PHYSIOLOGY OFGRED LOBULE

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The function of red blood cells is to transport oxygen from the lungs to the tissues and to transport part of the carbon dioxide from the tissues to the lungs.

1. ERYTHROPOIESIS(see chapter "Hematopoiesis")

Erythropoiesis is the medullary process leading from the hematopoietic stem cell to erythroid progenitors, Burst Forming Unit-erythroid (BFU-e) and Colony Forming Unit-erythroid (CFU-e), then to erythroblastic precursors (Proerythroblasts, erythroblasts (Eb) basophils, polychromatophilic erythroblasts, acidophilic erythroblates), and finally after enucleation of the latter to reticulocytes (immature RBCs) which will mature 24 to 48 hours before giving rise to RBCs. 4 cell divisions and perfect synchrony of nucleocytoplasmic maturation are necessary before arriving from CFU-e to acidophilic Eb. From the acidophilic Eb, there is no longer any dichotomous division. Terminal erythroid differentiation from CFU-e to acidophilic Eb lasts 5 to 6 days and complete terminal erythroid differentiation to RBC, 8 days.

The regulation of erythropoiesis uses proliferation cytokines with synergistic and multipotent effects (Stem Cell Factor (SCF), GM-CSF, IL-3, IL-6) and a lineage-specific differentiation cytokine, erythropoietin (EPO). This regulates terminal erythroid proliferation or late erythropoiesis. It is synthesized by the peritubular interstitial cells of the kidney. The main stimulus is hypoxia (Decrease in PO_2). EPO stimulates erythroid proliferation, inhibits apoptosis of erythroblastic precursors, accelerates the rate of hemoglobin (Hb) synthesis and the exit of reticulocytes from the bone marrow and thus ultimately increases the RBC pool. EPO and SCF act synergistically to inhibit apoptosis and the Fas/Fas Ligand system depending on the level of EPO is also involved in regulating the level of apoptosis of erythroblastic precursors. Finally, there must be a stable balance between the different players in erythropoiesis: both bone marrow (stroma and cytokines) and exogenous vitamin factors (B9, B12), iron (see specific courses) and different hormones such as thyroid hormones, IGF-1, androgens for a daily production of $2x10_{11}$GR/J.

2. STRUCTURE OF THE RED BLOOD CELL

The red blood cell (red blood cell, erythrocyte) is an anucleate cell in the shape of a biconcave disc (7 microns in diameter) and has a lifespan of 120 J (Figure 1). 2 $x10_{11}$red blood cells are produced every day. The red blood cell is a very deformable cell, because it has an excess of surface area compared to its volume, which allows it to cross, at the level of the microcirculation, capillaries with a diameter much smaller than its own, up to 3 microns (spleen capillary).

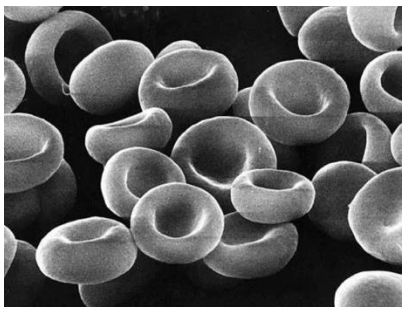

Figure 1: red blood cells in electron microscopy

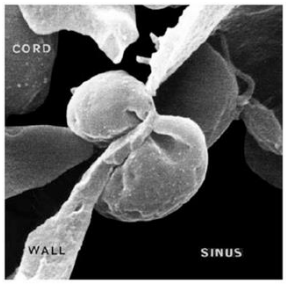

Figure 2: Cords of Bilroth

The red blood cell is made up of a membrane and contents.

2.1. Red blood cell membrane

The survival of the red blood cell is conditioned by its physical capacities: its ability to deform, its elasticity and its resistance. The red blood cell membrane (Figure 3) has an outer layer, or

lipid bilayer, formed by phospholipids, sphingolipids and cholesterol. This outer layer rests on a protein framework (cytoskeleton), itself constituted by a "horizontal" mesh anchored to the lipid bilayer by so-called "integral" proteins, anchored to the membrane, which cross the lipid bilayer and ensure exchanges between the red blood cell and the external environment.
Four main proteins form the framework mesh: spectrin, actin, band 4.1 protein and ankyrin.
Membrane proteins are divided into two complexes: complex 4.1 (horizontal connections) and Ankyrin complex (vertical connections).
Constitutional abnormalities of membrane proteins can lead to hemolytic anemia (example in pathology: hereditary spherocytosis secondary to alterations in vertical connections due to mutations in one of the proteins of the ankyrin complex).

