HEMATOLOGY
&LYMPH SYSTEM

physiology sheet

Number	3
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Regulation of erythropoiesis 00:10

Factors that regulate erythropoiesis (the production of RBCs) and keep their number relatively constant:

1- O_2 Supply (Oxygenation) .(hypoxia =decreased oxygenation or hyperoxia=increased oxygenation)
   Tissue oxygenation is the most essential regulator of red blood cell production. During hypoxia erythropoiesis increases whereas during hyperoxia erythropoiesis decreases.
2- Vitamins (B12,folate…etc)
3- Iron
4- Proteins
5- Trace elements(copper, cobalt)
6- Red bone marrow
7- Liver
8- Hormones

In animal foods, **iron** is often attached to proteins called **heme** proteins, and referred to as **heme iron**. In plant foods, **iron** is not attached to **heme** proteins and is classified as **non-heme iron**.

❒ **Iron**

- Iron is needed for the synthesis of the hemoglobin, myoglobin, cytochromes,and certain enzymes like (cytochrome oxidase, peroxidase, catalase and flavoproteins)→ Heme iron
- The total quantity of iron present in the body averages 4-5 grams(4g in females – 5g in males), about 65 percent of which is in the form of 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

About 4 percent is in the form of myoglobin, 0.1 percent is combined with the protein transferrin in the blood plasma, and 15 to 30 percent is stored for later use.

- Iron requirement for the individual is very little. The daily intake of iron range from 15-20mg , but only small proportions can be absorbed(about 4% which is 0.6mg).Iron intake is relatively high is the USA and Europe (20 mg per day)
- In nature, iron is found in ferrous (Fe^{+2}) and ferric (Fe^{+3}) forms. The body of the mammal can only utilize (benefit from) the ferrous iron which can be absorbed by the body.

I) 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 (ferroprotopophyrin) to separate ferrous (Fe^{+2}) and protopophyrin. Through Fe^{+2} carriers, then ferrous will be transported to the plasma.

B. Free ferrous

The liver secretes moderate amounts of apotransferrin into the bile, which flows through the bile duct into the duodenum. Here, the apotransferrin binds with free iron. This combination is called transferrin(TF). It, in turn, 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. Iron then binds to **apoferritin** to form **ferritin** and is transported through Fe^{+2} carrier across the basolateral membrane into the blood and later released into the blood capillaries beneath these cells. It immediately combines in blood plasma with β-globulin called **apotransferrin** to form **transferrin** which is then transported in the plasma. The iron is loosely bound in the transferrin and, consequently, can be released to any tissue cell at any point in the body. (Excess iron in the blood is deposited *especially* in the liver hepatocytes and less in the reticuloendothelial cells of the bone marrow). In the cell

cytoplasm, iron combines mainly with a protein, ***apoferritin***, to form ***ferritin.*** <u>This iron stored as ferritin is called *storage iron*</u>.

- The half life of epithelial cells of the intestine is about few days, 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. The lost iron range from 0.5-1 mg per day for adult male (about 0.6mg)

  - In the lumen of the intestines and in plasma → Fe^{+2} is bound to **transferrin**.
  - In the epithelial cells of the intestines and in the cell cytoplasm→ Fe^{+2} is bound to **ferritin**.

