Hematology

PHYSIOLOGY

☑ Sheet ☐ Slide ☐ Handout

Number: **3**

Subject: **Regulators of Erythropoiesis**

Done By: **Reem Akiely**

Corrected by: **Asma' Al-Kilani**

Doctor: **Saleem khraisha**

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Price:

**** This sheet was written according to the recording that belongs to section 1. Please pay attention that <u>the order of ideas in this sheet is a little bit different from that in the recording</u>.**

❖ Before we start,

It is important to always remember the regulators of Erythropoiesis:

(1). **Oxygen supply** (Hypoxia or Hyperoxia) – this factor was discussed in the previous lecture.

> <u>A quick reminder:</u>
>
> During hypoxia, erythropoiesis increases, whereas during hyperoxia, erythropoiesis decreases.

(2). **Vitamins** (B12, folate,...etc.), and Diet in general. *Vit.B12 and folic acid are the most important.*

a. <u>Vitamin B12</u> – the consequences of its deficiency were discussed in the previous lecture.

> <u>Another quick reminder:</u>
>
> - Absorption of Vitamin B12 occurs at the level of the **<u>lower ileum</u>**, most probably because there is no or little amount of bacteria.
> - Deficiency of vitamin B12 causes "**pernicious anemia**" or "megaloblastic anemia" characterized by :
>   - <u>Large oval</u> cells, with high amount of hemoglobin relatively, and short half life.
>   - <u>Reduced number</u> of cells (depending on the degree of vitamin B12 deficiency).
> - Deficiency of vitamin B12 also causes **neutropenia** (decreased number of neutrophils and sometimes platelets).

b. <u>Folic Acid</u> (or folate) – discussed in this sheet.

(3). **Iron** – discussed in this sheet

(4). **Proteins** – discussed in this sheet

(5). **Trace elements** (copper, cobalt).
(6). **Healthy bone marrow**. Bone marrow is the site of erythropoiesis.
(7). **Liver**. It's a multifunctional organ (storage, protein synthesis, hormone synthesis).
(8). **Hormones** (erythropoietin, androgens, thyroid hormones, corticosteroids).

❖ Topics of this lecture:

*Folic acid and the effects of its deficiency.

*The definite effects of vitamin B12 or folate deficiency.

*Iron and the effects of its deficiency.

*Hemoglobin; its structure, synthesis, and types.

❖ Folic Acid and The Effects of Its Deficiency

I. About folic acid (folate)

- Folic acid is the second vitamin essential for the maturation of RBCs.
- The role of folic acid in erythropoiesis is similar to that of vitamin B12.
- Folic acid has no role in the CNS → its deficiency does not affect the CNS.
- There are animal sources of folic acid and plant sources (from vegetables).
- Folic acid is absorbed through the **jejunum** because of the presence of the enzymes that are necessary for the absorption of folic acid.

II. Consequences of folic acid deficiency

- Deficiency of folic acid can cause anemia called "megaloblastic anemia".
  ➔ Megaloblastic anemia can be caused by either vitamin B12 deficiency or by folic acid deficiency.

  Note: Anemia due to deficiency of vitamin B12 is called megaloblastic anemia or pernicious anemia. On the other hand, anemia due to deficiency of folic acid can only be called megaloblastic anemia, meaning that the term "pernicious anemia" refers specifically to anemia caused by vitamin B12 deficiency.

- Cells produced by the deficiency of folic acid are similar to those produced due to deficiency of vitamin B12.
  ➔ Sometimes, we cannot distinguish between them unless we do further tests to figure out if these cells were produced due to deficiency of vitamin B12 or deficiency of folic acid.

III. Causes of folic acid/ folate deficiency

1. Inadequate dietary intake

2. Malabsorption. It can occur due to:
   - Celiac disease
   - Jejunal resection
   - Tropical sprue – acute or chronic diarrhea causes time to be insufficient for folic acid absorption.

3. Increased requirement, as in
   - Pregnancy
   - Premature infants
   - Chronic hemolytic anemias – when there is increased lysis of RBCs, there will be no sufficient amount of folic acid to compensate the destruction of RBCs.