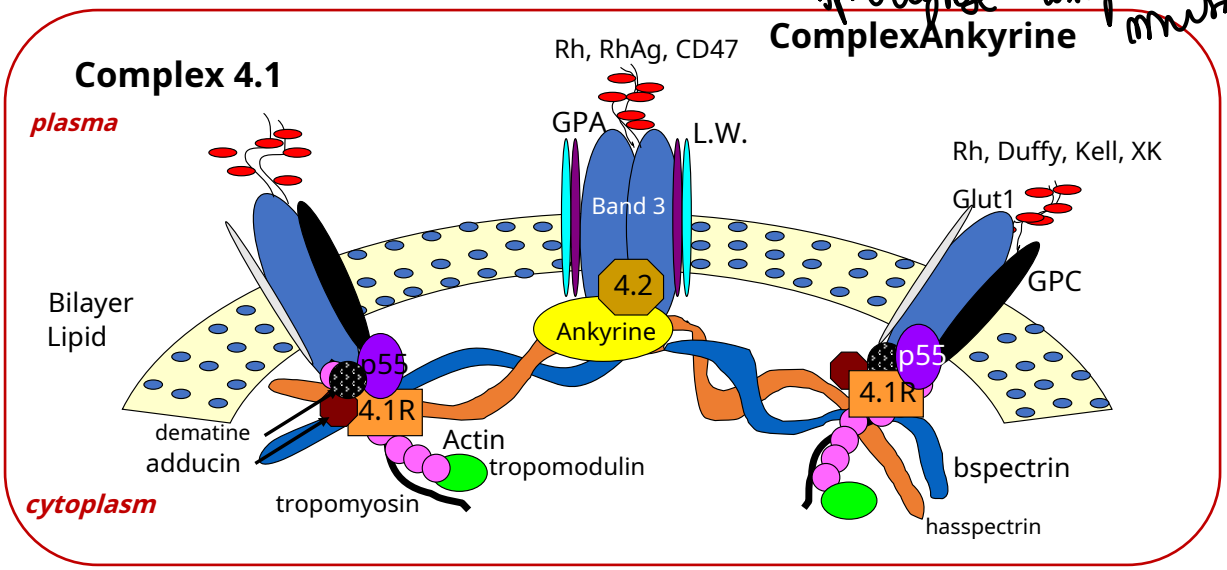

Figure 3: diagram of the red blood cell membrane

2.2. Red blood cell content

The cytoplasm of the red blood cell contains:

- some water,
- ions, in particular sodium and potassium,
- glucose,
- an enzymatic system, ensuring the metabolism of the red blood cell,
- hemoglobin, which alone represents 1/3 of the weight of the red blood cell (300x10$_6$mol Hb/GR).

2.2.1. Enzymatic system and red blood cell metabolism

The red blood cell does not contain a Krebs cycle, the only source of energy or ATP is that of anaerobic glycolysis. To ensure its function and survival, the red blood cell has an enzymatic system allowing it to constantly fight against hyperhydration and the oxidation of its essential constituents, iron and globin:

- the fight against hyperhydration is done by **system of "sodium-dependent ATP pumps"** constantly chasing sodium out of the cell.
- the fight against iron oxidation is done thanks to **methemoglobin reductases**:
  - main, whose coenzyme is NADH2
  - accessory, whose coenzyme is NADPH2

These methemoglobin reductases transform methemoglobin which contains iron in the oxidized ferric state, incapable of fixing oxygen, into hemoglobin containing iron in the reduced ferrous state, the only one capable of transporting oxygen.

- the fight against the oxidation of globin is done by **glutathione peroxidase** which ensures the elimination peroxides such as hydrogen peroxide (H2O2).

ATP, NADH2 and NADPH2 are produced in the red blood cell by the main pathway (triose pathway) and the accessory pathway, or pentose pathway, of intra-erythrocyte glycolysis (Figure 4). The red blood cell,

having no nucleus, is incapable of synthesizing new proteins and must therefore live with the enzymatic stock which was its own at birth.

