

## LET'S OBSERVE THE **BLOOD CELLS**

By Daniela Tagliasacchi and Giorgio Carboni, April 1997  
Drawings of Michele Pirazzini  
Thanks to Pasquale Chieco for his help in making the pictures  
Translation revised by David W. Walker

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### INTRODUCTION



What are blood cells? What do they look like? What functions do they perform? How can I recognize the different categories? This is a short description of the blood cells and includes a simple experiment which allows you to become familiar with the cells of this precious liquid.

The blood consists of a suspension of special cells in a liquid called **plasma**. In an adult man, the blood is about 1/12th of the body weight and this corresponds to 5-6 litres. Blood consists of 55 % plasma, and 45 % by cells called **formed elements**.

The blood performs a lot of important functions. By means of the hemoglobin contained in the

erythrocytes, it carries oxygen to the tissues and collects the carbon dioxide (CO<sub>2</sub>). It also conveys nutritive substances (e.g. amino acids, sugars, mineral salts) and gathers the excreted material which will be eliminated through the renal filter. The blood also carries hormones, enzymes and vitamins. It performs the defense of the organism by mean of the phagocitic activity of the leukocytes, the bactericidal power of the serum and the immune response of which the lymphocytes are the protagonists.

## THE PLASMA

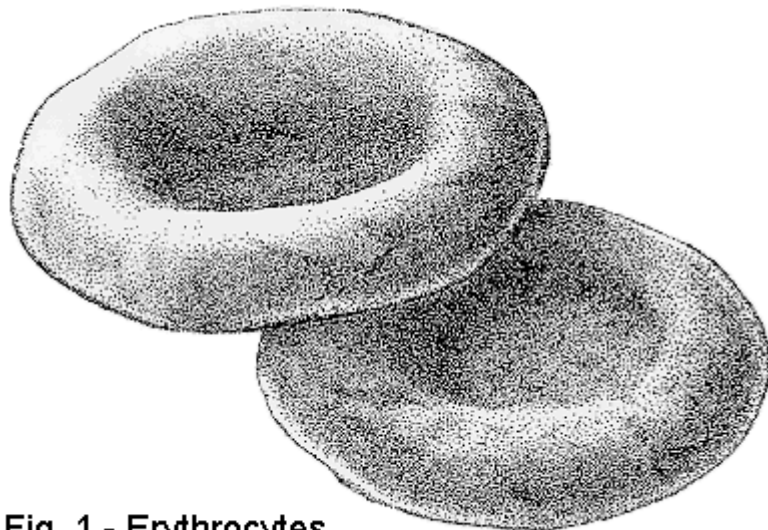
Cells free serum or plasma, can be obtained by centrifugation. The plasma is a slightly alkaline fluid, with a typical yellowish color. It consists of 90 % water and 10% dry matter. Nine parts of it are made up by organic substances, whereas one part is made up by minerals. These organic substances are composed of glucides (glucose), lipids (cholesterol, triglycerides, phospholipids, lecithin, fats), proteins (globulins, albumins, fibrinogen), glycoproteins, hormones (gonadotropins, erythropoietin, thrombopoietin), amino acids and vitamins. The mineral substances are dissolved in ionic form, that is dissociated into positive and negative ions.

## THE HEMATIC CELLS

In the blood are present special cells, classified in: **erythrocytes** and **leukocytes**. There are also **platelets** which are not considered real cells. In the following, we will deal the different categories of blood cells.

### ERYTHROCYTES (red cells)

The erythrocytes are the most numerous blood cells i.e. about 4-6 millions/mm<sup>3</sup>. They are also called red cells. In man and in all mammals, erythrocytes are devoid of a nucleus and have the shape of a biconcave lens. In the other vertebrates (e.g. fishes, amphibians, reptilians and birds), they have a nucleus. The red cells are rich in hemoglobin, a protein able to bind in a faint manner to oxygen. Hence, these cells are responsible for **providing oxygen to tissues** and partly for **recovering carbon dioxide** produced as waste. However, most CO<sub>2</sub> is carried by plasma, in the form of



**Fig. 1 - Erythrocytes**

soluble carbonates.

