic. A limited number of substances within cells and the extracellular matrix display basophil, react with basic dyes.

These include:

- Heterochromatin and nucleoli of the nucleus (both due chiefly to ionized phosphate groups in nucleic acids)
- Cytoplasmic components such as the ergastoplasm (also due to ionized phosphate groups in RNA)
- Extracellular materials such as the complex carbohydrates of the matrix of cartilage (due to ionized sulfate groups)

3. Neutral dye: obtained by a mixture of one basic and one acids coloring matters, they stain some cytoplasmic inclusion with neutral reaction (methylene azureozinate, methylene blue eosinate etc)

The most important dyeing methods

The dyeing techniques are classified after the following criteria:

a) The coloring matter numbers

- **Simple**: they are used with a single coloring matter, nuclear (hematoxylin) or cytoplasmic (eosin)
- **Combined**: the sections are successively obtained with two or more coloring matters (double hematoxylin-eosin, triple hematoxylin-eosin-methylene blue).

b) Using adjuvants

- **Directly**: the coloring matter is fixed by simple contact with the section (the hematoxylin-eosin staining)
- **Indirectly or by the mordant**: the mordant is necessary for fixing coloring matters on cellular compounds.

c) The staining intensity

- **Progressive:** we make successive attempt under microscopic observation until ????

The dyeing is stopped by washing the section (e.g. the hemalaun stains the cell nucleus by blue in 4-6 minutes, if we maintain the sections more time in contact with the coloring matter, the other cellular structures will be stained).

- **Regressive**: we make supra-staining and we stain other cellular structures than we wanted, after hat the coloring matter excess is removed by decoloring substances or differentiator (e.g. after staining the section with strong black ferric hematoxylin Heidenhein we introduce the lamina in a ferric ammonia alaun until only the nuclear chromatin remains stained, the other cellular elements-mitochondria, cellular center becoming uncoloured)

d) The material conditions

- **The vital dyeing**: it is made on living cell using coloring matters an it remains only for the time that the cell survives; it is used for the visualization of cellular organelles (mitochondria – green James B, or Golgi complex – neutral red) and some physiological cellular activities.
- The permanent dyeing: it is made by the dyeing of the permanent specimen

The are some points to notice:

I. Before dyeing

a) The Deparaffination

The paraffin section glued on the port-object lamina are deparaffinated by passing them through three solvent baths (xylol, benzene, toluene) within 5-10 min. It is a necessary step because the paraffin hinders the staining.

b) The Hydration Deparaffined Section

It is necessary operation when the colored solution used are aqueous; it is realized by passing them through five ethylic alcohol baths, with decreasing concentration (3 alcohol for baths of 100% then 96% and 70 %) within 1-2 minutes for each bath. After that they are passed in distilled water. When the alcoholic dyeing solution are use in, the section are introduced directly in the coloring solution, skipping the distilled water step.

II. The Dyeing

III. The Steps After Dyeing

a) **The washing**: Is made with distilled water to stop the dyeing and to eliminate the excess of coloring matters.

b) **The Dehydration**: It is realized by passing the colored section through five ethylic alcohol baths with increasing concentration (70%, 96% and three baths with pure alcohol – 100%); this step is necessary to obtain a permanent stained specimen.
c) **The clarification**: It remove the alcohol from the section and gives them a necessary transparency to allow the microscope examination; it is realized by passing the section through successive baths of xylol, toluel or benzene.
d) **The colored Section Mounting**: It is made by applying a layer of mounting substance on the pasted section. Then we cover it with a glass lamina and we eliminate air bubbles with a special pin. This step is necessary to protect the section from degradation, to keep the dyeing for a long time and to ensure the optimal homogeneity and transparency to the microscope examination.

The mounting substances can be:

- Glycerin – used for the undehydrated section (e.g. glycerin, gelatin, Apathy syrup, etc)
- Anhydrous (resinous) – for the dehydrated sections (Balsam of Canada).

e) **The labelling:** It is last step for obtaining the permanent specimen; it consists of pasting the label on the port-object, on the same face with the colored section. The specimens are kept in boxes, away from light and dust.

THE HEMATOXILIN-EOSINE DYEING TECHNIQUE

It is the most used topographic staining that allows a histological examination and a pathological exam.

It is net a cytological dyeing because there are cellular organells visualized. It is applied on fixed specimens or other fixing mixtures.

The steps are:

1. The fine liver section is introduced in a bath with hematoxylin for 5-10 minutes.
2. The washing with water for 5-10 minutes.
3. The differentiations in chlorydric alcohol when the nucleus is supra stained; them we transfer the section into a bath with saturated solution of lithium carbonate.
4. The washed section is introduced in a bath with aqueous eosin solution for 5 minutes.
5. The washing necessary to eliminate the coloring matter excess.
6. The dehydration, the clarification, the mounting in Balsam of Canada and the labelling. The operation of deparaffination, hydration, dyeing, dehydration and clarification are made in a staining complex formed of glass with plate bottom covered with lids, placed in a statistic with three steps.

Results:

The nucleus is stained in violet-blue with hematoxylin as the last is a basic coloring matter and the nuclear content is basophil. The cytoplasm is stained in pink with eosin as the eosin is an acid coloring matter and the cytoplasm is acidophile. Inside the cytoplasm there are basophile granulation (the Berg bodies) representing the endoplasmic granulated reticulum.

In the microscopic field appear the hepatic tissues dispersed in a ribbon separated by spaces where are sinuous capillaries and biliary interlobular ones.