HEMATOLOGY
&LYMPH SYSTEM

physiology sheet

Number	5
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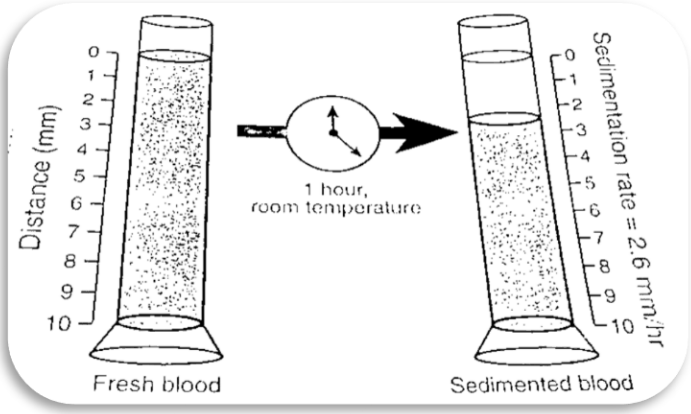
RBC's tests:-

1- Erythrocyte sedimentation rate (ESR) :-

Determination of the erythrocyte sedimentation rate (it measures the rate of RBCs sedimentation); this test is widely used in clinical medicine.

How is this test performed?

We take blood sample; fresh blood, anti-coagulated, and we put it in a graduated tube. We left it in the lab for about one hour then after one hour, we measure the clear plasma height. Sedimentation of the RBCs spontaneously occur, because this blood is anti-coagulated not centrifuged.



In one of the methods Westergren method, for example, **the height of the clear plasma in male ♂ up to 5mm and in female ♀ up to 15mm**, this height is called ESR. There are more than one method to measure ESR; the height of the tubes as well as the diameters are different therefore the ESR is different; because the height of the blood is different.

The methods are:-

1) Westergren method **2)** Wintrobe method **3)** Cutler method

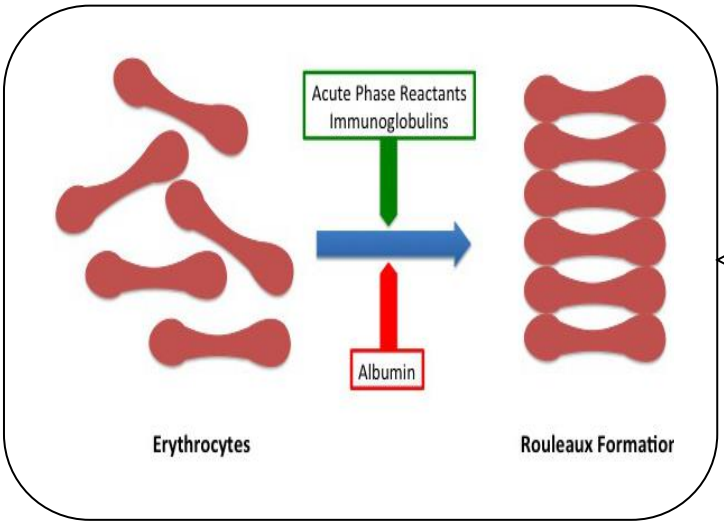
Actually, there are more than three methods, there is a fourth method for the measurement of ESR which is: **4)** Landau method

There are the normal levels of ESR in male ♂ and in female ♀ in these different methods:-

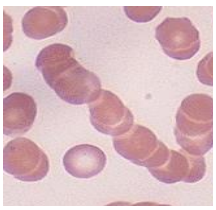
Gender / Method	Male ♂	Female ♀
Westergren	0-15	0-20
Wintrobe	0-10	0-20
Cutler	0-8	0-10
Landau	0-5	0-8

ESR in women more than in men.

The unit is mm/hr



RBCs form **rouleaux**;
"Aggregations of RBCs which form because of the unique discoid shape of the cells"
We call this process: **rouleaux formation.**



ESR affected by three factors:-

1) **Factors related to Erythrocytes;** SIZE OR MASS (the larger the particle the faster its rate of sedimentation, therefore macrocytic cells=high ESR and microcytic cells= low ESR); SHAPE, (you remember the shape of normal RBC's, they are circular and biconcave. Any change in this shape will alter the rouleaux formation such as in sickle cell anemia and in spherocytic anemia the ESR is low); and CELLS COUNT (in severe anemia ESR high because the RBCs level decrease and in polycythemia either normal or low).