In the red cells of the mammals, the lack of nucleus allows more room for hemoglobin and the biconcave shape of these cells raises the surface and cytoplasmic volume ratio. These characteristics make more efficient the diffusion of oxygen by these cells. In so-called "sickle-cell anaemia", erythrocytes become typically sickle-shaped. With the electron microscope, biologists saw that red cells can have different shapes: normal (discocyte), berry (crenated), burr (echinocyte), target (codocyte), oat, sickled, helmet, pinched, pointed, indented, poikilocyte, etc. The mean life of erythrocytes is about 120 days. When they come to the end of their life, they are retained by the spleen where they are phagocyted by macrophages.

## PLATELETS

The main function of platelets, or thrombocytes, is **to stop the loss of blood from wounds** (hematostasis). To this purpose, they aggregate and release factors which promote the blood coagulation. Among them, there are the serotonin which reduces the diameter of lesioned vessels and slows down the hematic flux, the fibrin which trap cells and forms the clotting. Even if platelets appears roundish in shape, they are not real cells. In the smears stained by Giemsa, they have an intense purple color. Their diameter is 2-3  $\mu\text{m}$  about, hence they are much smaller than erythrocytes. Their density in the blood is 200000-300000 / $\text{mm}^3$ .

## LEUKOCYTES (white cells)

Leukocytes, or white cells, are responsible for **the defense of the organism**. In the blood, they are much less numerous than red cells. The density of the leukocytes in the blood is 5000-7000 / $\text{mm}^3$ . Leukocytes divide in two categories: **granulocytes** and **lymphoid cells** or agranulocytes. The term granulocyte is due to the presence of granules in the cytoplasm of these cells. In the different types of granulocytes, the granules are different and help us to distinguish them. In fact, these granules have a different affinity towards neutral, acid or basic stains and give the cytoplasm different colors. So, granulocytes distinguish themselves in neutrophil, eosinophil (or acidophil) and basophil. The lymphoid cells, instead, distinguish themselves in lymphocytes and monocytes. As we will see later, even the shape of the nucleus helps us in the recognition of the leukocytes.

Each type of leukocyte is present in the blood in different proportions:

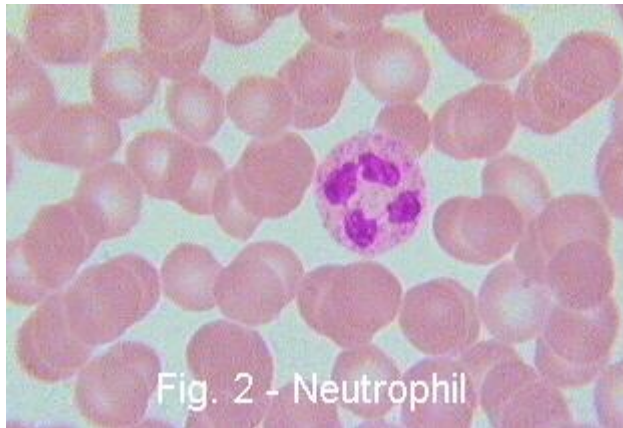
neutrophil 50 - 70 %

eosinophil 2 - 4 %

basophil 0,5 - 1 %

lymphocyte 20 - 40 %

monocyte 3 - 8 %



**Neutrophils** are very active in phagocytosing bacteria and are present in large amount in the pus of wounds. Unfortunately, these cells are not able to renew the lysosomes used in digesting microbes and dead after having phagocytosed a few of them.

