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

Histology practical No. 9

Topics:

1- STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF CELLS IN THE PERIPHERAL CIRCULATING BLOOD. (Pre-lab rev. ppt).

2- RECOGNITION OF VARIOUS TYPES OF BLOOD CELLS IN STAINED BLOOD SMEAR AND PC-MONITORED SLIDES OR PRINTED IMAGES.

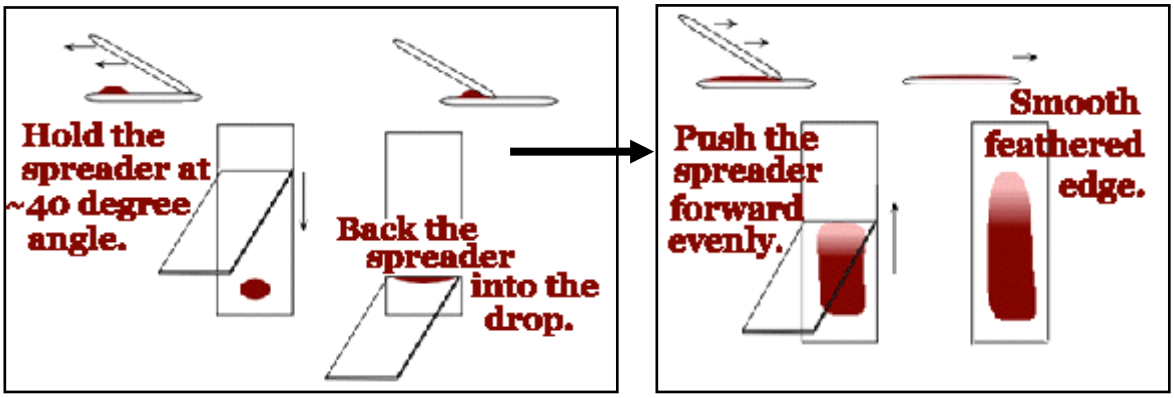
3- MAKING THE DIFFERENTIAL BLOOD COUNT OF WHITE BLOOD CELLS IN THE BLOOD SMEAR STUDIED.

4- UNDERSTANDING AND RECOGNITION OF ULTRASTRUCTURE OF SELECTED TYPES OF BLOOD CELLS IN PC-MONITORED SLIDES OR PRINTED EM IMAGES.

Methodical notes:

Preparation of the blood smear: (Not to be performed in this histology lab.)

1. Place a small drop of blood close to the frosted end of a clean slide that is on a flat surface.
2. With the thumb and forefinger of the right hand, hold the end of a second slide (the "spreader") against the surface of the first slide at an angle of 30-45 degrees. Slowly back the spreader into the

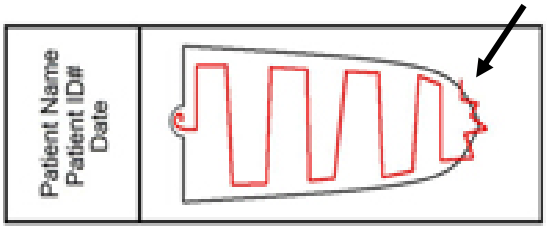


   blood drop.
3. Draw the spreader back to contact the drop of blood, allowing the blood to pool at the angle between the two slides.
4. Gently push the spreader slide at a moderate speed forward, spreading the drop of blood out into a thin film.
5. The end of the smear (the feathered edge) should be smooth and even.

Note: In a good film, there is a thick portion where the original drop spreads out (near the white, frosted end) and a thin portion that disappears into a feathered edge (on the left in the "good slide" picture at the end of the movie; the feathered edge is at the top in the cartoon). There should be a gradual transition from thick to thin sections of the smear. The smear should have a smooth, even appearance and should not have either thick sposts or holes. You should see a faint rainbow of color near the feathered edge.

The blood films (smears) are stained for hematology observations with a special stain called Giemsa stain or Wright's stain. This staining applies a mixture of basic stains (**Metylene blue** and **Azur 2**) and acid stains **(Eosin**) in order to visualize nuclei, nucleoli, cytoplasm and cytoplasmic granules in various types of blood cells.

Microscopic evaluation begins with a low power (10x or 20x) scan. **This step is not to be done in this practical, since all our microscopes today have the 100x oil immersion lenses fixed ready to use.** This initial scan allows rapid evaluation of the whole smear even though this scan should take no more than 30 seconds.



The high power evaluation (100x lens) is ideal for doing WBC differential counts in the monolayer of the smear. The monolayer is defined as the area of the smear where approximately half of the erythrocytes touch each other. Scan the blood smear in a meandering way, as shown in this figure, starting from the thinner edge of the blood film (arrow).