Note: deficiency of folic acid does not affect neurons while deficiency of vitamin B12 does. *(This is because vitamin B12 is needed for the normal function of myelin sheath in the nervous system, whereas folic acid does not affect myelination).*

Note: individuals who only eat vegetables lose weight easier than those who also eat meat. In addition, they are almost always not obese, and it's very rare to find obese individuals among them.

Additional pieces of information

(not mentioned by Dr. Saleem)

- **Celiac disease**: a disease in which the small intestine is hypersensitive to gluten, leading to difficulty in digesting food.
- **Jejunal resection** means the surgical removal of the jejunum that is necessary in certain conditions.
- **Tropical sprue** is a syndrome characterized by acute or chronic diarrhea, weight loss, and malabsorption of nutrients.

❖ The definite effects of vitamin B12 or folate deficiency

(note: the title says "OR" not "AND" because the presence of both deficiencies together is extremely unlikely to occur).

1. Megaloblastic anemia *(high MCV, sometimes reaching 150)*
2. Macrocytosis of epithelial cell surfaces
3. Neuropathy (for vitamin B12 only)
4. Sterility (severe anemia may cause sterility)
5. Rarely, reversible melanin skin pigmentation (usually on the face)

❖ Iron and the effects of its deficiency

I. About iron:

- The normal amount of iron present in the body is 4-5**g**.
- Iron is needed for the synthesis of hemoglobin, myoglobin, and certain enzymes like chytochromes (e.g. cytochrome oxidases).
- Iron requirement for the individual is very little, but the daily intake of iron ranges from 15-20 **mg**. The human body benefits only from 4% of the daily intake (that is about 0.6 **mg**).
- In nature, iron is found in ferrous (Fe^{+2}) and ferric (Fe^{+3}) states. The body of a mammal can only utilize (benefit from) the ferrous iron.

II. Iron absorption

Occurs through all parts of the intestines, but the **highest level of absorption is in the upper part (the duodenum),** then jejunum and then decreases gradually. We can say that absorption of iron mainly occurs in the duodenum and jejunum, very little absorption occurs in the ileum, and extremely little amount is absorbed through the colon.

Note: no absorption takes place in the stomach, except for water, alcohol, and aspirin.

Note: although the highest degree of absorption of iron is in the duodenum, **the jejunum is the part that mainly supplies the body with iron** (the jejunum is the part that provides almost all the iron to the body). This is due to the fact that the duodenum is very short compared to the jejunum.

Note 2: for the purpose of experiments, samples are taken from the duodenum because cells of the duodenum are very active.

Look at the figure in the next page and read the following points to understand the steps of iron absorption:

- Iron is ingested either as ferric iron (from vegetables)
  Or as heme containing ferrous iron (from animal sources)
- The acidity of the stomach converts ferric iron (Fe^{+3}) into ferrous iron (Fe^{+2}).

- Then,
  (a) **The heme** as a whole is taken up by the epithelial cells of the intestines. Inside the epithelial cell, certain enzymes will split the heme to separate ferrous (Fe^{+2}) from the protoporphyrin (the remaining structure of the heme). Through Fe^{+2} carriers, ferrous will be transported to the plasma.