**Eosinophils** attack parasites and phagocyte antigen-antibody complexes.

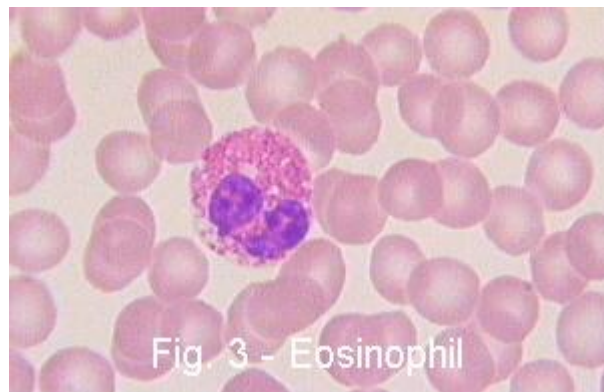
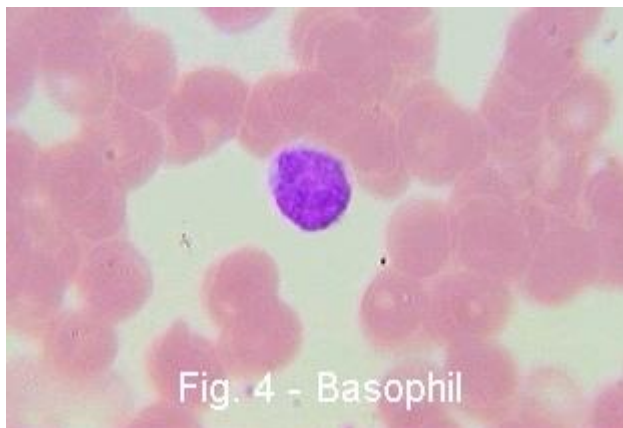


Fig. 3 - Eosinophil



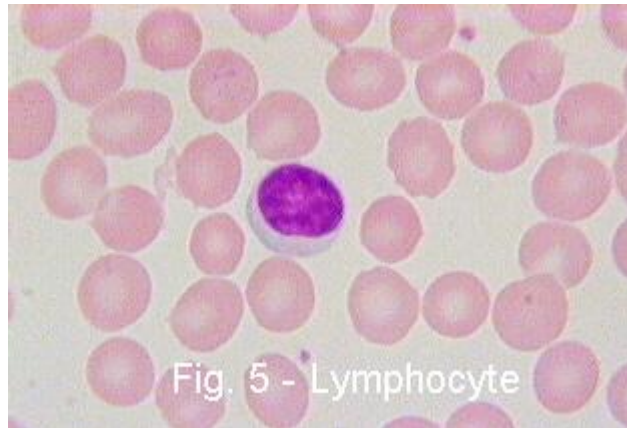
**Basophil** secrete anti-coagulant and vasodilatory substances as histamines and serotonin. Even if they have a phagocytory capability, their main function is secreting substances which mediate the hypersensitivity reaction.

Fig. 4 - Basophil

**Lymphocytes** are cells which, besides being present in the blood, populate the lymphoid tissues and organs too, as well as the lymph circulating in the lymphatic vessel. The lymphoid organs include thymus, bone marrow (in birds bursa), spleen, lymphoid nodules, palatine tonsils, Peyer's patches and lymphoid tissue of respiratory and gastrointestinal tracts.

Most lymphocytes circulating in the blood is in a resting state. They look like little cells with a compact round nucleus which occupies nearly all the cellular volume. As a consequence, the cytoplasm is very reduced. The lymphocytes of the lymphoid tissues and organs can be activated in a different amount following antigenic stimulation. In the blood, lymphocytes are 20-40 % of all leukocytes and are slight larger than red blood cells.

The lymphocytes are the main constituents of the immune system which is a defense against the attack of pathogenic micro-organisms such as viruses, bacteria, fungi and protista. Lymphocytes yield **antibodies** and arrange them on their membrane. An antibody is a molecule able to bind itself to molecules of a complementary shape called **antigens**, and recognize them. As for all proteins, even the antibodies are coded by genes. On the basis of a recombination mechanism of some of these genes, every lymphocyte produces antibodies of a specific shape.



Hence, lymphocytes perform an action which is called **specific** in that each of them recognize the complementary antigen only. Even if every lymphocyte is so selective to recognize only one molecule, the number of circulating lymphocytes is so large that they are able to recognize practically all substances which are in the organism, both its own and foreign. It is a question of recognizing hundreds of millions of different molecules.