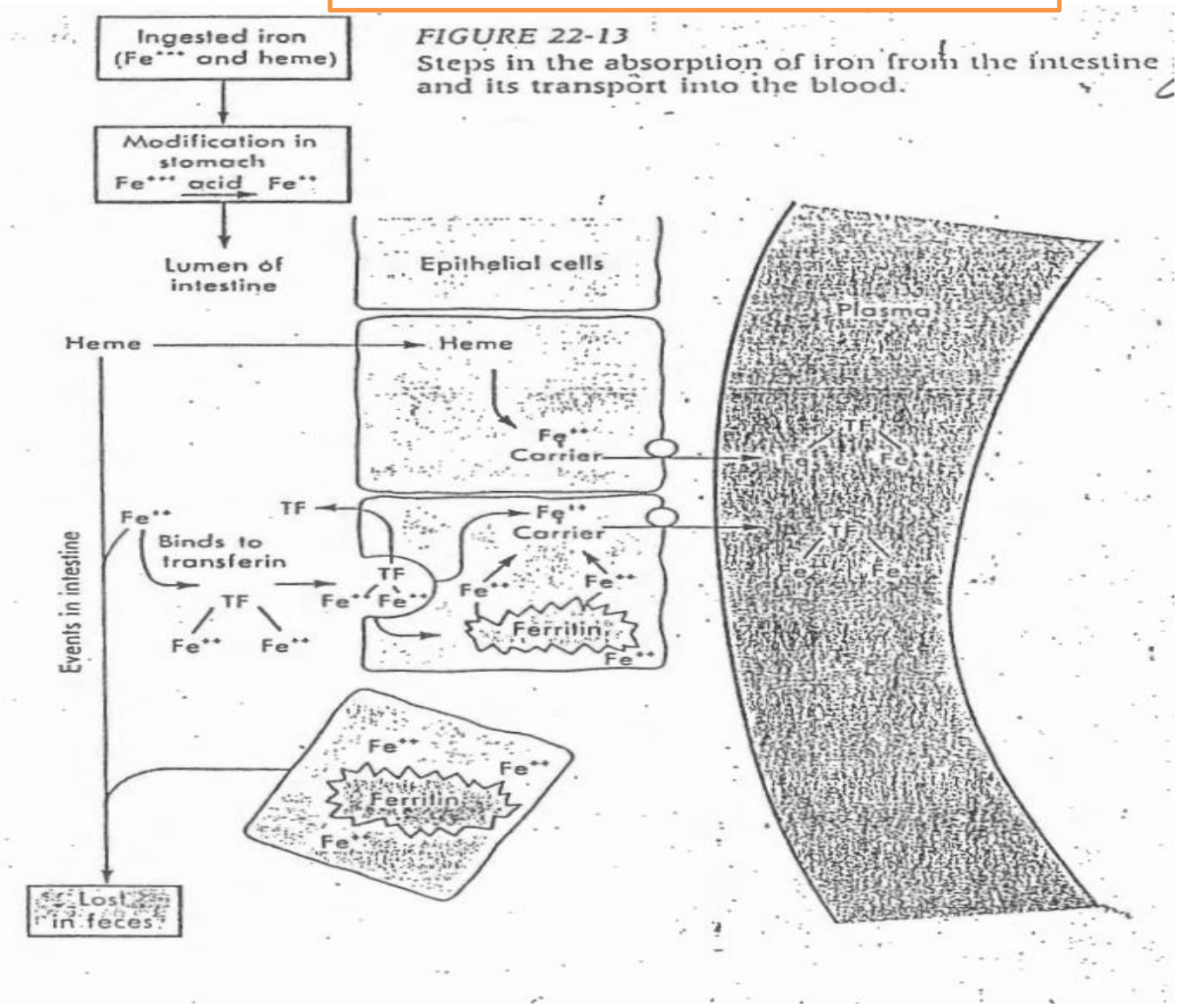


FIGURE 22-13
Steps in the absorption of iron from the intestine and its transport into the blood.

10-19:30

II) Iron requirements

- We mentioned earlier that the daily intake of iron is 15-20mg. the body benefits from only 4% of these 15-20 milligrams (about 0.6mg).
- A man excretes from 0.5-1mg/day (about 0.6mg of iron each day) mainly into the feces. Therefore, absorbed iron is almost equal to lost iron.
- 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 (table 2.3). 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	0.5–1				0.5–1
Post-menopausal female					
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, feces (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.

III) Factors favoring and factors reducing absorption

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

- Note that the first six factors favoring iron absorption are opposite to the first six factors reducing iron absorption. *(easier for memorization).*
- The Phytates present in cereals -especially bread- and **tannin**s present in tea reduce absorption of iron. 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

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

people more often that in richer groups (because poor people are highly dependent on tea and bread)

IV) 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 (hemorrhoids).