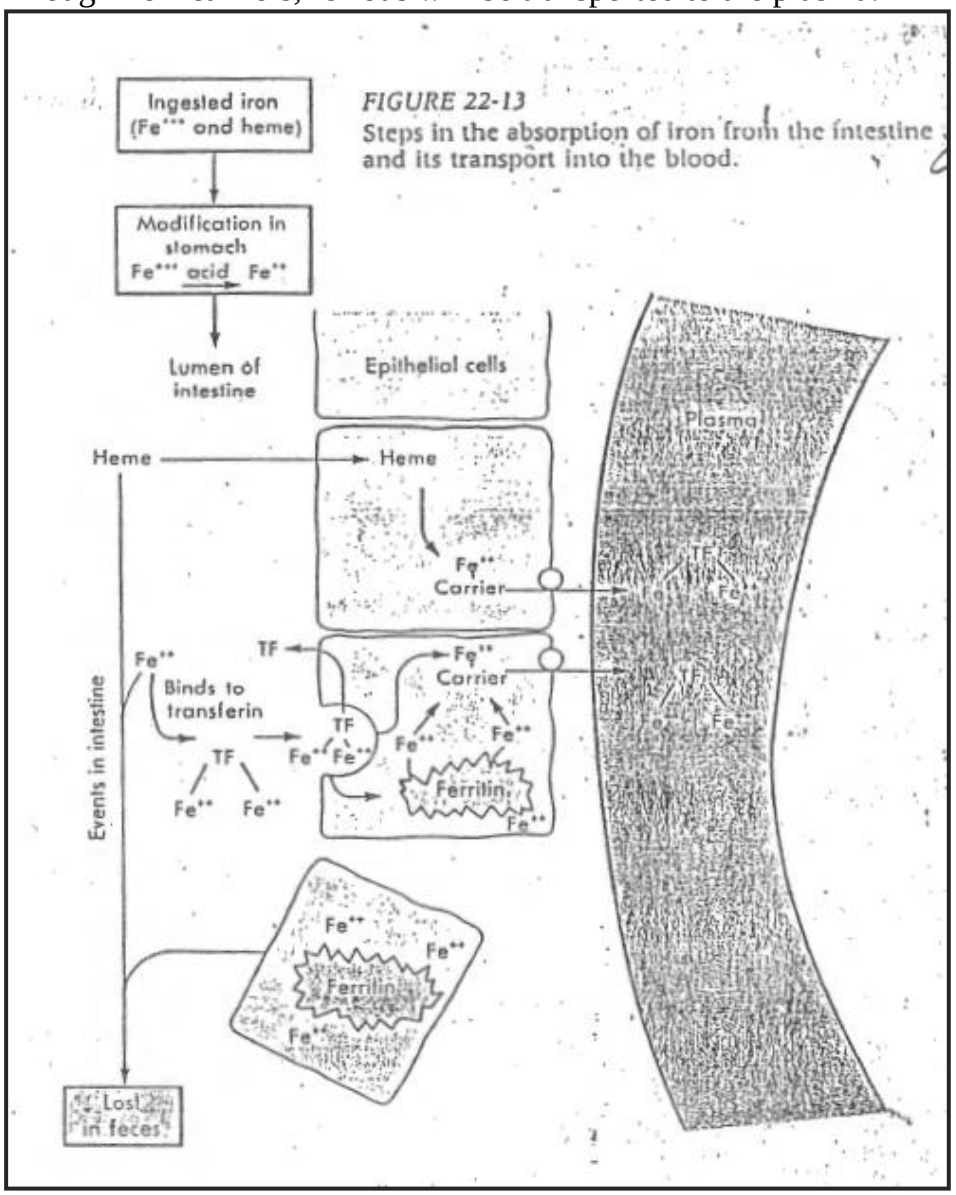


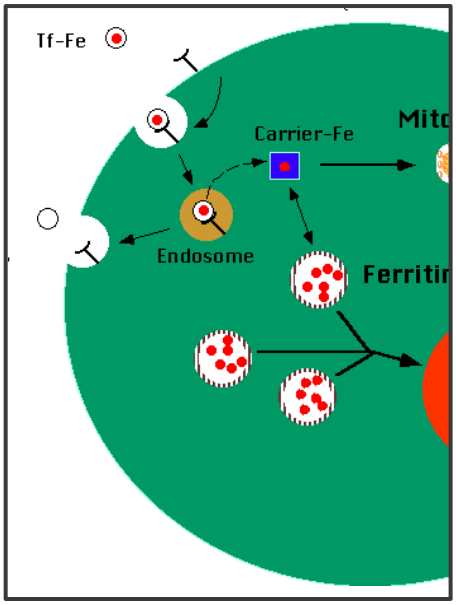
FIGURE 22-13
Steps in the absorption of iron from the intestine and its transport into the blood.

  (b) **Free ferrous** in the lumen binds to <u>transferrin</u> "TF" (a transferrin molecule binds to two Fe^{+2} molecules). After that, transferrin - bound to Fe^{+2}- will adsorb (adhere) to the epithelial cells of the intestine, and then will pass to the epithelial cells where ferrous will bind to <u>ferritin</u>.
  Again, through Fe^{+2} carriers, ferrous will be transported to the plasma.