2) **Plasma proteins;** is the single most important factor determining the ESR. Rouleaux of the RBCs are affected mainly by the plasma proteins level. We talked about the RBCs and the effect of RBCs in the ESR, also we said that plasma proteins affect many processes in the body; one of these is ESR.

   For example: Fibrinogen and Globulin affect the **viscosity**, also affect **ESR**, normal concentration or high concentration (higher than normal) of fibrinogens and globulins will increase the ESR.

3) **Mechanical and technical pathways;** such as holding **the tube in the racks**, it must be perpendicular (tilting the tube 3 degree will cause an error up to 30%) also the rack which hold the tube should not be subjected to any movement or vibration, **temperature** of the room (large changes in it will increase the ESR) and **the length and diamete**r of the tube also affect the final test result.

IN ALL INFECTIONS, ACUTE OR CHRONIC ESR INCREASES AND IN ALL CT DESTRUCTIVE DISEASES ALSO ESR INCREASES.

ESR not specific test; does not indicate directly the presence of specific disease but it indicates the presence of X disease.

It only Indicate something; the test tells us that there is a disease in that patient but does not specify the type of disease. So why do we use it? The Internist asks for this test also the surgeon asks for this test!

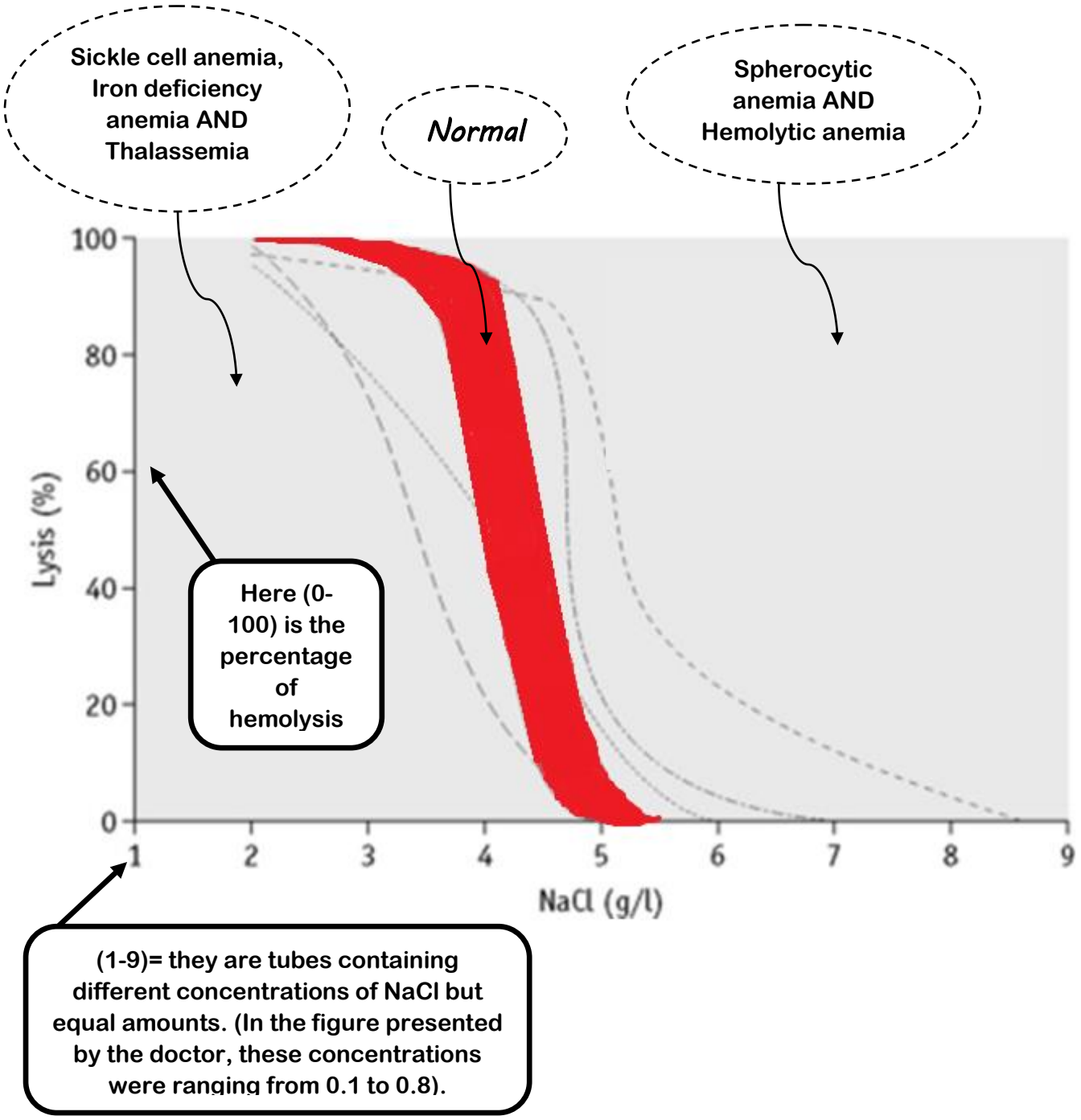
In infections, ESR increase specifically high in tuberculosis also increase in hepatitis (either infection or inflammation) and rheumatoid arthritis (connective tissue destructive diseases), in the presence of myocardial infarction the ESR increases especially in the acute myocardial infarction. But also the presence of normal ESR does not exclude the presence of organic diseases.