Slides:

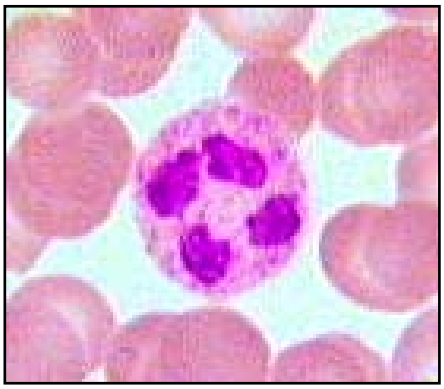
1. **<u>Blood smear (perifení krev, sl. n. 83), thin film., Giemsa stain.</u>**

In this practical session, the slide with the stained blood smear is already in place in the slide stage of your microscope, and the 100x oil immersion lens is in focus. Be very careful when using this high power lens, since it leaves extremely low gap between the front of the lense and the glass slide surface. As a thumb of the rule – never touch the macro-focussing knob for any focus-improving action. Never change the objective lense to any other magnification as the immersion oil on the blood film may contaminate the other lenses and reduce their optical quality.

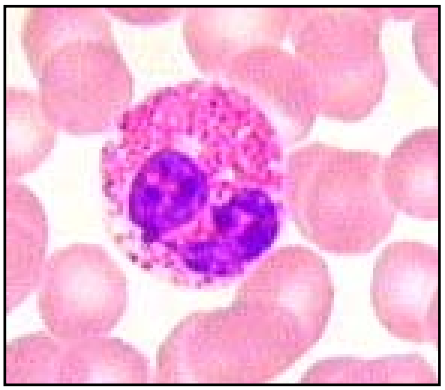
Scan the blood smear in a meandering way, as shown above, starting from the the thinner edge of the blood film. Continue systematically field by fieled in the direction of scanning and evaluate every white blood cell you find on this way. Classify each cell and make a mark into the proper column of the **Differential white cell count table**. For easy calculations, mark only ten cells in each columns of this table and continue the blood film evaluation so long until you count 100 cells all together. After summarizing the data in the whole table you will get the percentage of various types of cells in the evaluated blood sample. Be careful with classification of the unusual cell types that normally occur in a low percentage in normal blood samples (eosinophils, basophils). You may observe some atypical forms of white blood cells, that would be better to exclude from your observation, since we are interested today only in normal white blood cells.

DIFFERENTIAL COUNT OF LEUKOCYTES

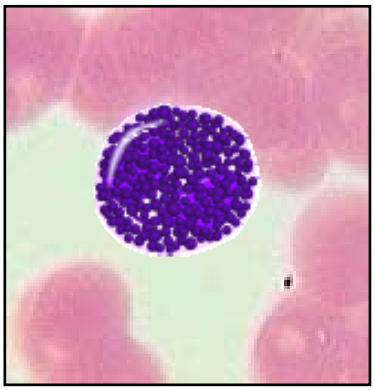
	WBC TYPES	1	2	3	4	5	6	7	8	9	10	Normal values	My results
Granulocytes	Segm. neutrophiles	///										**50 – 70 %**	
	Band cells											**2 – 5 %**	
	Eosinophiles											**1 – 4 %**	
	Basophiles											**0.5 – 1 %**	
Agranul.	Lymphocytes											**20 – 35 %**	
	Monocytes											**2 – 8 %**	
	Summary in columns	10	10	10	10	10	10	10	10	10	10	**100 %**	