The cells of the immune system, chiefly lymphocytes, cooperate amongst themselves to activate, boost or make more precise the immune response. To attain this scope, there exist different types of lymphocytes, with different functions: T and B lymphocytes. When the **B cells** are activated, they breed quickly (**clonal selection**) and they become **plasmacells** which secrete a great deal of antibodies in the blood stream (**humoral response**). When free antibodies meet micro-organisms with complementary shape (**epitopes**), they bind to them and form complexes which immobilize the micro-organisms. Later, other cells which are not specific, but which are able to recognize antibodies, phagocyte these complexes.

In their turn, the **T cells** are divided into three categories: Tc (cytotoxic), Th (helpers), Ts (suppressors). Even the **Cytotoxic lymphocytes** breed quickly when they are activated. They do not release antibodies in the bloodstream, but they keep the antibodies on their membrane and use them to recognize cells mainly of its own organism infected by virus or tumoral cells. The cytotoxic lymphocytes kill cells by means of the release of perforins, substances which produces lesions in the membrane of the target cell and cause its death by osmotic lysis (**cell-mediated response**). The **helper lymphocytes** are needed to activate both B and Tc lymphocytes which, even though they recognize extraneous agents, seldom enter into direct action. **Suppressor lymphocytes** reduce the intensity of the immune response.

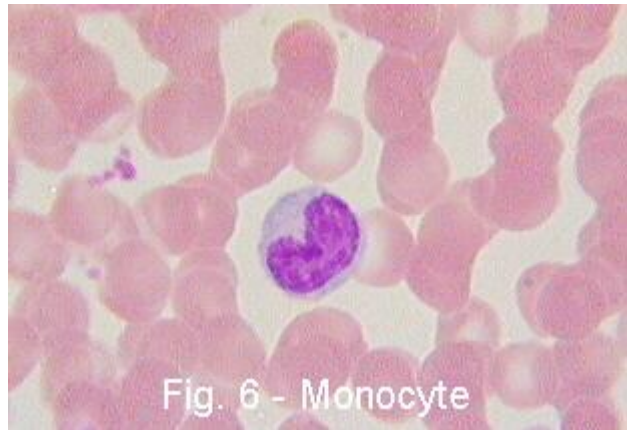
However, the immune system must not attack the cells of its body as the **autoimmune reaction** can damage the organism and lead to death. How does the immune system distinguish between self and not self? We have seen that B and Tc lymphocytes which have recognized an antigen, do not enter in action, but they need to be activated by a helper lymphocyte. A few times after the organism's birth, some of the new lymphocytes pass through the thymus where they become T lymphocytes. Here, these cells are compared with all antigens of the organism (autoantigens). It seems that lymphocytes which recognize an antigen, as they are still immature, will die. In this way, as the autoreactive Th lymphocytes are being killed, only the B and Tc lymphocytes which have recognized extraneous antigens can be activated. The system of cellular cytotoxicity mediated by Th cells is evolved as a defense against their own infected, modified or aberrant cells. In fact, B and Tc lymphocytes can activate themselves against bacteria even without the agreement of the helpers.

The B and Tc activated lymphocytes, besides to producing antibodies and killing foreign cells, multiply quickly. During the cellular division, rearrangements often occur in the sequence of the genes which code for the antibody. In this way, the antibody of the new cell takes a slightly different shape in comparison to that of its "mitotic parent". If the new shape matches the antigen better, this cell will be induced to divide more. The next generation of clones is therefore more efficient and, in its turn, can induce more selective varieties. This process and that of clonal selection make the immune response more effective. Finally, the immune system produces **memory cells**, i.e. deactivated lymphocytes ready to be reactivated on the occasion of further meeting with the same antigen.



