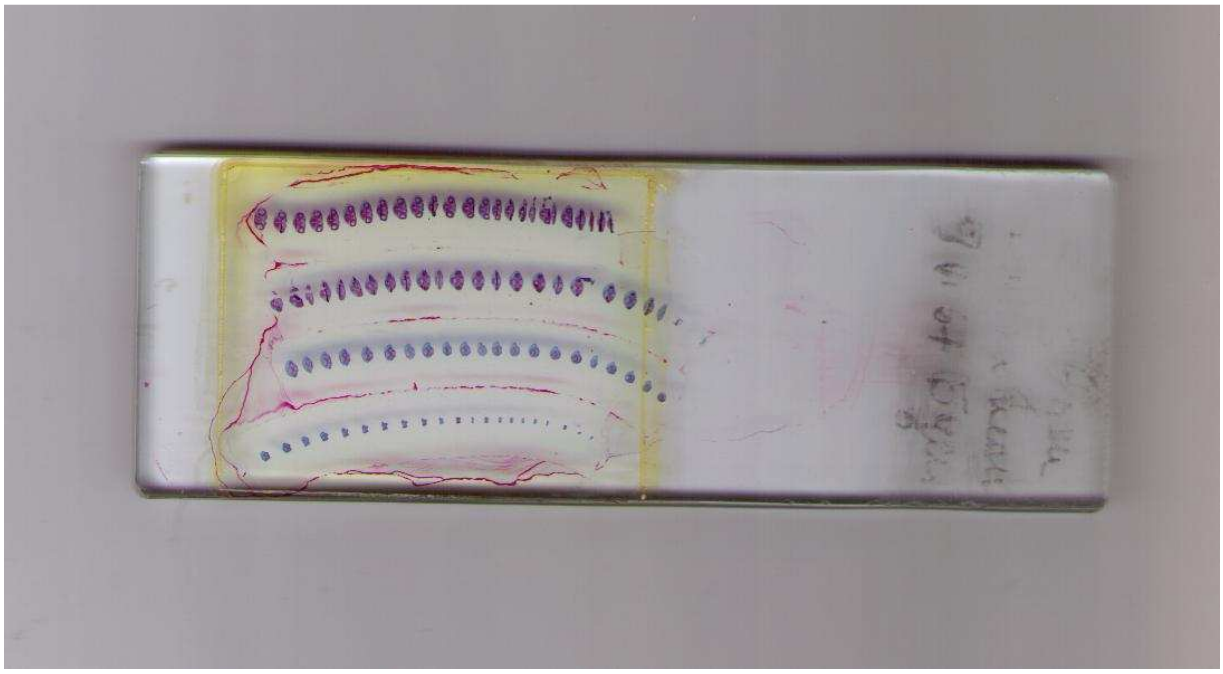
Immersion objective examination of a 1967 embryology course slide, or some thoughts on cellular development

By Veselin Andreev



In this article I would like to share with you some intriguing views in the head section of a rainbow trout embryo (Salmo irideus). Now, maybe the best thing to do first is to give an insight about this quite old slide. The only information I can gather from the pencil description is the date and type of fixation method that has been used. The specimen was fixed on the 9th of June 1967 (or at least it looks that way) and as regards the fixative, Bouin's fluid was the histotechnician's preference. There is some logic in that because Bouin's fixative is very suitable for whole embryo preservation. I acquired this slide along with some others in sets of relatively interesting histological collections. These

sets were handled to me by friends who haven't got any particular interest in microscopy whatsoever. Anyway they thought it would be great if someone could take advantage of these old slides. And after a rather long period of oblivion they were finally put under the objective.

There is no indication of the staining protocol but I presume that Heindenhain's azan stain was used. This method seems plausible because what you would like to depict and differentiate is the extracellular matrix from the actual cells. We would expect to find red nuclei and erythrocytes with well defined internal structure, blue collagen fibers and extracellular matrix also stained in blue. As regards the cytoplasm some cell are stained in blue and others in violet. All in all this is a rational staining method when we consider the nature of our specimen.