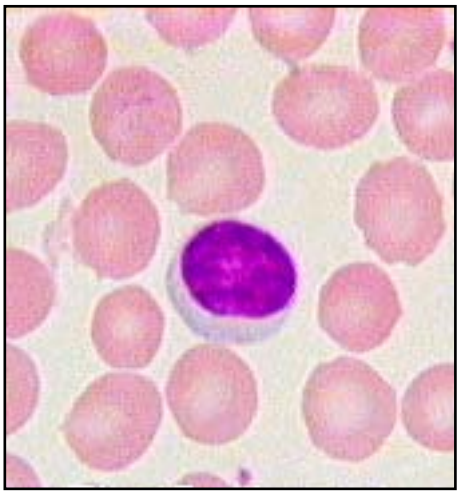
Neutrophil



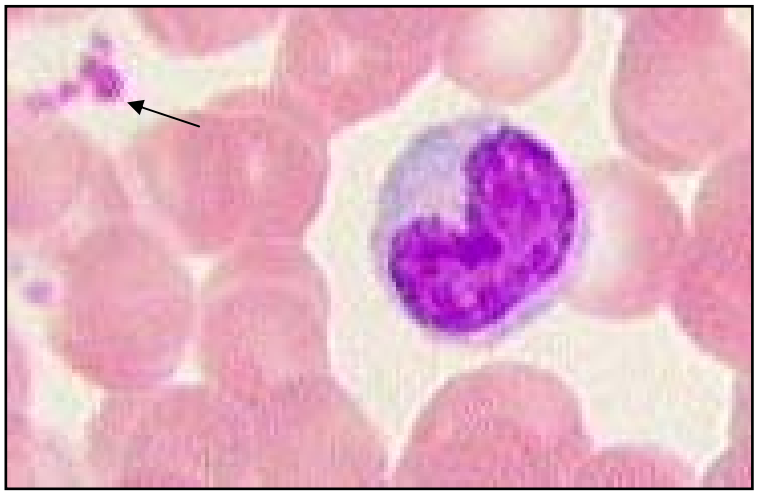
Eosinophil



Basophil

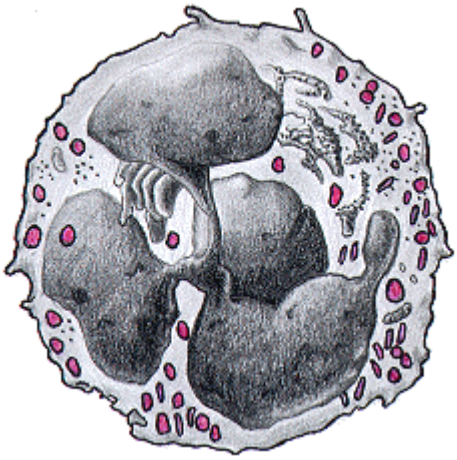


Lymphocyte

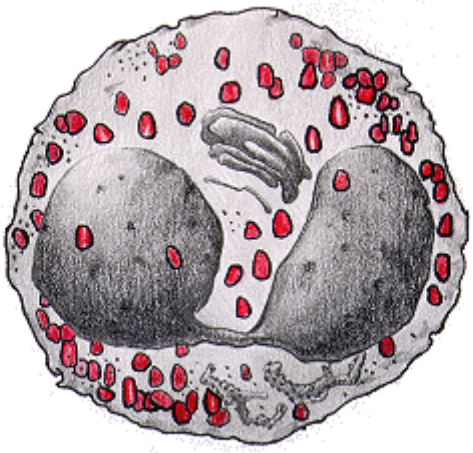


Monocyte (thrombocytes @ the arrow)

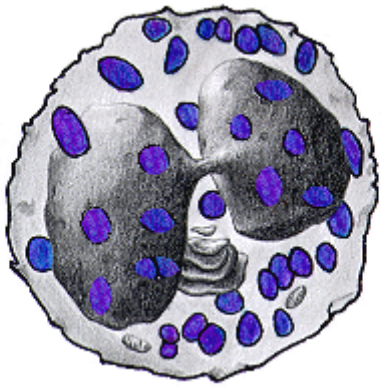
2. <u>In your selfstudy, revise the ultrastructure of specific granules in granulocytes.</u>
(Use the recommended textbook, atlas with CD-ROM and WWW)