According to Guyton and Hall Textbook, chapter 32 in the twelfth edition:

(the following two points are extra pieces of information to provide better understanding of the previous point)

- The iron is loosely bound in the transferrin and, consequently, can be released to any tissue cell at any point in the body.
- Transferrin (combined with Fe^{+2}) is attracted to and binds with receptors in the membranes of the intestinal epithelial cells. Then, by pinocytosis, the transferrin molecule, carrying its iron store, is absorbed into the epithelial cells.



- In plasma, ferrous iron binds to transferrin, and from there, it passes then to the bone marrow to participate in hemoglobin synthesis and erythropoiesis. Excess iron will be transported to the liver to be stored.

In the lumen of the intestines and in plasma → Fe^{+2} is bound to **transferrin**.

In the epithelial cells of the intestines → Fe^{+2} is bound to **ferritin**.

- The half life of epithelial cells of the intestine is about few days (Dr. Saleem said maybe 3-4 days, but he wasn't sure), meaning that they will be sloughed *(sloughing means shedding of dead tissue)* and new cells will form (renewal).
  Iron in the dead epithelial cells will either be excreted with the sloughed cells in stool, or will be transferred to the blood and then to the bone marrow.

III. Iron requirements

- As mentioned earlier, the daily intake of iron is 15-20 mg. The body benefits from only 4% of these 15-20 milligrams (about 0.6 mg).
- Iron requirement must be equal to the loss.
  The amount of iron required each day to compensate for losses from the body and growth varies with age and sex; it is highest in pregnancy and in adolescent and menstruating females. These groups, therefore, are particularly likely to develop iron deficiency if there is additional iron loss or prolonged reduced intake.

Table 2.3 Estimated daily iron requirements. Units are mg/day.

	Urine, sweat, faeces	Menses	Pregnancy	Growth	Total
Adult male Post-menopausal female	0.5–1				0.5–1
Menstruating female*	0.5–1	0.5–1			1–2
Pregnant female*	0.5–1		1–2		1.5–3.0
Children (average)	0.5			0.6	1
Female (age 12–15)*	0.5–1	0.5–1		0.6	1–2.5

*These groups more likely to develop iron deficiency.

- Notes concerning the previous table:
  - In an **adult male** or **post menopausal female**, the total requirement of iron ranges from 0.5 to 1 mg/day (we can say, about 0.6 mg as mentioned earlier), because the amount of iron lost through urine, sweat, and faeces ranges from 0.5 to 1 mg/day.
  - In a **menstruating female**, an additional 0.5-1 mg/day is lost due to menses, that's why the total requirement is 1-2 mg/day.
  - In a **pregnant female**, pregnancy consumes 1-2 mg/day, that's why the total requirement is 1.5-3 mg/day.
  - In **children**, the total requirement of iron is about 1 mg/day, to compensate the losses from the body (0.5) and growth (0.6).
  - In a **female aged between 12 and 15** (teenager), the total requirement is 1- 2.5 mg/day, to compensate the losses from the body through urine, sweat, faeces (0.5-1), and menses (0.5-1), in addition to growth (0.6).
  - Menstruating females, pregnant females, and females aged between 12 and 15 constitute the three groups that are more likely to develop iron deficiency because they need, relatively, higher intake of iron compared to other groups.
- Conclusion: iron requirements range from 0.6 to 3.0 mg/day depending on the age and sex.

IV. Distribution of iron in the body

Look at table 2.1 and pay attention to the following points:

- Note the difference between males and females. This is normal – as mentioned earlier in the lectures- because of the difference in the number of RBCs.
- Dr. Saleem focused on the last column (% of total) and on the fact that most of the body iron is found in hemoglobin.

Table 2.1 The distribution of body iron.