Besides the Th and B cells, there is a third population of lymphocytes in the peripheral blood and lymphoid organs which do not have receptors for antigens. These lymphocytes have a non-specific defense function which is not activated by Th lymphocytes. These cells represent the more ancient component of the immune system and they are characterized by their cytotoxic activity. For these reasons, they are named **NK, Natural Killer**. Apart from killing viruses, bacteria, infected and neoplastic cells, these lymphocytes also regulate the production of other hematic cells such as erythrocytes and granulocytes.

**Monocytes** are the precursors of **macrophages**. They are larger blood cells, which after attaining maturity in the bone marrow, enter the blood circulation where they stay for 24-36 hours. Then they migrate into the connective tissue, where they become macrophages and move within the tissues. In the presence of an inflammation site, monocytes quickly migrate from the blood vessel and start an intense phagocytory activity. The role of these cells is not solely in phagocytosis because they have also have an intense secretory activity. They produce substances which have defensive functions such as lysozyme, interferons and other substances which modulate the functionality of other cells. Macrophages cooperate in the immune defense. They expose molecules of digested bodies on the membrane and present them to more specialized cells, such as B and Th lymphocytes.



## PREPARATION OF THE BLOOD SMEAR

This experiment is intended for adults examining their own blood. If you want observe or let observe the blood of others (in school or other organization), you have to obtain the appropriate authorization to do so. You need to protect yourself and the others against the biohazard posed by; taking, working with and disposing of blood samples and you have to work according the suitable protocols.

In order to take a blood sample, you have to use latex gloves and special lancets which allow you to safely pierce the skin and take the sample. Following use, the lancets and glass slides must be disposed of in an appropriately labeled Sharps Bin. All materials such as tissues, wipes, stains etc that have been in contact with blood must be disposed of safely according to the protocols of the competent organization. In any case, read our page of [Warnings](#).

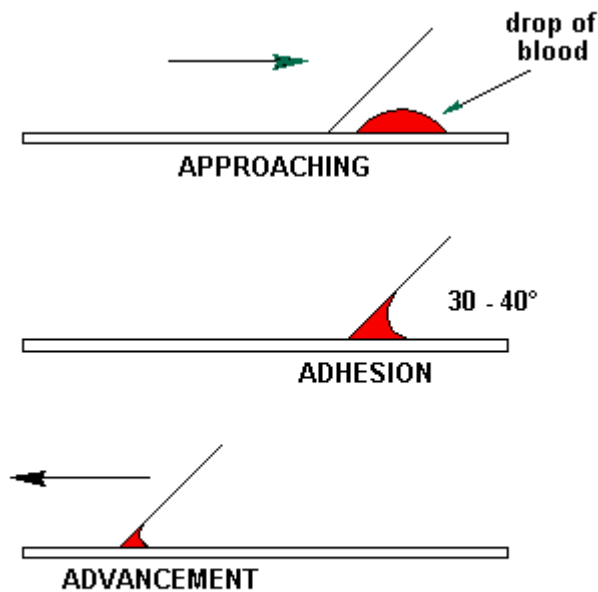
### MATERIALS

- sterilized lancets or needles
- 20 clean microscope slides and coverslips
- Canada balsam or other medium for permanent preparations
- 95% ethyl or methyl alcohol
- distilled water
- Giemsa stain
- low containers (you can make them with aluminum sheet also) or Petri dishes
- microscope which magnifies 200 times at least

## TAKING THE BLOOD

Cleanse a finger. With a sterile lancet, make a puncture on a fingertip. If you have difficulties in doing this, you can wait until you have a casual wound. In the meantime, keep all the materials needed ready and protected from dust, particularly the clean microscope slides.

## MAKING THE SMEAR



**Fig. 7 - How to prepare a blood smear**

Place a small drop of blood near an end of a slide. According to figure 7, bring the edge of another slide in contact with the drop and allow the drop to bank evenly behind the spreader. The angle between the two slides has to be 30-40 degrees. Now, push to the left in a smooth, quick motion. The smear should cover about half the slide. It is important that the quantity of blood is not excessive, otherwise the red cells could hide the leukocytes. So, if you succeed in making a gradual transition from thick to thin in your smear, you should get a zone with a satisfactory distribution of cells. With a single drop of blood, you can make several smears. In fact, to make a smear, it is enough to leave a spot of blood of 3 mm about in diameter on the slide. It is useful to perform many smears. In fact, not always they are successful, and with some attempts, it is easier to get one well prepared. To avoid producing clots, you must make each smear with fresh blood and straight after having deposited it. To this purpose, it is useful to be helped by another person where one deposits the blood, and the other makes the smears. With the microscope, you should observe the smears to check that some of them are properly made. The red cells must not overlap each other, nor be so scarce as to be too spread out.