The following microphotographs were taken with a handheld Nokia N95 mobile device comprising a 5 megapixel camera. As for the optical aspect I used an old vintage Carl Zeiss (Oberkochen) microscope and a polish PZO immersion oil objective (100x, 1.3NA). The overall optical magnification in all of the images is 100x12,5. It is not a bad idea to make an article about this microscope since I am quite sure that there are readers who are also interested in old Zeiss microscopes. I did some slight finishing touches with LView pro to enhance the contrast etc. I am not completely satisfied with the image quality, but in later articles I will show you some shots taken with the 40x objective, which gives great results when used in combination with a handheld digital camera

The way organisms develop is encoded in their genes. This is no mystery to anyone. What modern biology deals with is the intrinsic molecular machinery that makes up the denominators of life itself. This is a prosperous field since it can elucidate some developmental patterns that we share with lower organisms. Such research has implications in medicine to a certain degree. But in this article we will be dealing with only basic biological knowledge and leave the biomedical aspects aside.

One of the hallmarks of embryo cells is their ability to differentiate into a cell population with very specific structural and functional correlation. In figure 1

we see a group of chondroblasts (cartilage producing cells) surrounded by extracellular matrix composed of collagen and chondroitin sulfate (the blue stuff around cells in the center). In the marginal zones of the field one can spot elongated mesenchymal cells. They have the ability to lose part of their replication potential in order to carry out specific functions as in the case with matrix accumulation.