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

V)Hemoglobin; Its Structure, Synthesis, and Types

1. About Hemoglobin

- Hemoglobin is one of the parameters of the blood.
- It has two parts: heme part (4%) and globin part(96%), so Hb is mainly a protein.
- Hemoglobin consists of two parts:
  - Globin is the protein part (96%)→ it can bind carry CO_2, H^+, 2,3-BPG, 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 14g/100mL blood. We say 15 (as average).

additional note:

Hemoglobin comprises 1/3 of the red blood volume which is 45% of 100mL of blood sample

2. 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.

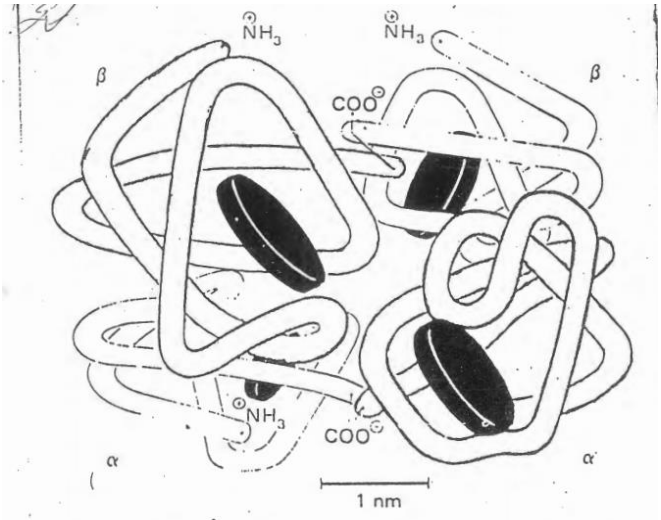


Figure 27–10. Diagrammatic representation of a molecule of hemoglobin A, showing the 4 subunits. There are two α and two β polypeptide chains, each containing a heme moiety. These moieties are represented by the disks. (Reproduced, with permission, from Harper HA et al: *Physiologische Chemie*. Springer-Verlag, 1975.)

*Each α = 141 a.a
*Each β = 146 a.a

3. Hemoglobin synthesis

- 65% of hemoglobin synthesis occurs in the Erythroblast stage (these cells in the in the bone marrow contain nuclei). The remaining (35%) occurs in the reticulocyte (these cells contain fragments of nucleus).
  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)*