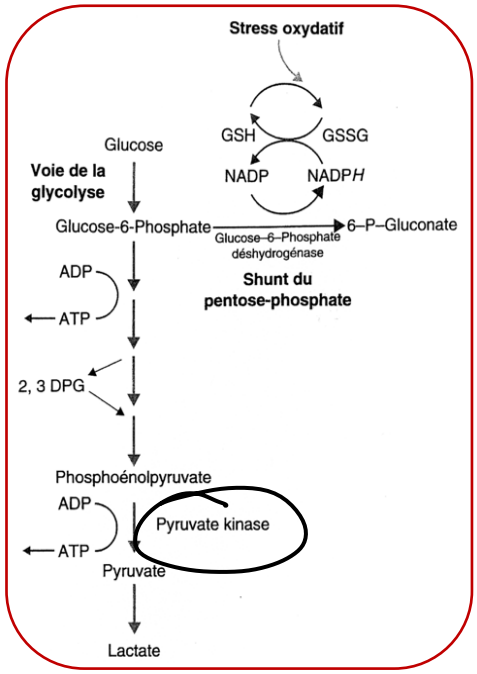

Figure 4: Intra-erythrocyte metabolism

We see in Figure 4 the consequences that a G6PD deficiency will have, for example, leading to a failure of the globin anti-oxidative system and the precipitation of oxidized globin in the form of Heinz bodies.

2.2.2. Hemoglobin

Hemoglobin is a respiratory pigment that reversibly binds oxygen. It is the essential constituent of the red blood cell. It performs its function which is the transport of oxygen from the lungs to the tissues.

Genetics of globin synthesis

The - genes are located on chromosome 16 and the non - genes on chromosome 11 (Figure 5). Family -contains three genes: the embryonic gene - and 2 genes -. The non-family has five: the gene embryonic -, 2 fetal genes -G and -A, and 2 adult genes - and -.

Hemoglobin synthesis

- the synthesis of protoporphyrin IX takes place in erythroblasts. Heme is formed after fixation of an iron atom on protoporphyrin IX.

- the synthesis of globin is stimulated by heme, hence a synchronization of synthesis of the parts heme and globin of hemoglobin.

Structure of hemoglobin

A hemoglobin molecule contains four heme molecules and four globin chains.

- **heme**is composed (Figure 6):
  - a protoporphyrin IX, formed of 4 pyrrol nuclei and 8 side chains, methyl, vinyl or propionic acid.
  - an iron atom, in the center of the protoporphyrin, capable of fixing an oxygen molecule.

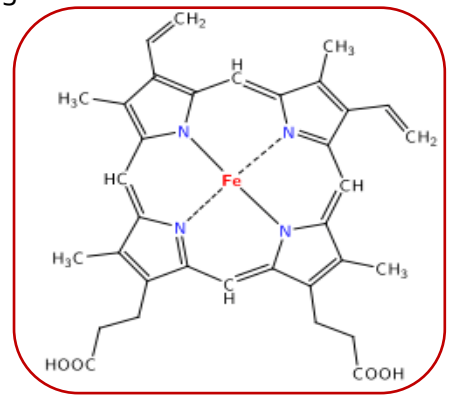

Figure 6: Heme

-**globin**:each hemoglobin molecule consists of 4 globin protein chains two by two identical (Figure 7):
- 2 chains - and 2 chains - for adult hemoglobin A (HbA) α β
- 2 chains - and 2 chains - for fetal hemoglobin F (HbF) α γ
- 2 chains - and 2 chains - for hemoglobin A2 (HbA2) α ε

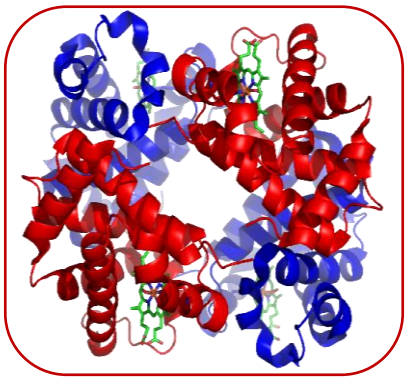

Figure 7: Representation of the 4 subunits of a hemoglobin A molecule

Each heme-globin set is a subunit: 1 hemoglobin molecule therefore has 4 subunits.

