Department of Histology and Embryology
Faculty of Medicine, PU, Olomouc, CZ

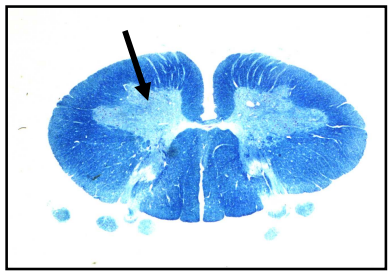
Histology practical No. 2

Topics:

1- IDENTIFICATION OF CELLS, THEIR SHAPES, SIZES, STAINING.
2- OBSERVATION OF CELL ORGANELLES AND INCLUSIONS IN LIGHT MICROSCOPY SLIDES. (Demo: Hamamatsu cell video of living organelles.)
3- OBSERVATION OF CELL ORGANELLES IN ELECTRON MICROGRAPHS.
4- CELL SURFACE SPECIALIZATIONS IN L.M. AND E.M.
5- MITOSIS – projection of time-lapse video-microscopy of mitosis in cultured cells (Demo: Hamamatsu cell video).

Contents:

1. Spinal cord (mícha, sl. no. 61) t.s., H&E stain.



In this section of spinal cord the large neurons are centrally located in gray matter. Observe these multipolar nerve cells with several processes. Note the very pale (euchromatic) nucleus and the dense, central nucleolous in some cells. The small nuclei scattered between large neurons belong to the supporting neuroglial cells.

2. Spinal cord (mícha, sl. no. 61A) t.s., Kluver-Barrera stain.

This is the same section of the spinal cord (like slide n. 60) stained with a special method to visualize selectively all basophilic structures in cells. In the cytoplasm and some processes of large nerve cells, find the scattered irregular purple bodies known as Nissl bodies (substance).Why are these bodies basophilic? What other cell structures are basophilic?

3. Golgi complex (dorsal root ganglion, spinální ganglion sl.n. 65A) l.s., Silver impregnation.

This section contains two parts – longitudinally cut nerve bundles and groups of large, rounded nerve cell bodies of various sizes. Observed with a high power lens, the neurons appear rounded or oval and may contain large central nuclei. In the cytoplasm of these neurons, there are dark brown to black granules, curved rods or irregular structures, that constitute the Golgi apparatus as seen by light microscopy.
Why do some of the neurons appear not to contain nuclei?

4. Lipofuscin in cardiac muscle cells (myokard lipofuscin, sl.n. 1) s., H&E stain.

Observe these cardiac muscle cells with high power lens and find the blue nuclei located in centers of muscle cells. At their poles, look for light yellowish or brown refractile granules of the lipofuscin pigment bodies. This is their natural color (not stained). Why did they accumulate in these locations?

5. Brown fat, hedgehog (hědý tuk, ježek, sl.n. 72) semithin sect., Toluidine blue stain.

This kind of fat tissue (multilocular fat) is composed of cells that contain in their

cytoplasm many lipid droplets of various sizes. At high magnification, observe single fat cells and locate their nuclei and the droplets of lipids stained in pale gray/blue color. The irregular-shaped, empty-looking spaces among these cells are blood capillaries.

6. Spinal cord, neurofibrils (mícha, sl. n. 61B) t.s., Bodian stain.

This is the same section of the spinal cord (like slide n.60) stained with a special method to visualize selectively bundles of neurofibrils in nerve cells. Locate again the bodies of large multipolar nerve cells and their processes to observe the neurofibrils. They appear as thin, dark brown or black colored lines. The thin processes (axons) of nerve cells, located everywhere in the section, are completely filled up with these structures. How do we call this kind of structures in the cytoplasm?

7. Trachea, stereocilia (trachea, sl.n. 34) t.s., H&E stain.

This slide contains one or more transverse sections of the wind pipe (trachea) that has its wall composed of several layers of different tissues. Locate the external surface covered with a pseudostratified columnar epithelium, that has many columnar cells with clearly visible kinocilia on their apical surfaces. Note, that the kinocilia are absent on the surface of pale or empty-looking cells, called goblet cells. What is the function of kinocilia in this location? What is their ultrastructure? Draw a simple diagram of the axoneme.

8. Ductus deferens, stereocilia (ductus deferens, sl.n. 42) t.s., H&E stain.

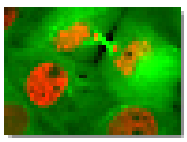
This slide contains a section of a tube-like structure, called ductus deferens as well as sections through several large blood vessels around. Locate the ductus deferens, that can be recognized by absence of red blood cells in the lumen, and by its thick muscular wall. The tall columnar epithelial cells lining the lumen have many thin, hair-like projections on their apical surfaces. These are the (non-motile) stereocilia.

9. Jejunum, microvilli (jejunum, sl.n. 27) l.s., H&E stain.

This slide contains one or more sections through the wall of the small intestine (jejunum) that is composed of several layers of tissue. The most superficial layer, that folds into many villi, is covered with simple columnar epithelial cells and some goblet cells. Using the highest power objective lens, observe the apical surface of these epithelial cells and locate the "brush border" that represents the layer of microvilli. What is their ultrastructure and function?