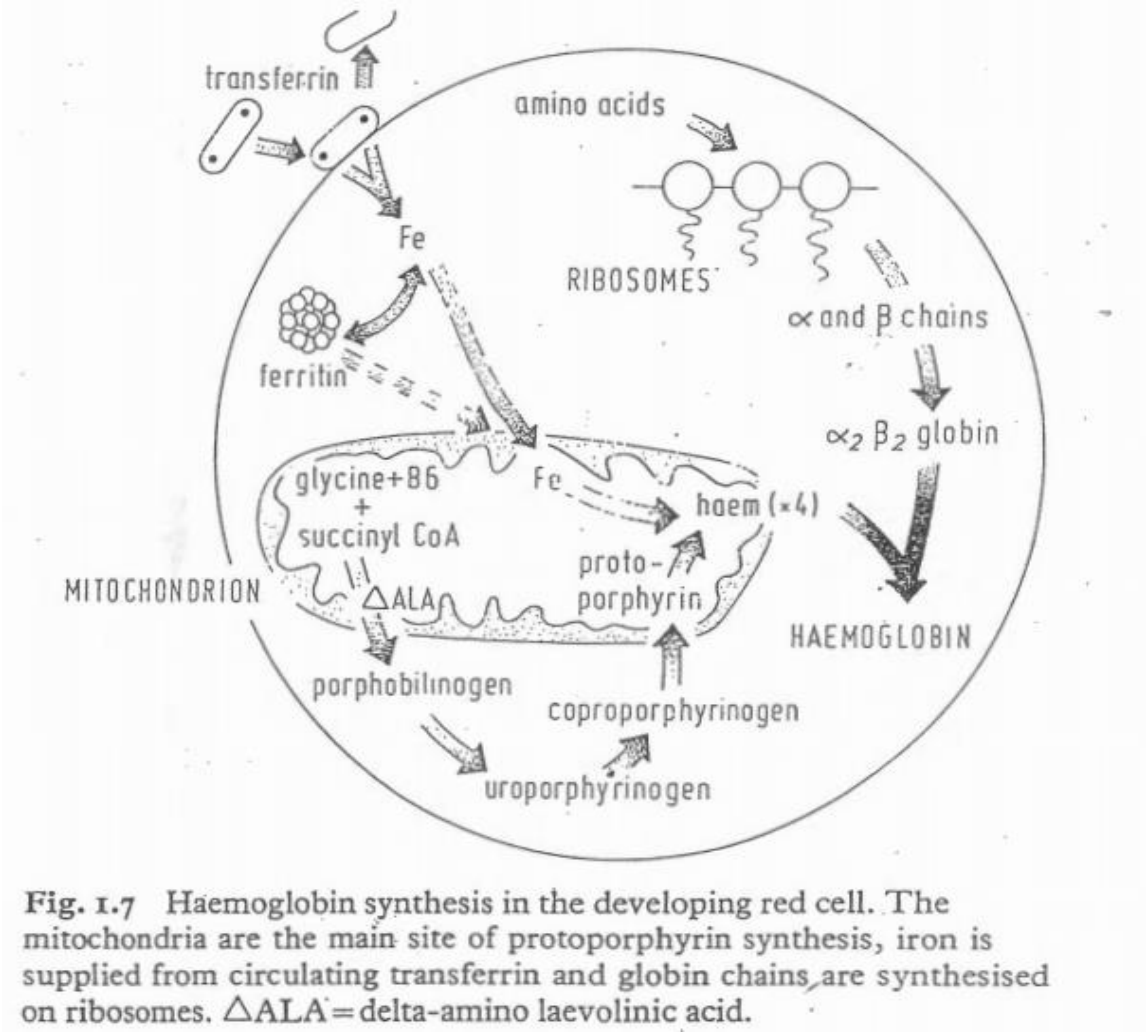


Fig. 1.7 Haemoglobin synthesis in the developing red cell. The mitochondria are the main site of protoporphyrin synthesis, iron is supplied from circulating transferrin and globin chains are synthesised on ribosomes. ΔALA = delta-amino laevolinic acid.

- ➢ Iron is obtained from plasma of the blood(in the form of transferrin) and then go to the cytoplasm of the mitochondria to form ferritin as we mentioned earlier. Iron can be obtained from the RBCs themselves or plasma.
  - ➢ Heme(ferroprotopohyrin) = 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.

- 4 Protoporphyrin will bind to 4 iron to form 4 hemes

- Finally, 4 heme molecules unite with globin (2 α subunits and 2 β subunits) to form the one hemoglobin molecule.

VI)Consequences of iron deficiency

1- Sometimes problems occur in the iron absorption or in the iron amount in the body, such as iron deficiency or inflammation.

2- Sometimes there is a problem in the protoporphyrin synthesis. This is called sideroblastic anemia.

→ so the problem is in the heme is either in protoporphyrin or in the iron

3- Sometimes there is a problem in the globin synthesis such as in thalassaemia

- All these 1,2,3 produce *hypochromic microcytic anaemia*, these cells contain low hemoglobin (hypochromia) and the result is small cells →MCV is less than 70 fL or 70 μm^3

- So these are the causes of hypochromic microcytic anaemia and any one of them will cause hypochromic microcytic anaemia.