	Amount of iron in average adult		
	Male (g)	Female (g)	% of total
Haemoglobin	2.4	1.7	65
Ferritin and haemosiderin	1.0 (0.3–1.5)	0.3 (0–1.0)	30
Myoglobin	0.15	0.12	3.5
Haem enzymes (e.g. cytochromes, catalase, peroxidases, flavoproteins)	0.02	0.015	0.5
Transferrin-bound iron	0.004	0.003	0.1

V. Factors favouring and factors reducing iron absorption

Look at table 2.2 and pay attention to the following points:

- Note that the first six factors favouring iron absorption are opposite to the first six factors reducing iron absorption. *(easier for memorization).*
- Phytates are present in cereals -especially bread-. That's why consuming bread and tea in high amounts makes it more likely for the individual to develop iron deficiency anemia. This is seen in poor groups of people more often that in richer groups (because poor people are highly dependent on tea and bread).

Table 2.2 Iron absorption.

Factors favouring	Factors reducing
1 Ferrous form	1 Ferric form
2 Inorganic iron	2 Organic iron
3 Acids—HCl, vitamin C	3 Alkalis—antacids, pancreatic secretions
4 Solubilising agents—e.g. sugars, amino acids	4 Precipitating agents—phytates, phosphates
5 Iron deficiency	5 Iron excess
6 Increased erythropoiesis	6 Decreased erythropoiesis
7 Pregnancy	7 Infection
8 Primary haemachromatosis	8 Tea
	9 Desferrioxamine

VI. Causes of iron deficiency

Dr. Saleem read all the information in table 2.4, but in the examples on blood loss through the gastrointestinal tract, he only focused on: peptic ulcer, aspirin ingestion, and piles.

Table 2.4 Causes of iron deficiency.

1 BLOOD LOSS
Uterine.
Gastrointestinal. e.g. oesophageal varices, hiatus hernia, peptic ulcer, aspirin ingestion, partial gastrectomy, carcinoma of stomach or caecum, colon or rectum, hookworm, angiodysplasia, colitis, piles, diverticulosis, etc.
Rarely haematuria, haemoglobinuria, pulmonary haemosiderosis, self-inflicted blood loss.

2 INCREASED DEMANDS (see also Table 2.3)
Prematurity.
Growth.
Child-bearing.

3 MALABSORPTION
e.g. gastrectomy, coeliac disease.

4 POOR DIET
A contributory factor in many countries but rarely the sole cause.

VII. Consequences of iron deficiency

- Iron deficiency causes iron deficiency anemia (IDA).
- In iron deficiency anemia, the cells are small (microcytic), and their content of hemoglobin is low.
  ➔ These cells are called **hypochromic microcytic cells**.
- Note: similar to these cells are the cells produced due to deficiency or problem in Protoporphyrin *(protoporphyrin is the substance that carries iron in the heme)*, and the disease is called sideroblastic anemia.
- To make things more clear:
  - Hypochromic microcytic anemia is a type of anemia in which the RBCs are small (microcytic), and contain low amounts of hemoglobin (hypochromic).
  - **Causes of hypochromic microcytic anemia** *(see the figure in the next page):*
    (1). Iron deficiency (lack of iron), or lack of iron release from macrophages to serum (anemia of chronic inflammation or malignancy).
    (2). Failure of protoporphyrin synthesis (sideroblastic anemia).
    (3). Failure of globin synthesis (α or β thalassaemia)