In young men ESR is low, and in old men ESR is high, why?
And why ESR low on polysythemia and high in anemia?

2- Osmotic fragility test :-

We will see this test in our lab.

We use this test to measure RBCs resistance to hemolysis when they are exposed to different concentrations of NaCl solution.



Equal amounts of NaCl solutions but different concentrations with equal drops of bloods in these tubes (5-6 drops in our lab) from the same patient if the RBCs begin to hemolyze in tube 0.5 or 0.55 and complete the hemolysis in 0.3 or 0.1 and maybe 0.25 then this sample is normal; the RBCs is normal " normal curve ".

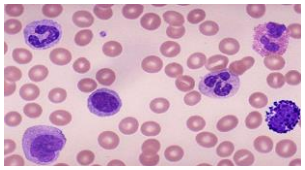
When the cells hemolyze in higher concentration we say the **osmotic fragility increases** for these cells such as in hemolytic anemia and in hereditary spherocytic anemia, sometimes the cell begins to hemolyze in lower concentration we say **the osmotic fragility decrease** such as in sickle cell anemia, iron deficiency anemia and in thalassemia.

As we see the osmotic fragility test is used to diagnose different types of hemolytic anemia and it affected by the:-

Shape of the RBCs (spherocytic cells, sickle cells, Iron deficiency) which in turn affected by the **Volume** (small cells, iron deficiency anemia, sideroblastic anemia and thalassemia), **Surface area** (sickle cell), and **Functional state** of the RBCs (G6PD deficiency, pyruvate kinase deficiency in the RBCs).

Also **Extracorpuscular defects** such as blood groups incompatibility **Drugs** such as penicillin and **Infections** such as in malaria.

Usually, internist and hematologist use this test.



WBCs

Leukocytes, range from 4000 to 11000, below 4000=leckopenia and above 11000=leckocytosis.

Either:

1) granular: Neutrophils + Basophils + Eosinophils. They have granules in the cytoplasm.

2) non-granulocytic: monocytes + Lymphocytes there are no granules in the cytoplasm (sometimes there are granules but they are very little).

All of them are larger than the RBCs, they have nuclei, they are too much less in number, the half-life is much shorter; the WBC ½ life ranges from hours to days whereas the RBC has a lifespan of 120 days.

There is no difference between male and female, but even in the same person, there is a change in the count whatever male or female let us say maximum in the evening minimum in the morning, why? There are differences in the activity, WBCs increase after a meal, and excitement, exercise, also they increase in pregnancy and this is the only difference between male and female.

They are active cells they move through the capillary and also they leave the capillary into tissues.

There is another classification:

1) Granulocytes, **2)** Lymphocytes, and **3)** Monocytes

There are 60% neutrophils, 4% eosinophils, 1% basophils, 30% lymphocytes and 5% monocytes.

From the past papers

Wrong about eosinophils? **With basophils form 10% of WBCs (not sure)**

But if we count a hundred cells and we find the percentage of the WBC types this is called DLC (differential leukocyte count); 100 cells: 60 neutrophils, 34 lymphocytes, 4 monocytes, 3 eosinophils, and basophils 0.5. Sometimes you may not get any results when you count 100 cells.

These cells we counted is just **50%** of the WBCs in the body the other **50%** still adhered to the inner surface of the blood vessels, this called **marginal pool**. They are released when needed such as in hemorrhage or hemolysis. This is very rare but not in every person neutrophils are the highest, sometimes the lymphocytes.