The quaternary structure provides a central pocket in the center of the hemoglobin molecule, where 2,3 DPG is located. There is competition for binding at the hemoglobin level between oxygen and 2-3 DPG. It plays a role in the affinity of hemoglobin for oxygen and in the immediate response mechanism to anemia by decreasing the affinity of Hb for oxygen (Figure 8).

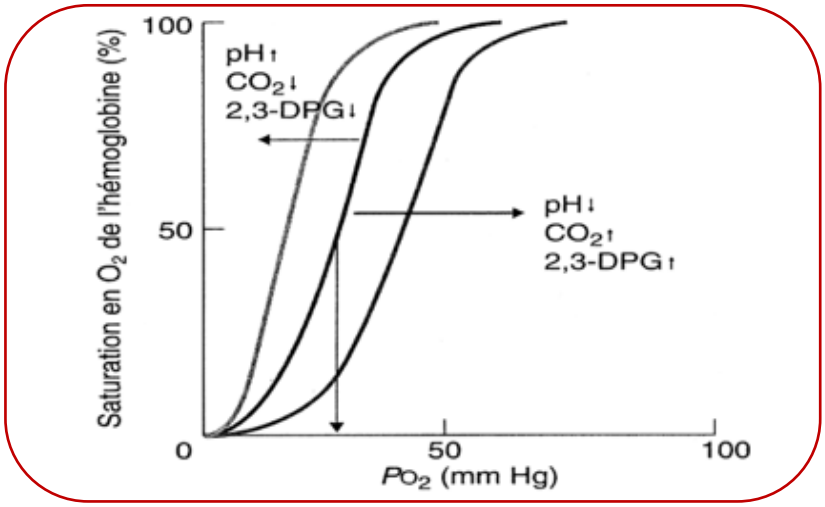

Figure 8: Hemoglobin saturation as a function of oxygen partial pressure

Functions of hemoglobin

The main one is its capacity to fix 4 molecules of oxygen per molecule of hemoglobin: the fixing of one molecule of oxygen leads to a modification of the structure of hemoglobin (Figure 8). At P02 of lungs, where it captures atmospheric oxygen, hemoglobin is completely saturated with oxygen while it is largely desaturated in the tissues, where it releases its oxygen. Finally, hemoglobin F has a greater affinity for oxygen than hemoglobin A.

Ontogenetic evolution of human hemoglobins

Different forms of hemoglobin occur one after the other throughout life. They are distinguished by the globin chains constituting them:
- the chain - is produced throughout gestation and postnatal life,
- the non - string is:
    - essentially the chain - in the fetus,
    - essentially the chain - in adults,
- the chain - is produced in small quantities at all stages of development.

The study of hemoglobin is done using hemoglobin electrophoresis which makes it possible to highlight certain abnormal hemoglobins such as HbS in sickle cell anemia.

3. PHYSIOLOGICAL AND PATHOLOGICAL HEMOLYSIS

Physiological hemolysis (intratissular hemolysis) occurs after 120 days for aging RBCs by bone marrow and liver macrophages.. It results in the release of globin, iron, CO, stercobilinogen and urobilinogen, resulting from the metabolism of bilirubin itself resulting from the catabolism of heme.