## FIXING

If you apply the stain to a smear without having fixed it beforehand, the cells will explode because of the so-called **osmotic** or **hypotonic shock**. This happens because the saline concentration inside the cells is much higher than that of staining fluid which is diluted in distilled water. In the attempt to equal the internal saline concentration to the values of the external one, the cells undergo swelling by **osmosis**. To attain the same saline concentration of the external liquid, the cells should swell more than their membrane allows, in fact they explode. The cell contents are released, and the preparation becomes unusable. To avoid this, before staining, you have to **fix** the smear. This operation hinders the inflation of the cells which keep sound when they are stained.

A simple and effective fixing technique consists of dipping the smear in a vessel containing 95% ethyl or methyl alcohol for 3-5 minutes. In order to put alcohol on the smear, you can also use a dropper or a bottle dispenser.

## STAINING

If you observe the smear as it is after fixing, you will see very little because all cells are very transparent. The erythrocytes are slightly visible, but the leukocytes are too pale, almost invisible and you will not see anything inside them. To be able to observe and recognize the different kinds of leukocyte, you must stain them. For this purpose, normally **Giemsa stain** is used. It is a mixture of stains, based on methylene blue and eosin. It is cheaply available commercially in volumes of 100 cc. It consists of a concentrated solution which you have to dilute in the proportion 1/10, that is one part of Giemsa in nine of distilled water, or buffer solution (pH = 6,8-7,2). You can buy the stain in a store of chemicals and laboratory equipment.

To stain a smear, take a slide with a fixed and dry smear. Put on the slide a drop of stain until it is fully covered. Stain for about 16 minutes, renewing the stain about four times. Then rinse the slide with distilled water at room temperature. Drain off the water and leave the slide to dry.

## CHECKING

With the microscope, verify that the cells are well stained. If necessary, apply the stain for a few more minutes. If you were planning to mount the slide with Canada balsam, the staining has to be stronger.

## COVER-SLIPPING

At this point, your smear is ready to be observed, but if you want to keep it for a long time, you should make the preparation permanent. To this purpose, after drying the slide, place a drop of **Canada balsam** or another medium mountant on the smear, then mount the coverslip. If the balsam is too viscous, you may heat a few of the slides (but not over 40 degrees C) to help the balsam flow between the slide and coverslip.

## OBSERVATION

A magnification of 200 times is enough to allow you to observe and identify the different types of cells. If you use a higher power, you can also see the cells details better. You can examine either with dry objectives or with the oil immersion technique. In this last case, if you have put on a coverslip, you must wait a day to allow the balsam to set, otherwise, when you move the slide, oil will displace the coverslip.

## -- ERYTHROCYTES



The red cells are very numerous in the blood. Usually, they measure 6,6-7,5  $\mu\text{m}$  in diameter. However, cells with a diameter higher than 9  $\mu\text{m}$  (macrocytes) or lower than 6  $\mu\text{m}$  (microcytes) have been observed. In the observation field of the microscope, you will see a lot of erythrocytes and, sometimes, some isolated leukocytes. Erythrocytes are without nucleus (among vertebrates, only the red cells of mammals are lacking a nucleus). Their typical shape is that of a cake depressed in the center (fig. 1). Under the microscope, they look like pink discs clearer in the middle (fig. 2-6: pink cells around the leukocytes). Sometimes, they are piled up like coins. As we saw, the red cells can also have different shapes from those we described. Sometimes, this is normal, other times, this is due to diseases or to defective process of preparation and staining of the smear.