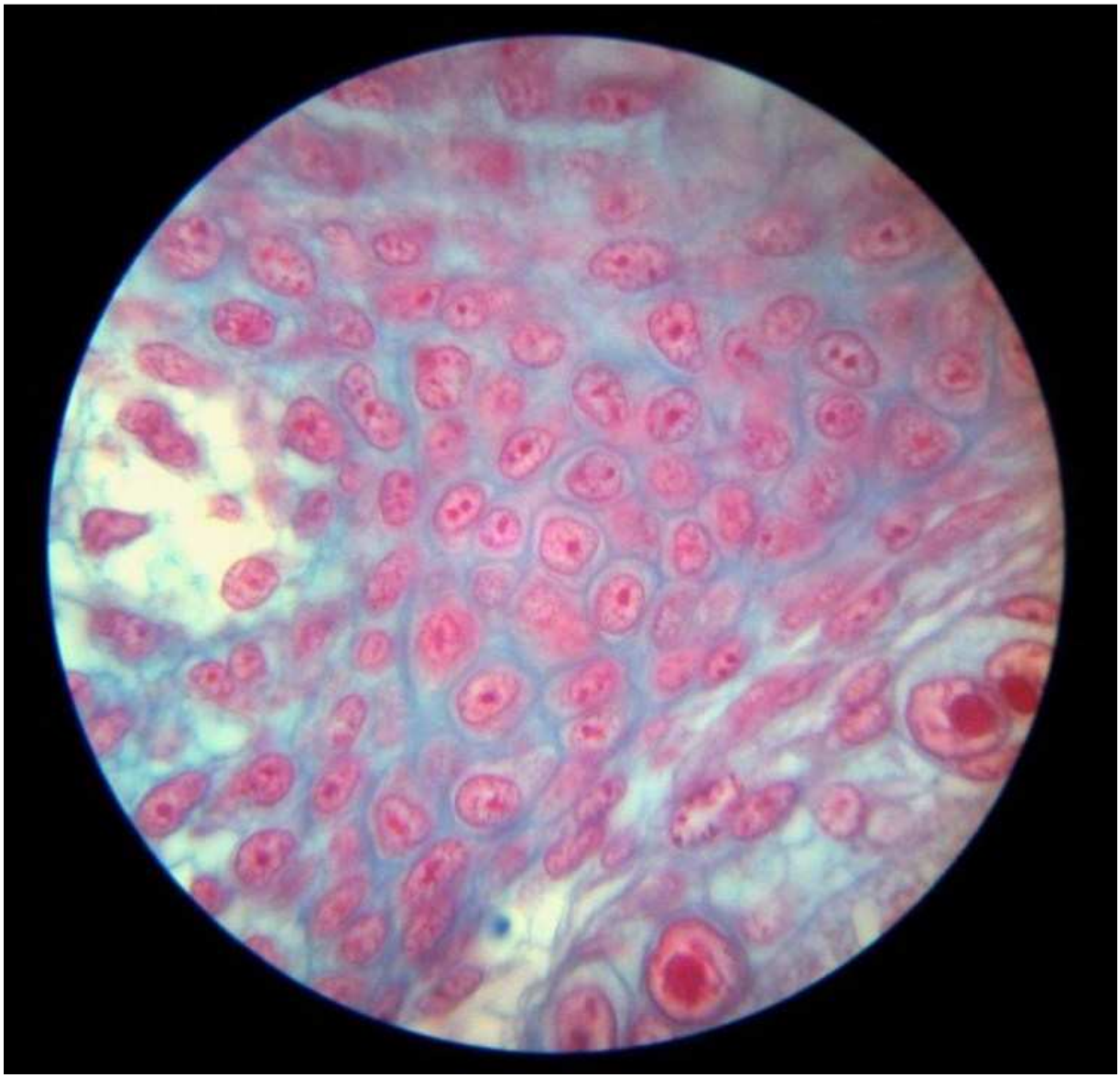


Figure 1 Chondrogenesis

Due to the high pace of tissue development a network of blood vessels is required to supply nutrients and oxygen as well as to carry hormones around and take away metabolic waste products. The process of angiogenesis (growth of new blood vessels) starts again with the mesenchymal tissue but under the influence of other differentiating signals. In the following microphotograph one can see an endothelial cell in the structure of a capillary. The origin of these cells can be traced back to mesenchymal tissue that basically fills up the intermediary spaces in the embryo.

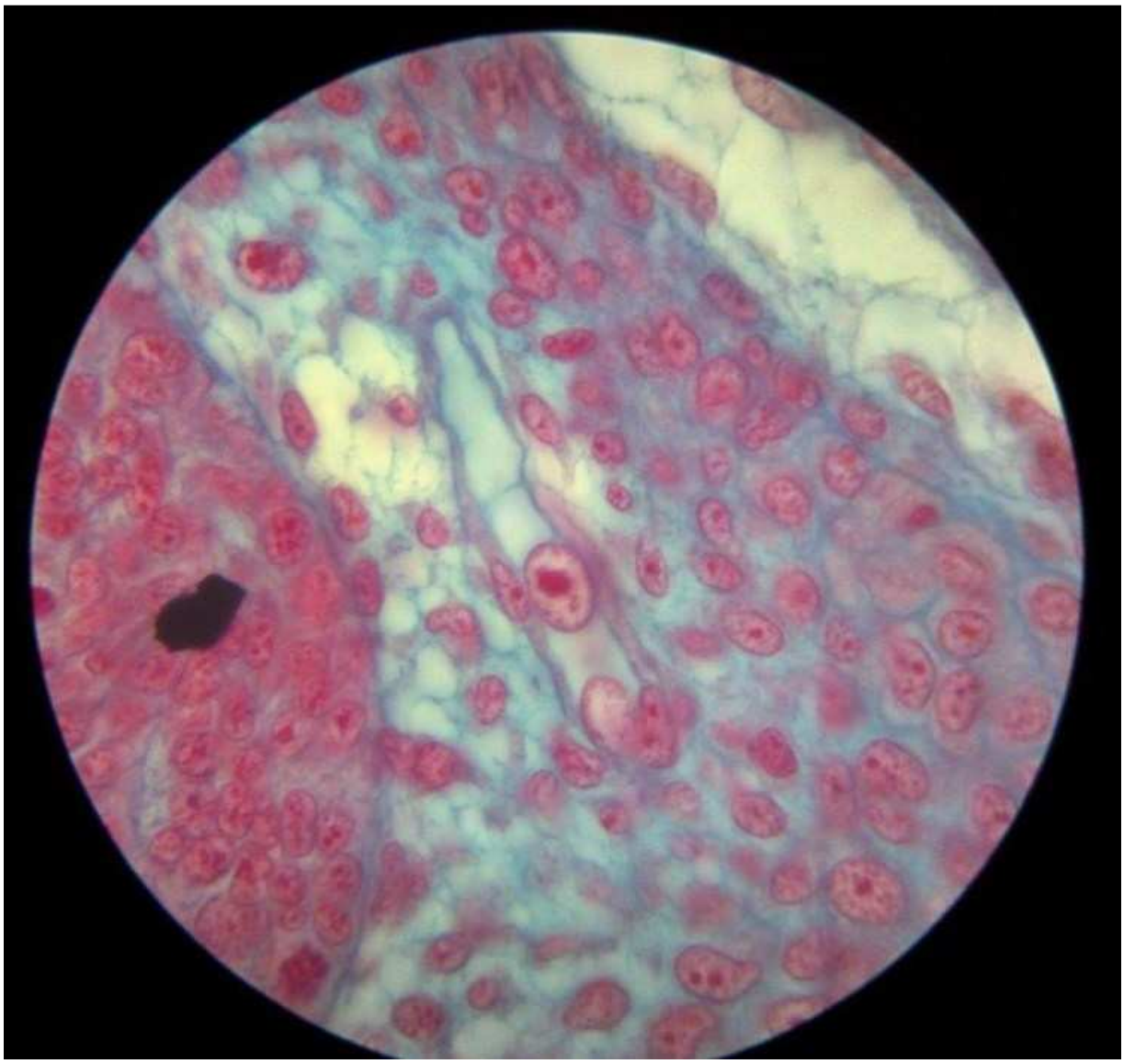
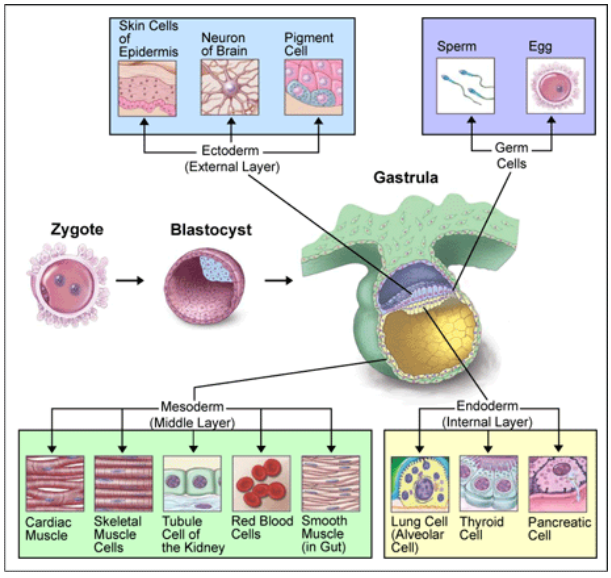