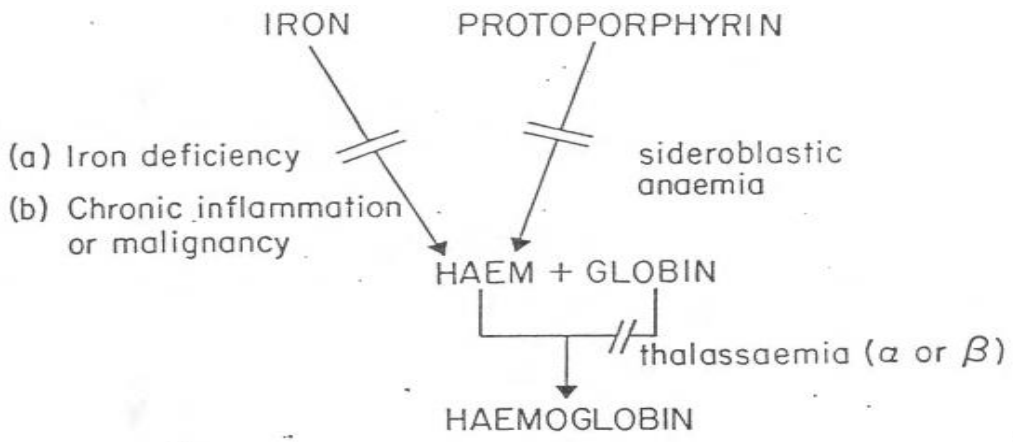


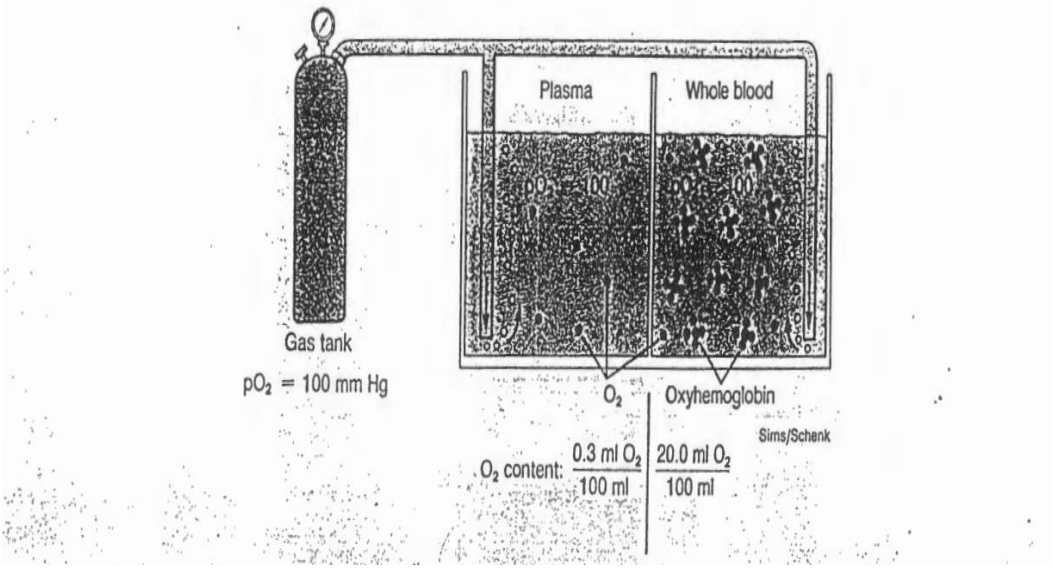
Fig. 2.1 The causes of a hypochromic microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic inflammation or malignancy), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (α or β-thalassaemia).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

VII) Few notes *(the doctor read them from a slide he had)***30-40 min**

- Iron deficiency anemia 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 hypochromic microcytic anemia.
- Some discrimination indices calculated from RBCs indices -*Dr. Saleem said that the will explain this later*- are defined and used for rapid discrimination between 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.

- 1 gram hemoglobin carries 1.34mL Oxygen (almost constant).
  It means 100 mL **blood** (has RBCs) carries 20mL Oxygen.
- While 100mL **plasma** (no RBCs) carry 0.3 mL of Oxygen

Figure 14.24 Plasma and whole blood that are brought into equilibrium with the same gas mixture have the same pO_2 and thus the same amount of dissolved oxygen molecules (shown with black dots). The oxygen content of whole blood, however, is much higher than that of plasma because of the binding of oxygen to hemoglobin.