## -- PLATELETS

Platelets are not true cells. They gemmate from big leukocytes called **megakaryocytes**. They are small sized diskettes about 3 $\mu\text{m}$  in diameter. They appear a purple color and are more intense than red cells (you can see some platelets in figures 5 and 6).

## LEUKOCYTES

Unlike red cells, leukocytes have a nucleus. It is easily visible under the microscope, but only after having stained the smear. The nucleus of these cells can show multiple lobes, or be indented or kidney-shaped (reniform). Usually, the shape of the nucleus of various kind of leukocytes is different. Together with the different colors of granules, the shape of nucleus helps us to recognize these cells. Leukocytes are divided into granulocytes and lymphoid cells. In the drawings which follow, besides nuclei and granules, you can see even mitochondria, Golgi apparatus, endoplasmic reticula and ribosomes.

## - GRANULOCYTES

They come from the marrow bone. Their cytoplasm is rich in granules which take typical colors which help their recognition. The nucleus is condensed in a little masses or **lobes**. In the blood, there are immature cells as well. They distinguish themselves by having a less segmented nucleus. As we have said, there are three types of granulocyte: neutrophil, eosinophil, basophil.

## -- NEUTROPHIL Granulocytes

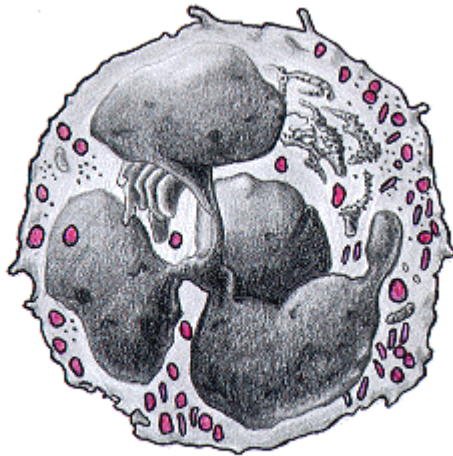


Fig. 8 - Neutrophil

The neutrophil are the more common leukocytes. They have a diameter of 12-15  $\mu\text{m}$ . You can recognize them as their nucleus is divided into 2 - 5 lobes connected by a fine nuclear strand or filament (fig. 8). The cytoplasm is transparent because its granules are small and faintly pink colored. Immature neutrophils have a band-shaped or horseshoe-shaped nucleus and are known as **band cells**. In the nucleus of the neutrophil of cells from females, you may see an appendage like a little drumstick (Barr body). It is the second X chromosome, inactivated.

## -- EOSINOPHIL Granulocytes

The eosinophils are quite rare in the blood. They have the same size as the neutrophils. Generally their nucleus is bi-lobed. But even nuclei with three or four lobes have been observed. The cytoplasm is full of granules which assume a characteristic pink-orange color (fig. 9). As for the neutrophil, the nucleus is still easily visible.

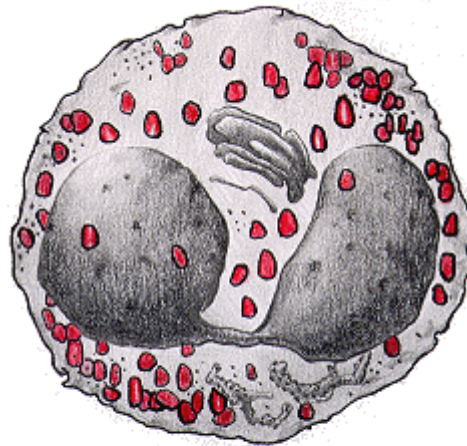


Fig. 9 - Eosinophil

## -- BASOPHIL Granulocytes

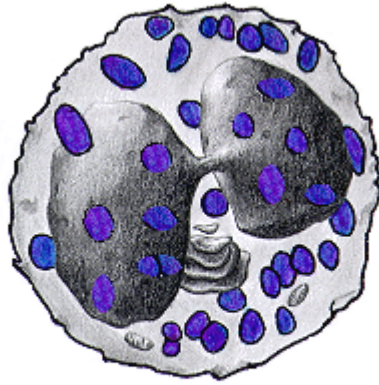


fig. 10 - Basophil

Basophils are the rarest leukocytes: less than 1 %. They are quite small: 9-10  $\mu\text{m}$  in diameter. Cytoplasm is very rich in granules which take a dark purple color. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of granules which hide it (fig. 10).