Figure 2 Capillary

An elliptical erythrocyte is seen inside the capillary. An interesting fact about red blood cells is that all vertebrates, except mammals, have nucleated erythrocytes. They resemble a flattened ellipsoid with a small bulge in the center where the nucleus is situated. The chromatin is highly condensed (pyknotic) and it is functionally inactive. The nucleus is supported by a microtubule apparatus attached to the flattened edge of the erythrocyte. In mammals however, the erythrocytes have lost their nuclei to meet a simple cytoarchitectonics condition. With the nucleus being more and more condensed and finally eliminated from the differentiating blood cell, there is more space for hemoglobin-rich cytoplasm. Also with the nucleus away so is the central bulge and this is an opportunity for a central depression to form. Although this depression decreases the overall hemoglobin quantity, it increases the surface/volume ratio of the erythrocyte. This is a general principle in evolutionary morphology that shapes fluid exchanging structures. As the S/V ratio increases so does the intensity of gases, liquids and nutrients diffusion. Just think of the "fuzzy" inner surface of the intestines. There are relatively large protrusions of absorptive epithelium called villi. The cells covering those structures show an even "fuzzier" surface. Now when you apply this principle to the mammal erythrocyte it is obvious why it is better for the nucleus to be removed. In this manner the level of metabolic gases exchange is increased. This is just one of the many neat examples of cellular differentiation and its subsequent benefits.



In the center of the diagram are three of the early steps in the development of a mammal. On the top and bottom are some of the fully-differentiated cell types that will eventually form in the adult.