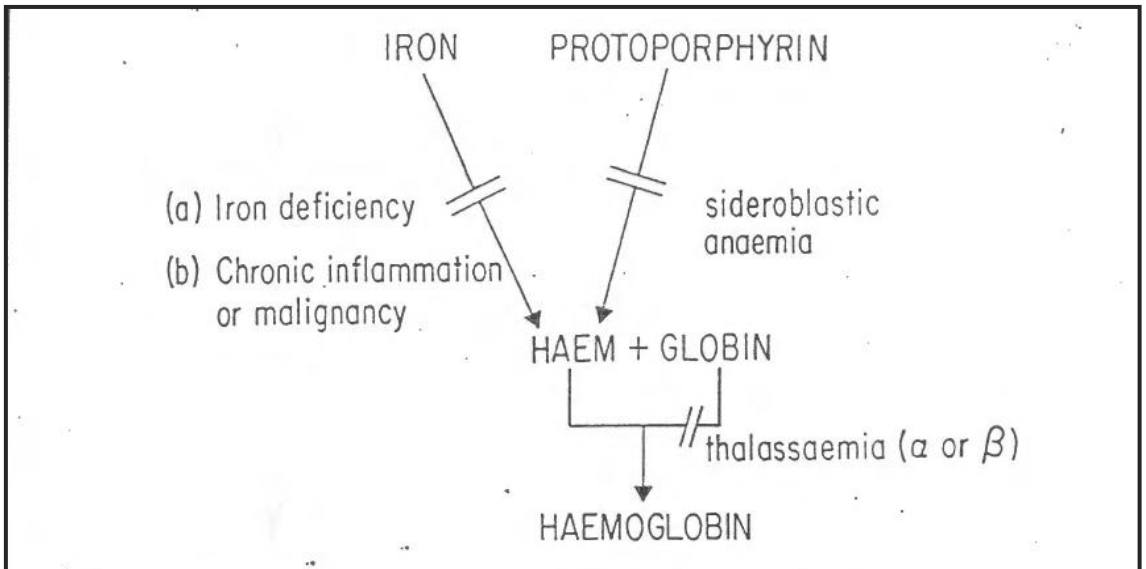


Fig. 2.1 The causes of a hypochromic microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic inflammation or malignancy), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (α or β-thalassaemia). Lead also inhibits haem and globin synthesis.

VIII. Few notes *(the doctor read them from a slide he had)*

- Iron deficiency is estimated to affect about 30% of the world population.
- Iron deficiency anemia is still the most important deficiency related to malnutrition.
- Iron deficiency anemia (IDA) and thalassaemia (TT) are the most common forms of microcytic anemia.
- Some discrimination indices calculated from RBCs indices -*Dr. Saleem said that he will explain this later*- are defined and used for rapid discrimination between TT and IDA.
- IDA is a common clinical problem throughout the world and an enormous public health risk in developing and even in industrialized countries.
- Traditionally, several methods other than serum ferritin were used to assess IDA.

❖ Hemoglobin; Its Structure, Synthesis, and Types

I. About Hemoglobin,

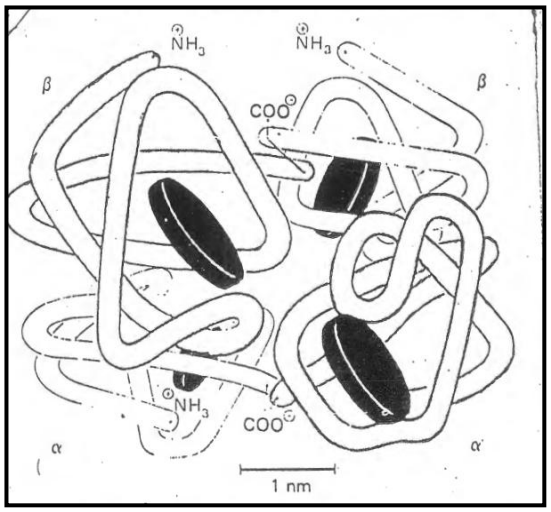
- When we mentioned the factors regulating erythropoiesis, the fourth factor was "proteins".
- 96% of the hemoglobin molecule is protein (we can say that hemoglobin is a protein).
- Substances that hemoglobin in RBCs can carry:
  oxygen, CO^2, hydrogen ions, 2,3-DPG (2,3- Bisphosphoglycerate)

- Hemoglobin consists of two parts:
  - Globin is the protein part (96%)→ it can bind carry CO^2, H^+, 2,3-DPG, and maybe other substances.
  - Heme is the non-protein part (4%) → only binds oxygen (O^2)
    ** ***reversible binding*****

- Hemoglobin concentration in males is 16 g/100mL blood, while its concentration in females is 14 g/100mL blood.
  (If we don't want to mention the sex, we can use the value **15** g/100mL blood).

II. The structure of hemoglobin

- A hemoglobin molecule is a tetramer consisting of 4 subunits (2 α and 2 β, in the case of adult hemoglobin, *as shown in the figure to the right*).
- Each subunit contains a heme moiety.
- Each α subunit contains 141 amino acids, while each β subunit contains 146 amino acids.