Additional note: how the last point was calculated

In 100mL blood there is 15% hemoglobin which is 15g and each gram will carry 1.34mL of Oxygen >>> so 15 X 1.34 = 20

VIII)Types of hemoglobin

- Types include adult hemoglobin -also known as adult hemoglobin A or A1, fetal hemoglobin, and other types.
- Look at table 4.4,
  - There are 6 types of hemoglobin in human beings.
  - 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 A2
  80 % → fetal Hb

- In adults:

  97 % → adult Hb

  2.5 % → Hb A2

  <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

- **Fetal hemoglobin is replaced by adult hemoglobin in the sixth month**

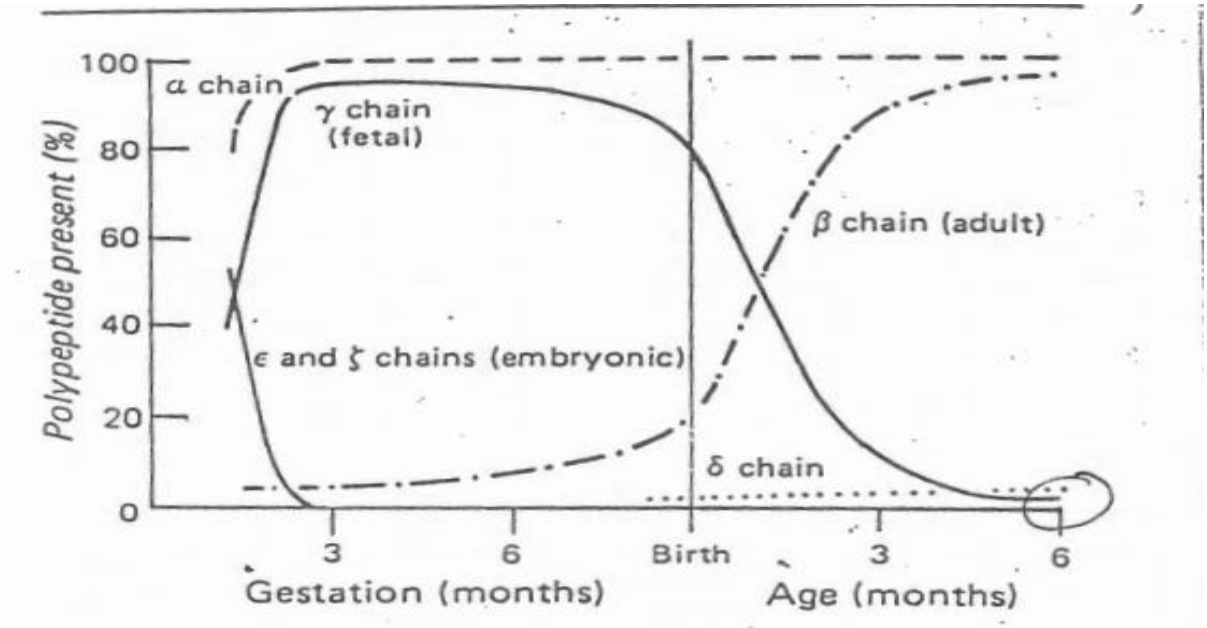


Figure 27–14. Development of human hemoglobin chains.

Look at the figure above and note that:

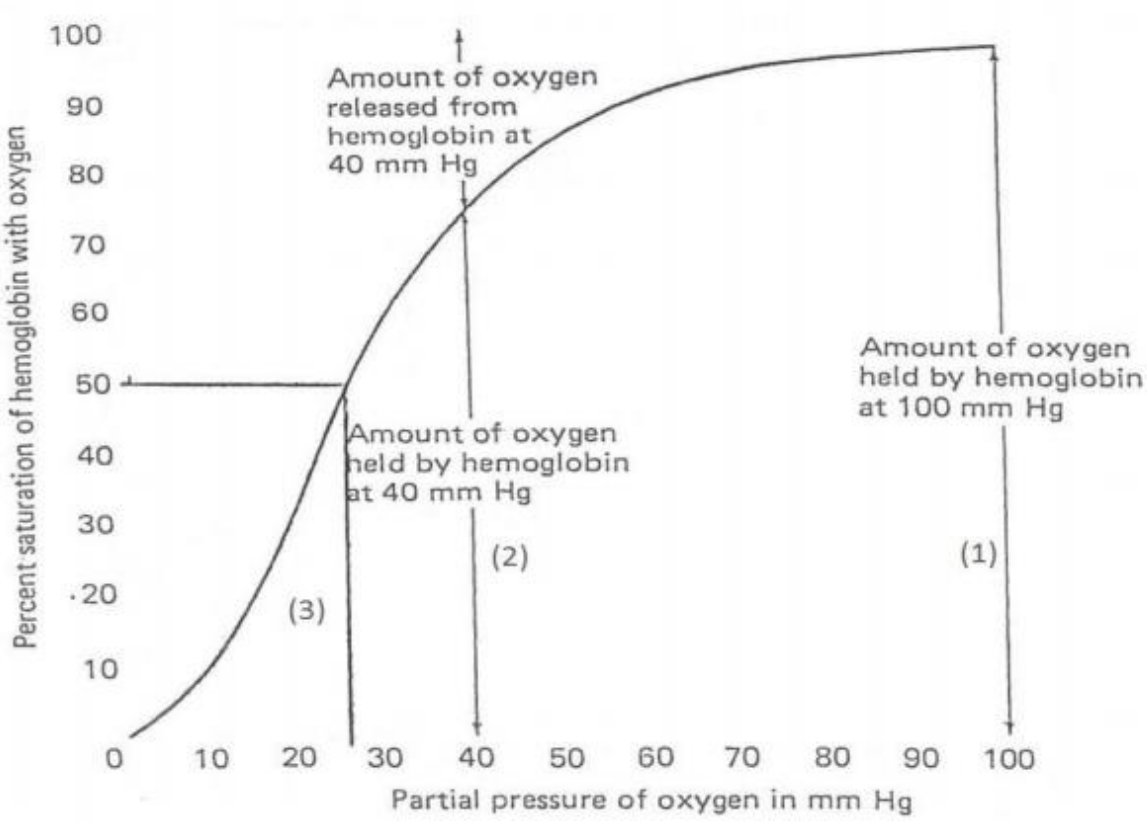
- After birth, fetal hemoglobin is gradually replaced by adult hemoglobin. (β chain replaces γ chain)
- By the 6th month, fetal hemoglobin will be almost totally (not 100%) replaced by adult hemoglobin. The proportion of the remaining fetal hemoglobin is less than 1% as mentioned in the table above.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

- Besides hemoglobin, heme is part of the structure of other proteins like:
  - **Myoglobin** → an oxygen binding pigment found in red (slow) muscle.
  - **Neuroglobin** *(we don't know much information about it)* →an oxygen binding globulin in the brain (it carries oxygen in the nervous system).

IX)Hemoglobin-Oxygen dissociation curve of an adult human: ¬

- The figure below shows the extent to which hemoglobin picks up and releases oxygen as the oxygen pressure in the blood changes.
  1- • When blood passes through the lungs, where the partial pressure of oxygen is about 100 mm Hg, the hemoglobin becomes about 97% - 98% (not 100%) saturated with oxygen.
  2- • When blood passes through distant tissues, where the partial pressure of oxygen is ordinarily about 40 mm Hg, the hemoglobin releases about 25% of its oxygen (75% remains bound).

  3-• When blood passes through very distant tissues, where the partial pressure of oxygen is about 26 mm Hg (P_{50}), hemoglobin releases 50% of its oxygen (half the oxygen content is bound and the other half is bound.



- When we plot oxygen content against Po_2 depending on the hemoglobin concentration, we get different figures, as seen below (you have to read what is written below the figure)