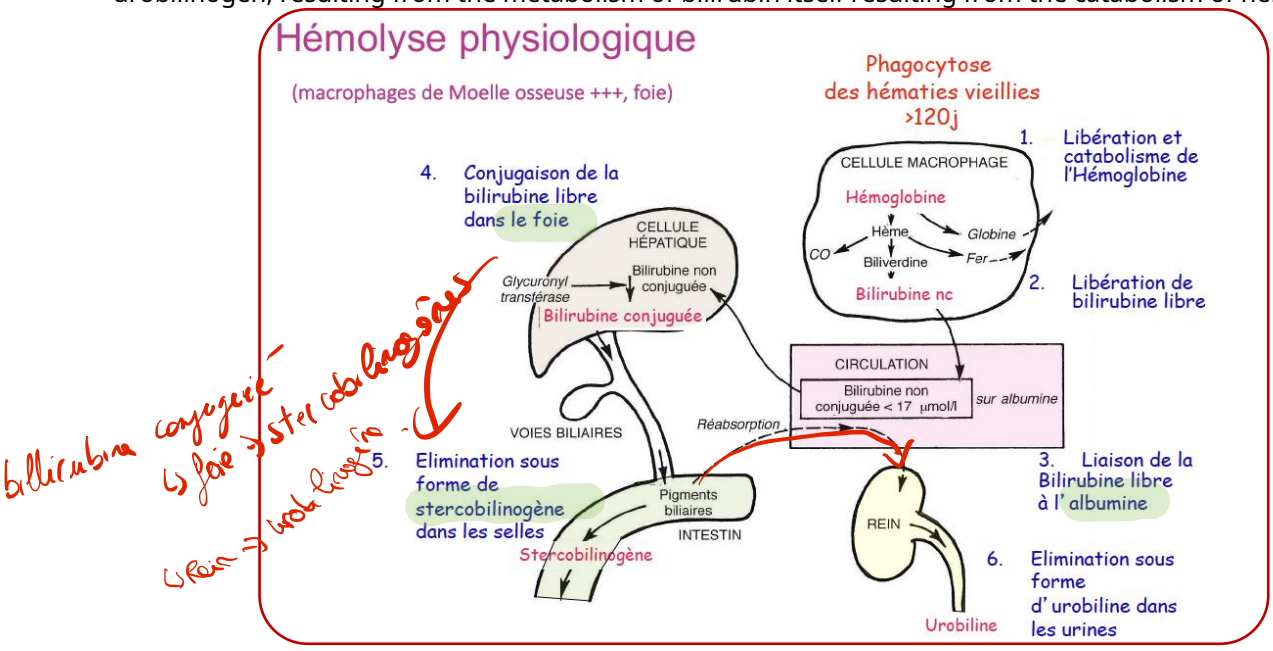

Figure 9: Physiological hemolysis

Pathological hemolysis occurs mainly in the macrophages of the spleen, bone marrow and liver. Intravascular rupture of RBCs will lead to intravascular hemolysis which can be extremely serious and life-threatening. Cell lysis of RBCs results in the release of erythrocyte enzymes including LacticoDesHydrogenase (LDH). Globin dimers bind the inflammatory protein haptoglobin synthesized by the liver, hence the fall in haptoglobin classically observed in adults in cases of pathological hemolysis and the elimination of globin in the urine (hemoglobinuria) which must be distinguished from hematuria, which is the passage of blood in the urine. The catabolism of GR in spleen macrophages then follows the same sequence as during physiological hemolysis.