## - LYMPHOID CELLS (or agranulocytes)



Because usually these cells appear lacking in granules, they are also named **agranulocytes**. They have a compact nucleus and a transparent cytoplasm. There are two types of lymphoid cells: lymphocytes and monocytes. Their look is similar, but their origin is different. In fact, whereas lymphocytes spring from lymphatic organs, monocytes have the same origin as the granulocytes.

## -- LYMPHOCYTES

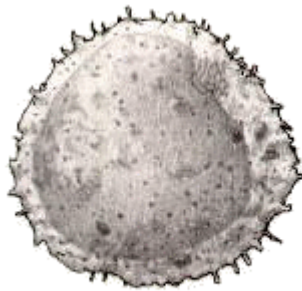


Fig. 11 - Lymphocyte

Lymphocytes are quite common in the blood: 20-40%, 8-10  $\mu\text{m}$  in diameter and generally they are smaller than the other leukocytes but they are still a few larger than red cells (fig. 11). The cytoplasm is transparent. The nucleus is round and large in comparison to the cell and it occupies most of it. In any case, some of the cytoplasm remains visible, generally in a lateral position. According to the quantity of cytoplasm, lymphocytes are divided into small, medium and large. With Giemsa stain, we cannot distinguish the different types of lymphocyte (B, T, NK), either in the blood because they are not activated, or because it would be necessary to perform special immunochemical staining.

## -- MONOCYTES



Monocytes are the biggest leukocytes: 16-20  $\mu\text{m}$ . They have a great reniform or horseshoe-shaped nucleus, in some cases even bi-lobed. The cytoplasm is transparent, but with an appearance of "ground glass" (fig. 12).



Fig. 12 - Monocyte

## CONCLUSION



Now that you have learned this technique, you can apply it to analyze the blood of other animals. For example, observe the blood of earthworms, which are easy to find. What you see even in the blood of simple animals is very interesting. As the blood of vertebrates is the fruit of a long evolution, as you descend the evolutionary ladder, you will see simpler types of blood, but you will also find a continuity which will help you to understand the blood of different animals.

In the more primitive beings, the liquid which flows among the cells has a composition very close to that of the water and performs modest functions. If you go up the evolutionary tree, this liquid assumes new and more complex functions. While in the invertebrates, the blood, called **hemolymph**, wets the organs and only in a part flows inside vessels, in the vertebrates blood flows in a vascular system which is entirely contained by walls and the cells are wetted by lymph instead. In the vertebrates, the blood also carries out complex functions of transport, homeostasis and defense.

As for the immune system, even in the protists there is a very rudimentary form of recognition of what is extraneous. Of course, in this case it is too far removed to be called an immune system! However, in the comparatively simple multicellular organisms, such as annelids and arthropods, cells do exist with defensive functions but they do not perform a specific action. Often, they are generically named phagocytes, other times with more specific terms. In the vertebrates, lymphocytes occur, defensive cells endowed with specific roles.

We hope this experiment has helped you to become familiar with the blood and the methods for the microscopic observation of its cells. This may be useful as a science experiment during school, or even as general knowledge. To an amateur microscopist it may be a stimulating experiment. However, what you will observe during these experiments will help you to follow the development of life in our planet, even if from an unusual point of view.

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A useful introduction on sample processing and staining methods is: Animal Tissue Techniques;  
Gretchen L. Humason, W.H. Freeman

Here you can see other pictures of blood cells: <http://www.md.huji.ac.il/gabi/blood/bloodmain.htm>

Browse the web with a search engine, using words as the following: "blood smear", lymphocyte,  
"immune system".

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