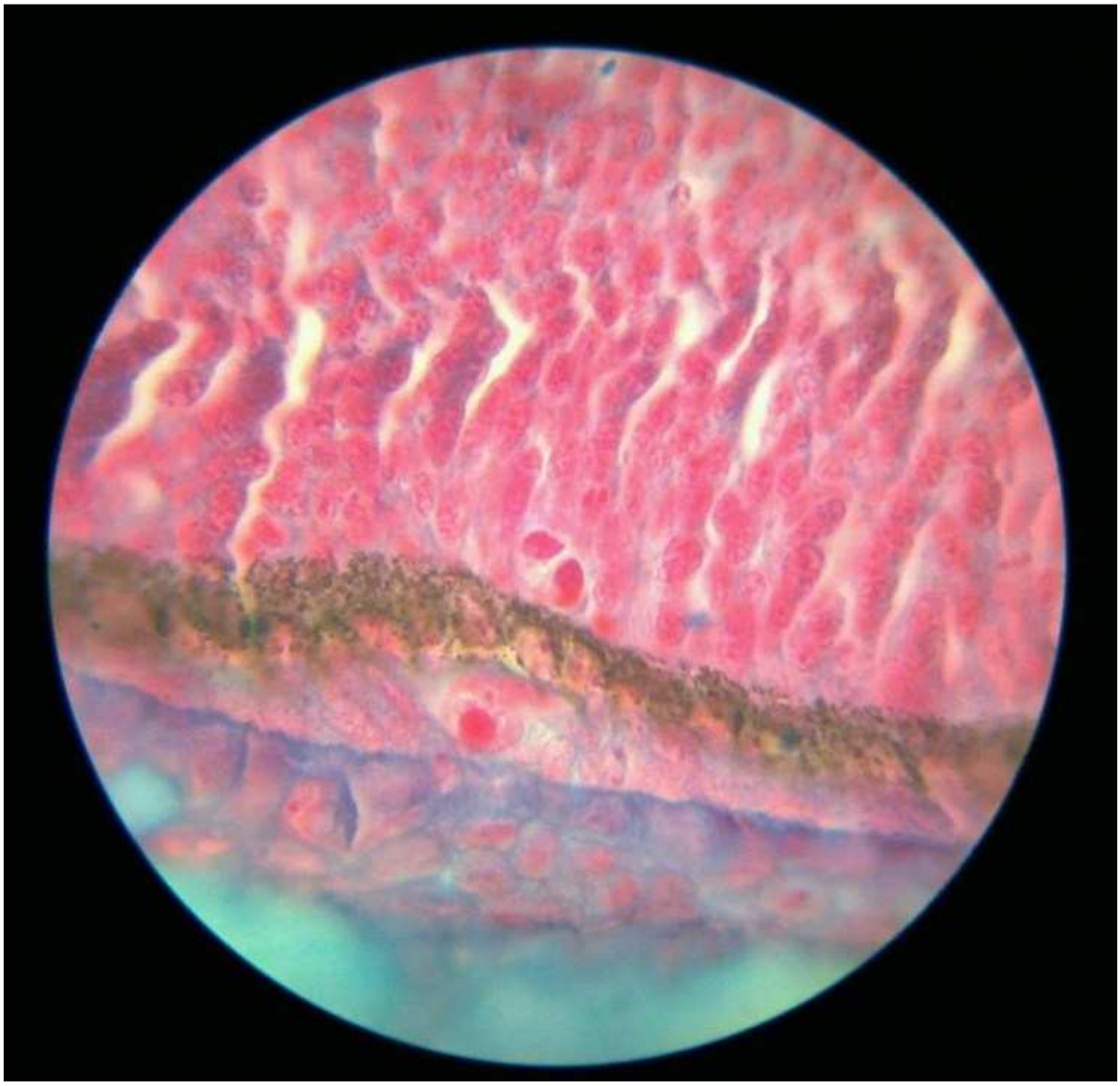


Figure 3 Retinal pigment epithelium.

Following strict instruction the cells quickly find their way around the body of the future adult organism. Each cell is in contact with surrounding tissue and regulation on the basis of chemical communication occurs. In this microphotograph (fig.3) we see cells that have been instructed to produce and store melanin granules in the retinal pigment epithelium. These cells nourish the photoreceptor cells and play a role in the absorption of UV radiation. They are also involved in a crucial biochemical step in the vitamin A cycle. Right above the pigment epithelium you can see a cell in the last phase of mitosis (telophase).