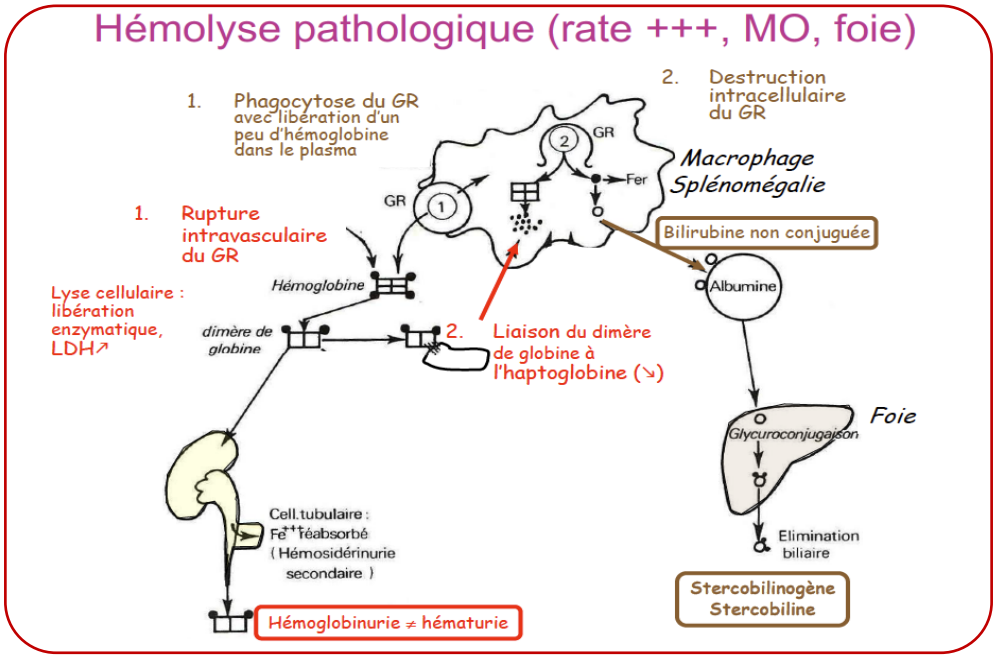

Figure 10: Pathological hemolysis

4. PATHOLOGICAL SITUATION: ANEMIA

4.1. Definition and clinical signs:

Anemia is defined by a decrease in hemoglobin (Hb) concentration.(<13 g/dL in men, <12g/dL in women, <10.5 g/L in pregnant women, <14g/L in newborns (first month of life)) **and not by the reduction in the number of red blood cells.**Adaptive mechanisms, initially intraerythrocytic, are put in place (increase in 2.3 DPG to reduce the affinity of Hb for O_2) and then extra-erythrocytic (vasoconstriction of non-noble organs (vessels of the skin and mesentery) and increase in cardiac output. The effects of stimulation of erythropoiesis take longer to achieve (3 days).

Clinical signs of anemia **at first** are there **classical triad**: **mucocutaneous pallor, asthenia, exertional dyspnea** possibly associated with palpitations indicative of tachycardia with anorganic systolic murmur (increased cardiac output). **If anemia sets in** and does not correct itself, the **asthenia increases**, there **dyspnea** manifests **from rest and** of the **signs of cerebral anoxia** appear (headaches, dizziness, tinnitus, myodesopsia and at most coma and death).

4.2 Characterization of anemia:

Anemia, if initially clinical, must be verified and confirmed in order to assess its depth and therefore severity by a study of the **erythrocyte and reticulocyte indices** (Blood count with absolute reticulocyte count and examination of the blood smear under the microscope after staining with May-Grunwald-Giemsa (see chapter "Hemogram").

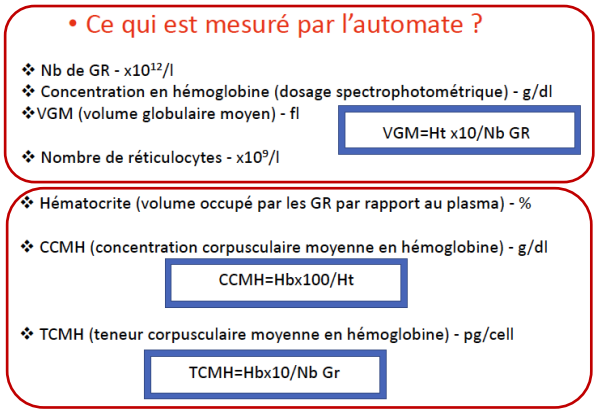

Figure 11: Red blood cell indices and reticulocyte counts

Once identified after elimination of false anemia (hemodilution (pregnancy from 2thtrimester, hyperhydration), hypersplenism, monoclonal immunoglobulins), and assessment of the depth of anemia, it is appropriate to analyze the **Mean Globular Volume (MCV)** and the **Mean Corpuscular Hemoglobulin Concentration (MCHC)** and reticulocyte count, as well as other cell lines (white blood cell and platelet counts).

Accordingly, anemia will be said to be isolated if only Hb is reduced or associated with another cytopenia or other abnormalities of platelets and/or white blood cells.

A reticulocyte count >120x10⁹/L defines regenerative anemia while a count <120x10⁹/L defines aregenerative anemia.

The following table describes the different possibilities for describing anemia according to these indices. This analysis is crucial because it will:

- **to guide the mechanism** occurrence of anemia (peripheral origin if >120x10⁹/L or central to the reverse)
- **to guide the etiology** or diagnosis and additional examinations to consider to confirm the diagnostic hypotheses.

VGM	**< 80 fL = microcytosis**	**> 100 fL = macrocytosis**
CCMH	**< 32 g/dL = hypochromia**	**> 36 g/dL = hyperchromia**
Reticulocytes	**< 120 G/L = regeneration**	**> 120 G/L = regeneration**