Neutrophil

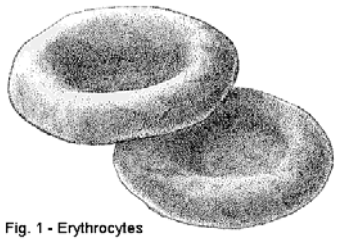


Eosinophil



Basophil

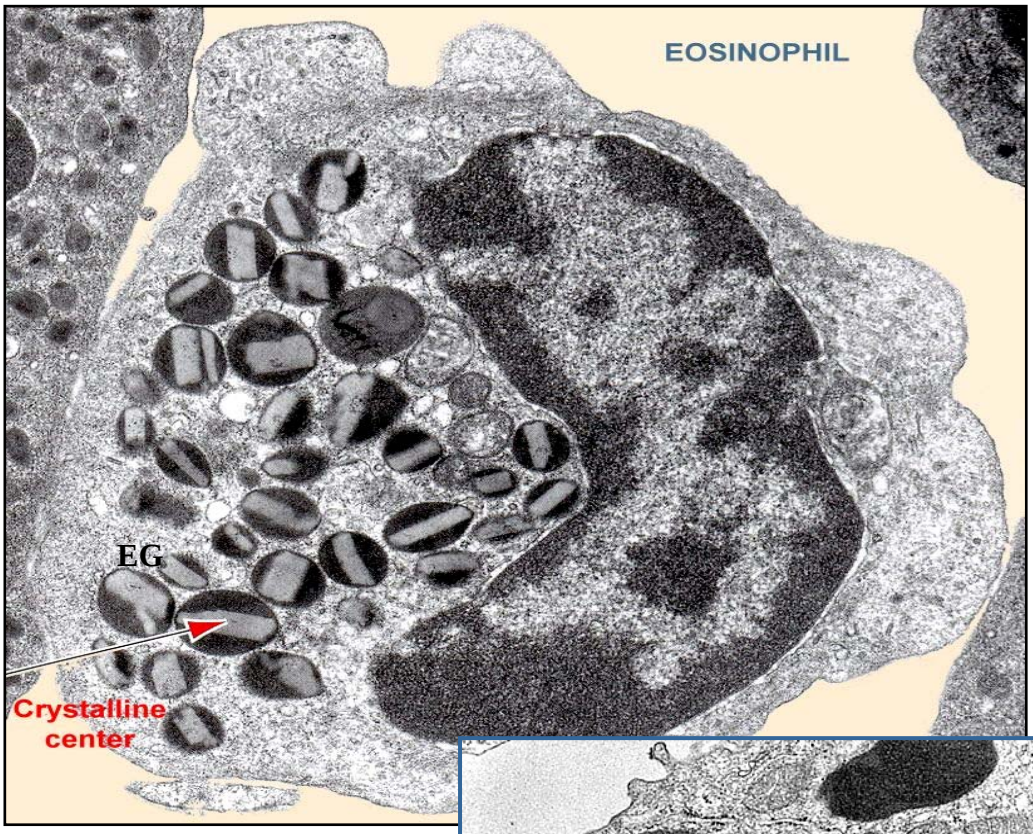
Lymphocyte



Erythrocytes

Monocyte

Pictures from: http://www.funsci.com/fun3_en/blood/blood.htm#5

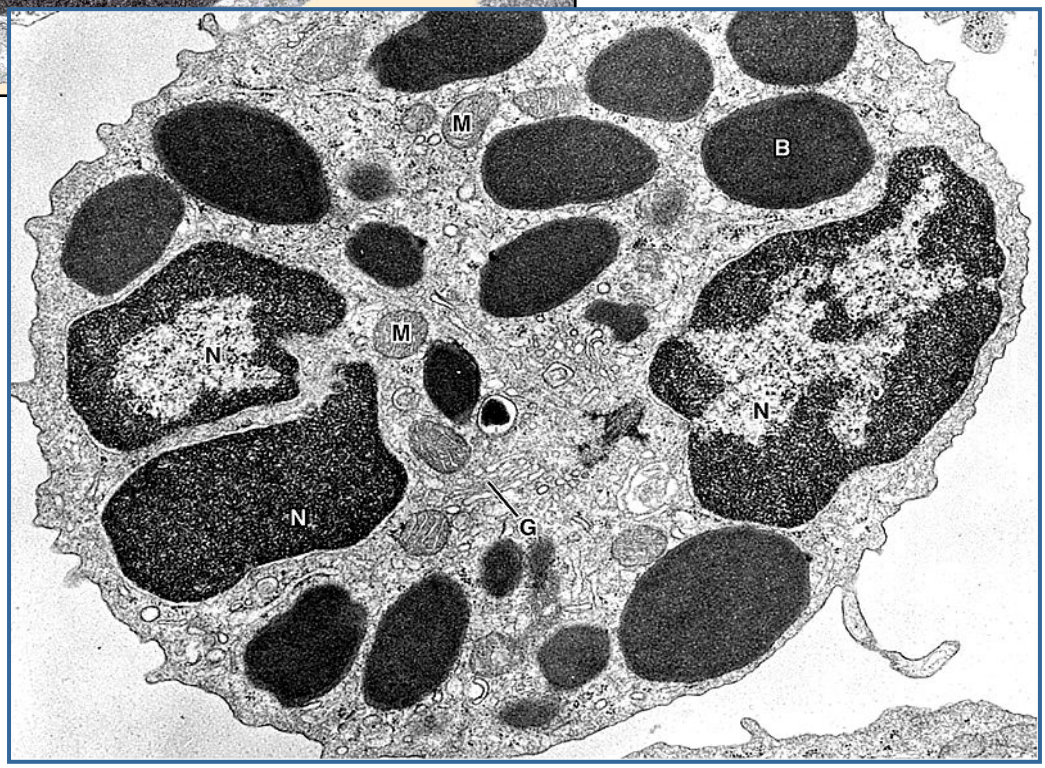


EOSINOPHIL

Study the ultrastructure of the eosinophilic granules (EG, arrow).

BASOPHIL

Note the size and structure of the basophilic granules (B).
M – mitochondria
G – Golgi apparatus
N – nucleus



Websites:

http://web.indstate.edu/thcme/PSP/labtests/bloodsmear.htm
http://www.greatscopes.com/act001.htm
http://beloit.edu/~gravisd/courses/immuno/BloodSmearPreparation.pdf
http://www.scribd.com/doc/8801750/Preparation-of-Peripheral-Blood-Smear-Staining-With-Wrights-Stain
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