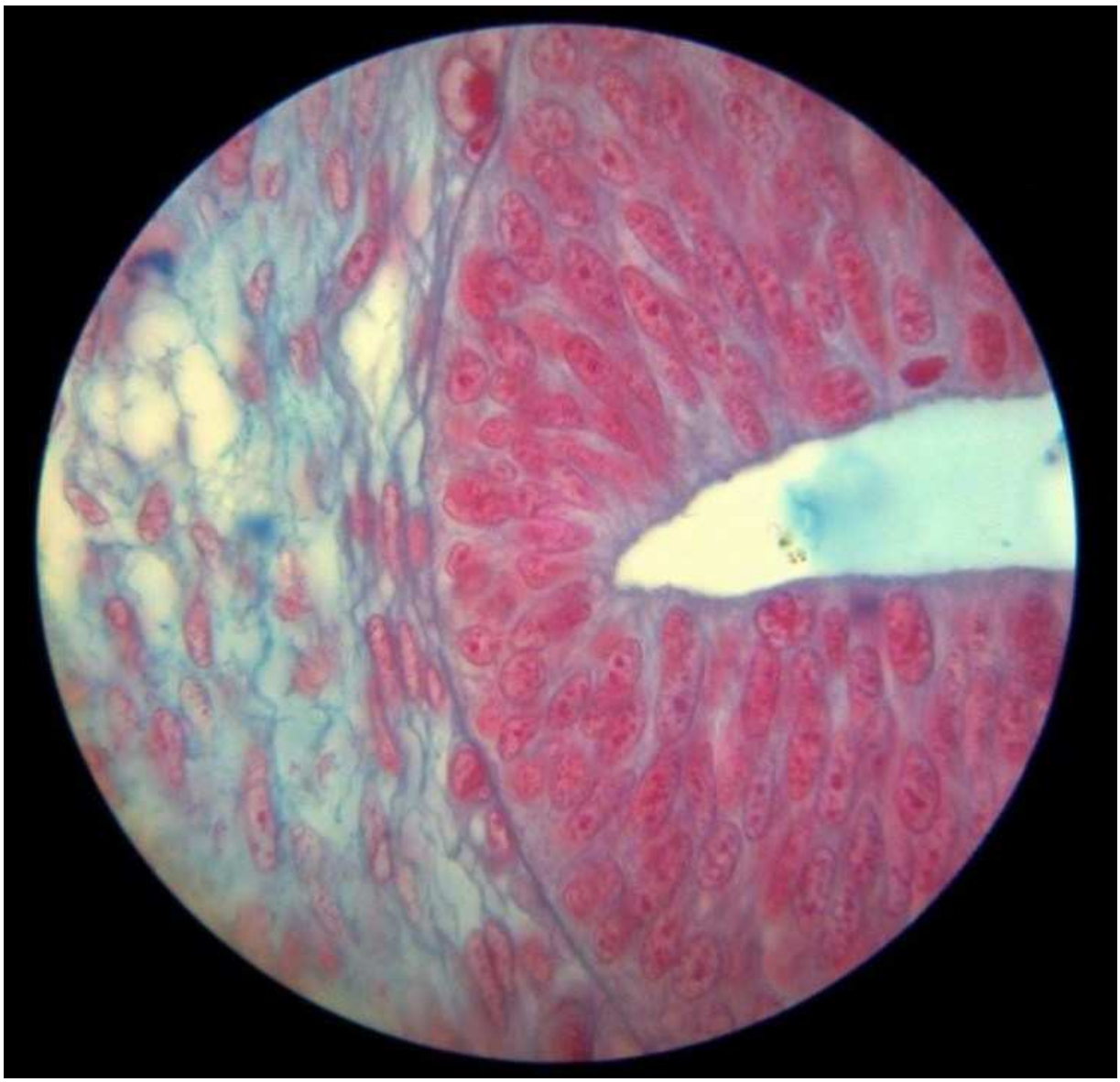


Figure 4 Cerebral ventricle (brain cavity)

As we browse around the head section it is unavoidable to find the brain. Probably many of you agree that it is one of the most complex structures in the body and this is nowhere far from the truth. In figure 4 we see some mesenchymal tissue in the left which specializes when it comes in contact with the brain itself. I am not quite sure which part of the brain this is but it can be seen that this is a region where the two hemispheres are being divided. There are elongated nuclei with prominent nucleoli some with more than one. It is

important to note that the number of nucleoli depends on the level of protein biosynthesis and also the mitotic activity of the cell. The nucleolus is involved in the assembly of ribosomes (these are the tiny protein factories in each cell). In order to explain this we must first examine the structure of the nucleolus. From a cytological point of view it is formed by the condensation of RNA and RNA-protein complexes (ribosomal subunits) in certain chromosomal parts called nucleolar organizing regions (NOR's). Since azocarmine has a binding affinity to nucleic acids it stains the nucleolus (high ribosomal RNA concentration) more intensely than some other parts of the nucleus. The number of NOR's differs among species but when a cell is in a state of rest the nucleoli fuse into one large nucleolus. This is very obvious in neurons who have sacrificed their proliferative potential at the expense of a total structural-functional commitment. In figure 4 we can also spot a mitotic figure (prophase) in the upper right part of the brain.

This concludes our analysis of this 'ancient' slide retrieved from the dust covered boxes in some forgotten locker of the developmental biology department.

I'll be happy to receive any questions and remarks on my article. Please feel free to contact me on my email: sswaffe@abv.bg

References:

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