III. Hemoglobin synthesis

- 65% of hemoglobin synthesis occurs in the Erythroblast stage. The remaining (35%) occurs in the reticulocyte stage.
  No hemoglobin synthesis takes place in the mature RBCs.
- As we mentioned earlier, hemoglobin consists of
  - Globin (protein) → synthesized on the ribosomes.
  - Heme → synthesized in the mitochondria.

- The detailed steps of heme synthesis will be mentioned in biochemistry, but in brief, the steps include the following: *(see the figure below)*
  - Iron is either obtained from plasma of the blood or from the RBCs themselves.
  - Heme = protoporphyrin + iron
  - Heme synthesis that takes place in the mitochondria begins by the condensation (binding) of glycine with succinyl CoA, under the effect of delta-amino levulinic acid (delta-ALA). Vitamin B6 is a coenzyme here.
    This step is stimulated by erythropoietin and inhibited by the heme.
  - The previous step is followed by further few steps that eventually result in the formation of protoporphyrin.
  - Protoporphyrin will bind to iron.
  - Finally, 4 heme molecules unite with globin (2 α subunits and 2 β subunits) to form the adult hemoglobin molecule.

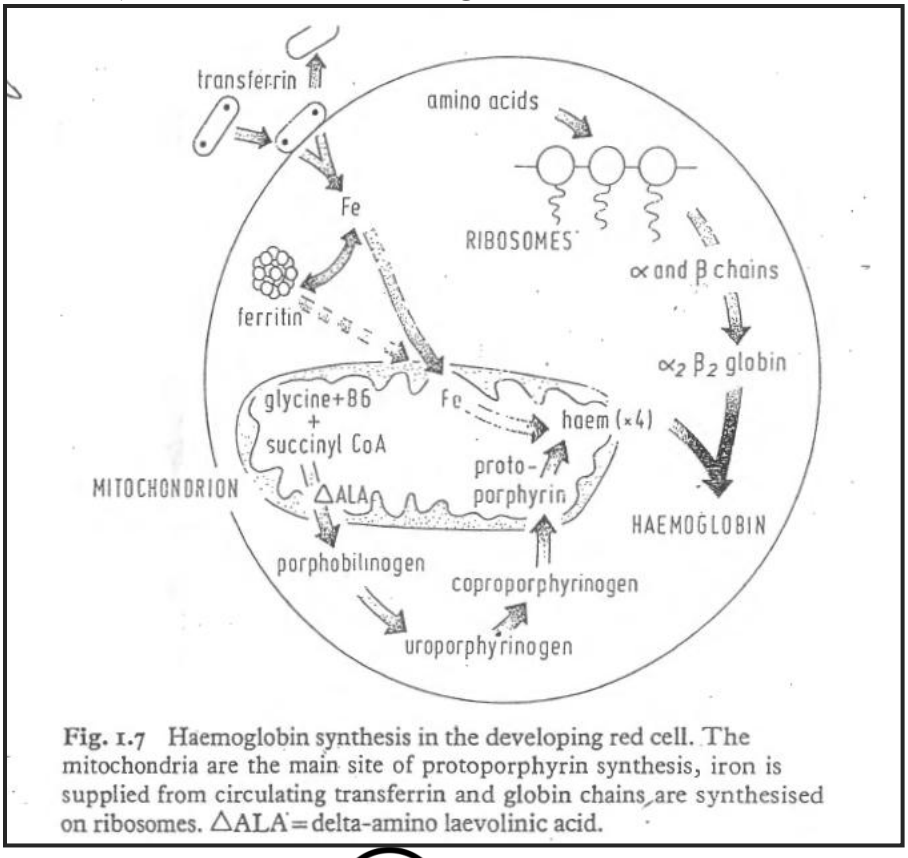


Fig. 1.7 Haemoglobin synthesis in the developing red cell. The mitochondria are the main site of protoporphyrin synthesis, iron is supplied from circulating transferrin and globin chains are synthesised on ribosomes. ΔALA = delta-amino laevolinic acid.