Leukopoiesis activity is similar to the erythropoiesis activity in the duration (3-6 days) also in the WBCs production it is the same, but in WBCs production when they are produced they remain in the bone marrow for about 6 days then they are released, why? **To be computerized in order to activate them.**

All the WBCs are produced in the bone marrow but as far as lymphocytes they are produced in the lymphoid tissues (lymph nodes, spleen, and thymus).

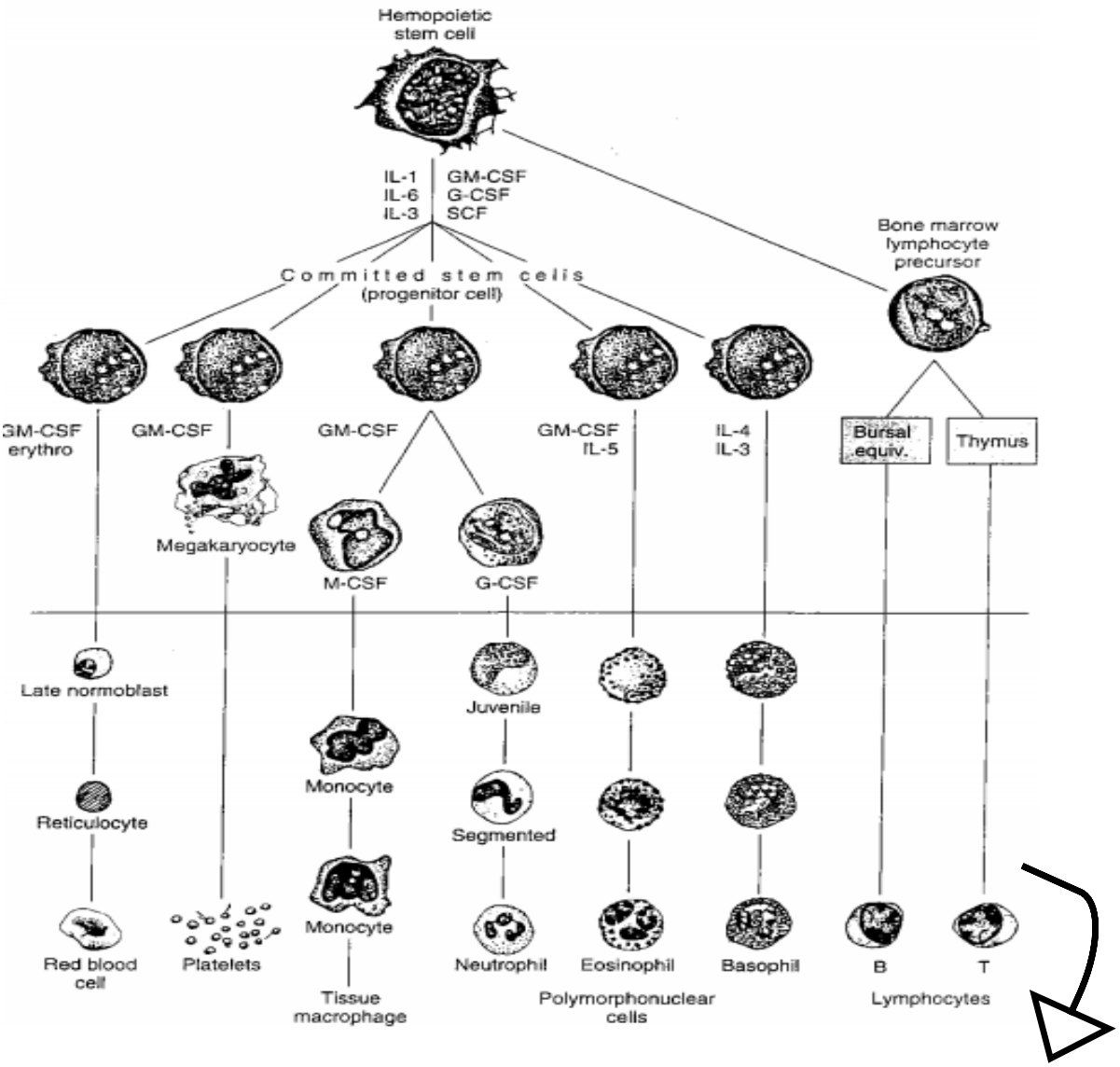
All the WBCs contain enzymes to digest and kill the bacteria but don't forget that the basophils contain a very important coagulant in the body **heparin** and also they contain **histamine** (vasodilator) and **serotonin** (vasoconstrictor).