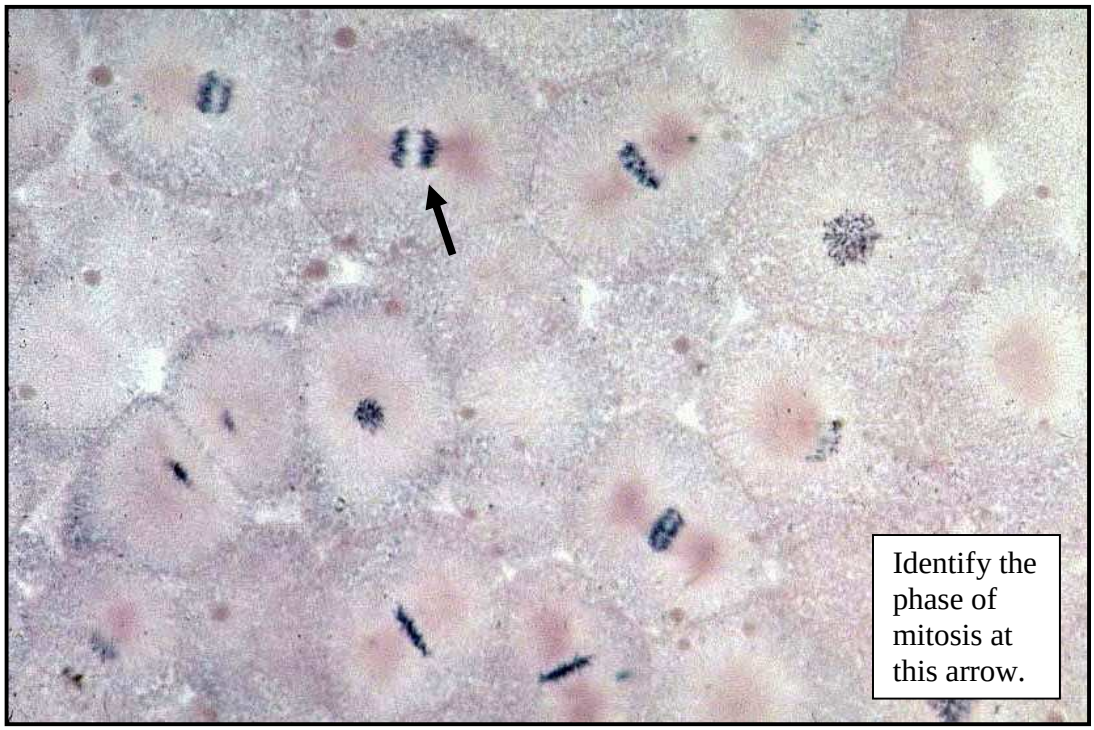
10. Observing Chromosomes and the Spindle in Mitosis.

(Demo: Hamamatsu cell video)



Mitosis of pig kidney cells in tissue culture.
The digital video sequences presented in this section feature epithelial cells undergoing mitosis as visualized through the highly dynamic interactions between fluorescently labeled **red histones** and **green tubulin**. Cells enter mitosis through **prophase** and proceed through division to create a pair of daughter cells. The labeled histones enable visualization of the condensed chromatin as it progresses through various phases, while the tubulin can be seen forming the **mitotic spindle** as well as the **midbodies** at **telophase.** Almost immediately after the **metaphase** chromosomes are aligned at the metaphase plate, the two halves of each chromosome are pulled apart by the **spindle apparatus** and migrate to the opposite spindle poles. The **kinetochore** microtubules shorten as the chromosomes are pulled toward the poles, while the

polar microtubules elongate to assist in the separation. **Anaphase** typically is a rapid process that lasts only a few minutes. When the chromosomes have completely migrated to the spindle poles, the kinetochore microtubules begin to disappear, although the polar microtubules continue to elongate. This is the junction between **late anaphase** and early **telophase**, the last stage in chromosome division. In the digital video presented in this section, pig kidney epithelial cells expressing mCherry fluorescent protein fused to histone H2B (red fluorescence) and mEGFP fused to tubulin (green fluorescence) were imaged in 7-minute intervals during mitosis.



11. <u>Selfstudy of electron micrographs showing various cell components of the nucleus and cytoplasm.</u>

Identify the organelles labeled in micrographs and record your observations in writing.

Websites:

http://learn.hamamatsu.com/galleries/digitalvideo/spinningdisk/llcpk1lumen/LLC-PK1-EGFP-Tubulin-mCherry-H2B.html
http://www.histology-world.com/contents/contents.htm#cells
http://visualhistology.net/Visual_Histology_Atlas/VHA_Chpt1_Cells.html
http://www.kumc.edu/instruction/medicine/anatomy/histoweb/cytology/cytology.htm (Root tip - phases of mitosis)
http://www.anatomyatlases.org/MicroscopicAnatomy/Section01/Section01.shtml
http://www.meddean.luc.edu/lumen/MedEd/Histo/frames/h_frame2.html
http://casweb.cas.ou.edu/pbell/Histology/Outline/cell.html
http://www.path.uiowa.edu/cgi-bin-pub/vs/fpx_gen.cgi?slide=115&viewer=java&lay=histo