IV. Types of hemoglobin

- Types include adult hemoglobin -also known as adult hemoglobin A or A_1 - *(the figure in page 11, containing 2 α subunits and 2 β subunits),* fetal hemoglobin, and other types.
- Look at table 4.4,
  - There are 6 types of hemoglobin in human beings and you should know the subunits that constitute each type.
  - The last 3 types; Portland, Gower I, and Gower II, are called **embryonic hemoglobins**, and they are present only in the embryo.
    (Note that their proportions in adults and newborns equal zero)

  - In the newborns : 20 % → adult Hb
    0.5 % → Hb A_2
    80 % → fetal Hb
  - In adults: 97 % → adult Hb
    2.5 % → Hb A_2
    <1 % → fetal Hb (higher than 1% is pathological)

Table 4-4. Normal Human Hemoglobins—Genetic Variants

Name	Designation	Molecular Structure	Proportion in Adults	Proportion in Newborns
Adult hemoglobin	A	$\alpha_2\beta_2$	97%	20%
Hemoglobin A_2	A_2	$\alpha_2\delta_2$	2.5%	0.5%
Fetal hemoglobin	F	$\alpha_2\gamma_2$	<1%	80%
Portland		$\zeta_2\gamma_2$	0	0
Gower I		$\zeta_2\epsilon_2$	0	0
Gower II		$\alpha_2\epsilon_2$	0	0

- Look at the figure in the following page and note that:
  - After birth, fetal hemoglobin is gradually replaced by adult hemoglobin. (β chain replaces γ chain)
  - By the 6th month, fetal hemoglobin will be almost totally (not 100%) replaced by adult hemoglobin. The proportion of the remaining fetal hemoglobin is less than 1% as mentioned above.
  - It's important to remember that ε (epsilon) and ζ (zeta) chains are only present in the embryo as shown in the figure.

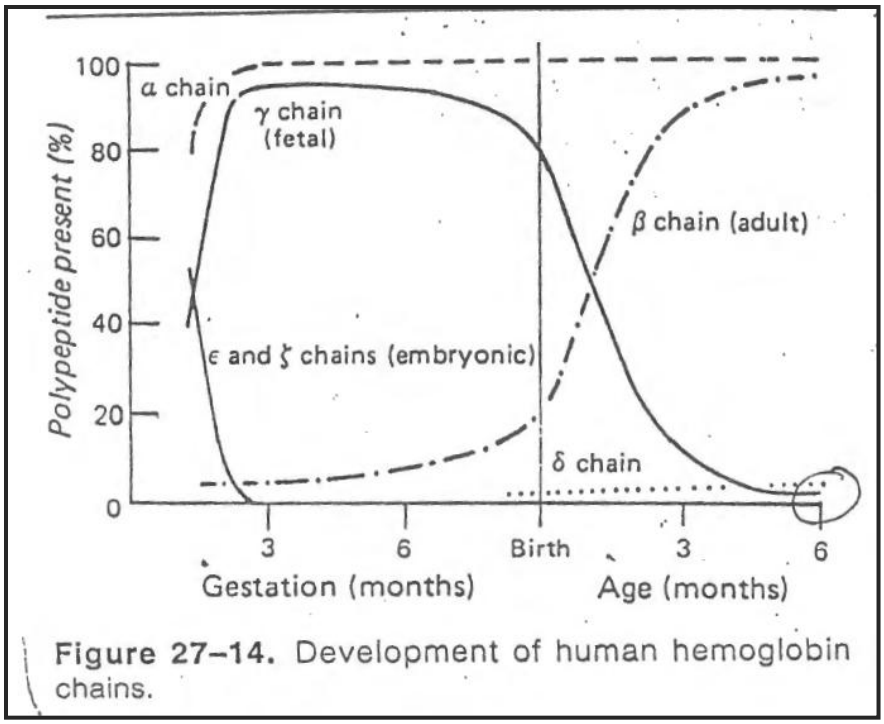


Figure 27–14. Development of human hemoglobin chains.

Final note:

Besides hemoglobin, heme is part of the structure of other proteins like:

- **<u>Myoglobin</u>** → an oxygen binding pigment found in red (slow) muscle.
- **<u>Neuroglobin</u>** *(we don't know much information about it)* →an oxygen binding globulin in the brain (it carries oxygen in the nervous system).

I apologize for any mistake I may have made.

Wish you all best of luck **:D**