75% of the cells in the bone marrow belong to the WBC production and only 25% are maturing RBC, why? Because WBCs have a very short half-life (The average lifespan of the WBC is short).

Vitamin B12 affects the neutrophils (actually all the WBCs, but we talk about neutrophils because they are the highest).

From the past papers

Wrong about B12? **Its deficiency mostly affects WBCs**



We will have an idea about the leukopoiesis (the production of WBCs). As you see, it is the most complicated process in our body. Why? As you see in this figure there are many stages involved from the mother stem cell to the production of the mature cells, many factors are involved as you see about 10 factors involved in the production of WBCs also many cells are involved and affected even the RBCs and platelets they are involved during the production of WBCs. Sometimes more than one factor affect the production of one cell, sometimes more than one factor affect the same cell in more than one stage. For example, IL-3 affects the production of the megakaryocyte in addition to another cell.

If we delete any of the factors, the production of WBCs will be affected, and there are overlaps between the products of these factors, why we call

them factors? We call them factors because we don't know everything about these factors (The general name, these are called cytokines, chemicals), each one of these factors is produced by many cells or tissues.

We see here multifunctional factor called colony stimulating factor (CSFs), we see colony in more than one stage, what is this mean? This means when we add this factor to the culture in the same media this produces cells in colonies, therefore, these factors are colony stimulating factors. In the culture when we add any one of these factors this factor causes colonies (cells in colonies (groups)). This is an idea about the leukopoiesis so these are produced and remain in the bone marrow for 6 days then computerized and released to the circulation.

All cytokines are cell line stimulated, the same cytokine stimulates **many cells in many stages** and **the cytokines sources**, if you can see it is multiple cell types so for any one of these cytokines there are products from many cells.

Cytokine	Cell Lines Stimulated	Cytokine Source
IL-1	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
IL-3	Erythrocyte Granulocyte Megakaryocyte Monocyte	T lymphocytes
IL-4	Basophil	T lymphocytes
IL-5	Eosinophil	T lymphocytes
IL-6	Erythrocyte Granulocyte Megakaryocyte Monocyte	Endothelial cells Fibroblasts Macrophages
IL-11	Erythrocyte Granulocyte Megakaryocyte	Fibroblasts Osteoblasts
Erythropoietin	Erythrocyte	Kidney Kupffer cells of liver
SCF	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
G-CSF	Granulocyte	Endothelial cells Fibroblasts Monocytes
GM-CSF	Erythrocyte Granulocyte Megakaryocyte	Endothelial cells Fibroblasts Monocytes T lymphocytes
M-CSF	Monocyte	Endothelial cells Fibroblasts Monocytes
Thrombopoietin	Megakaryocyte	Liver, kidney

All leukocytes have many properties which related to their functions in the body:

Diapedesis

They pass capillaries into tissue spaces in accordance with the local need

Amoeboid motion

They have the ability to move even through tissues or capillary

Chemotaxis

They are attracted to the infected area by certain chemical substances released by bacteria or by inflamed tissues

Phagocytosis

The ability to engulf and digest or kill bacteria and products of cell death

From the past papers

 # Which of the following statements regarding leukocytes are correct?

1. They move out to the tissues by a process called emigration **2.** Neutrophils and microphages are required in phagocytosis **3.** Inflammatory cells are attracted by bacterial molecules and inflamed tissue by a process called chemotaxis **4.** Leukopenia is an increase in the number of WBCs in the circulation ???

(a)1 and 2 only (b) 2, 3, 4 **(c) 1, 2, 3** (d) 1, 2, 3, 4 (e) 3 and 4 only

These 4 characteristics found in all types of WBCs but with varying degrees mostly seen in neutrophils then lymphocytes.

Eosinophil, have a special characteristic which is a granule with **orange color**. It is the only cell which has this color; with special stain, it looks bright orange color.

Basophils, the granules cover the nucleus.

Neutrophils have nucleus fragmented, segmented sometimes with more than one segment.

"Why do we even try when the barriers are so high and the odds are so low? Why don't we just pack it in and go home? It'd be so, so much easier.

It's because, in the end, there's no glory in easy.

No one remembers easy. They remember the blood and the bones and the long, agonizing fight to the top. And that is how you become... Legendary".

— *Amelia Shepherd*

"اللّٰهُمَّ انْفَعْنَا بِمَا عَلَّمْتَنَا، وَعَلِّمْنَا مَا يَنْفَعُنَا، وَزِدْنَا عِلْمًا إِلَى عِلْمِنَا"