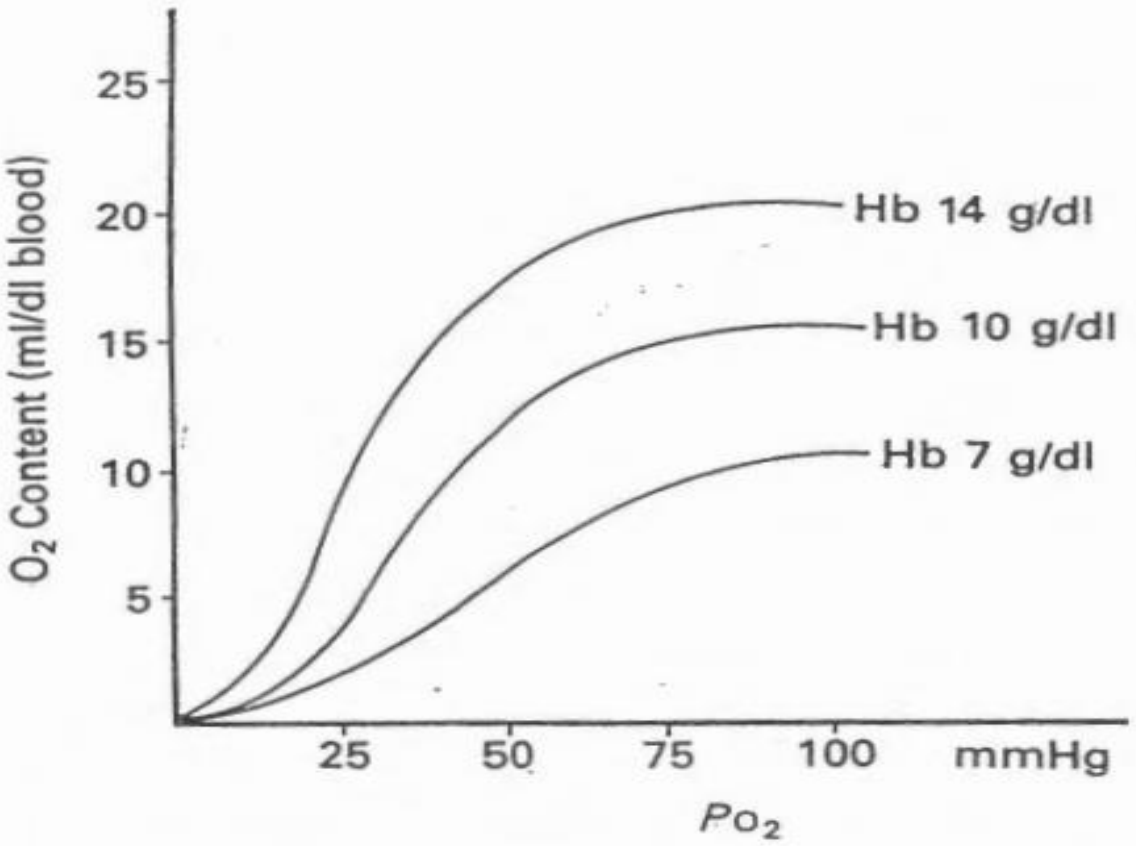


Fig. 6.12 Effect of anaemia on oxygen content of the blood at different Po_2 values.

It is evident that the **quantity** of oxygen carried in a volume of blood is dependent on the Po_2 as well as the haemoglobin concentration. The percentage saturation of haemoglobin with oxygen is dependent on Po_2 and totally independent of **haemoglobin concentration**. If oxygen content (instead of percentage saturation of haemoglobin with oxygen) is plotted against Po_2, the level of the curve will be dependent on the haemoglobin concentration of the sample of blood (Fig. 6.12). But when plotting **percentage saturation** against Po_2, as is usually done, the curve will always be the same, whatever the haemoglobin concentration is, if other factors remain the same.

*The figure above clearly shows that the amount of oxygen carried in a volume of blood is dependent on PO2 as well as hemoglobin concentrations. The higher the PO2, the higher the content of oxygen in the blood. The higher the hemoglobin concentration, the higher the content of oxygen in blood.

On the other hand, the percentage saturation of hemoglobin with oxygen is dependent on PO2 and totally independent of hemoglobin concentration, as presented in the first figure. Thus, if oxygen content was plotted against PO2, the level of the curve will be dependent on hemoglobin concentration of the sample of blood, and so, the curve will not be the same for all people as it depends on the hemoglobin concentration variations. (second figure) But when the percentage saturation of hemoglobin is plotted against PO2, the level of the curve will always be the same, whatever the hemoglobin concentration is. Therefore, the plot will always be the same for all people, males and females. (first figure)

The end

Sorry for mistakes if any

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