HEMATOLOGY
&LYMPH SYSTEM

Biochemistry sheet

Number	8
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Minimal Daily Iron Requirement:

	Amount that must be absorbed (mg)	Minimal amount that must be ingested (mg)
Infants	1	10
Children	0.5	5
Young, non-pregnant women	2	15-20
Pregnant women	3	30
men and post-menopausal women	1	10

Note: - we absorb 5-10% of the amount ingested.
- During pregnancy iron requirement is increased.
- Requirement of infants equals that for adults because of the fast growth of infants.

*****Iron deficiency: (mostly from the slides)***

-Iron deficiency is the most common nutritional deficiency worldwide.

-There is no excretory mechanism for iron, which slowly accumulates in the body throughout life. Iron is absorbed in small quantities from the diet. It is called a *one way substance*, due to its absorption and lack of excretory mechanisms, so it enters but it doesn't exit (one-way).

-Only about 1 mg of iron is absorbed per day in men- about the same amount is lost by the desquamated cells of the skin and intestinal mucosa, bile, urine, and sweat.

-Iron deficiency is **rarely** caused by dietary deficiency alone. Typical situations include:

1-*Acute massive hemorrhage*: 1 liter of blood loss = 500- 550 mg of iron loss
If enough body iron storage is mobilized afterwards→the hematocrit returns to normal within a few weeks.

2-*Chronic hemorrhage*:
→young women lose 20-40 ml blood/menstrual cycle (~11-22 mg iron)
→Occult blood loss from chronic bleeding in the GIT; hemorrhoids or tumors.

3-*Growth*

4-*Pregnancy and lactation*: amount of loss is as follows:
*Fetus during pregnancy → 250-300 mg (maximum loss during 3rd trimester)
*Placenta, cord blood, blood loss during delivery → 80-400 mg.
*Lactation → 180 mg iron is lost.

Important: 1-Iron deficiency anemia is a **microcytic, hypochromic anemia**.

2- The most important **differential diagnosis** for microcytic, hypochromic anemia is to exclude *thalassemia* before beginning iron therapy- thalassemia patients already have overload of iron.

Note: Dr.Nayef said he explained what is next previously, so you can read them on your own.

-Iron deficiency anemia's **most prevalent groups** include:

1-Growing children
2-Menstruating females
3-Pregnant women

-**Treatment**: administer ferrous sulphate and ascorbic acid.

-**Prevalence** of the disease:

*It is the most common nutritional deficiency.
* 2-10% of the population in developed countries
*10-50% of the population in developing countries.

-Common **causes** include: excessive menstrual flow, multiple births, and GI bleeding.

-Normal body iron levels:

*Total body iron = 3-4 g (for a 70 kg adult male), of which 2.5 g are in Hb.
*0.1% (3.5mg) of iron is in plasma- in tests, it is 50-160 μg/dL.
*Transferrin saturation percentage ~ 33%
*Ferritin: in males: 5-30 μg/dL, while in females: 1.2- 10 μg/dL.

-The doctor talked about this:

Initial stage of iron deficiency:

1- Depletion of iron stores in the body
2- Plasma ferritin level is decreased.
3- Level and percent of saturation of plasma transferrin ~ normal.

Second stage:

1- Hb level begins to fall.
2- Morphological changes.
3- Fall in serum iron.
4- Rise in transferrin level.
5- Decrease in transferrin saturation (< 16%).

Third stage:

1- Depletion of iron containing enzymes with pronounced metabolic defects.

**Measurement of ferritin level in serum is a useful indicator of iron deficiency.

-Many of these indices are mentioned in the below table, it is useful in revision.

TABLE 24.2

Biochemical indices of iron deficiency and iron overload

Index	Normal	Changes in: Iron deficiency	Changes in: Iron overload
Hematocrit			
Male	43%-49%	Decreased	Normal
Female	41%-46%		
Blood hemoglobin			
Male	14%-18%	Decreased	Normal
Female	12%-16%		
Total plasma iron	50-160 μg/dL	Decreased	Increased
Total iron binding capacity	250-400 μg/dL	Increased	Increased
% Transferrin saturation	20%-55%	Decreased	Increased
Serum ferritin			
Male	5-30 μg/dL	Decreased	Increased
Female	1.2-10 μg/dL		

00:00-8:00

Metabolism in the mature RBC:

- Mature RBCs lack intracellular organelles (e.g. nucleus/ mitochondria), and is left out only with its cytosolic enzymes. An RBC needs to circulate in the blood for 120 days. In order to survive that long and also be able to perform its function (O2 transport mainly, but also CO2 and H+), it needs its metabolic machinery to keep its membrane integrity, and to repair any oxidative stress that it might encounter.

- So, RBCs' metabolic enzymes present in mature cells functions include:

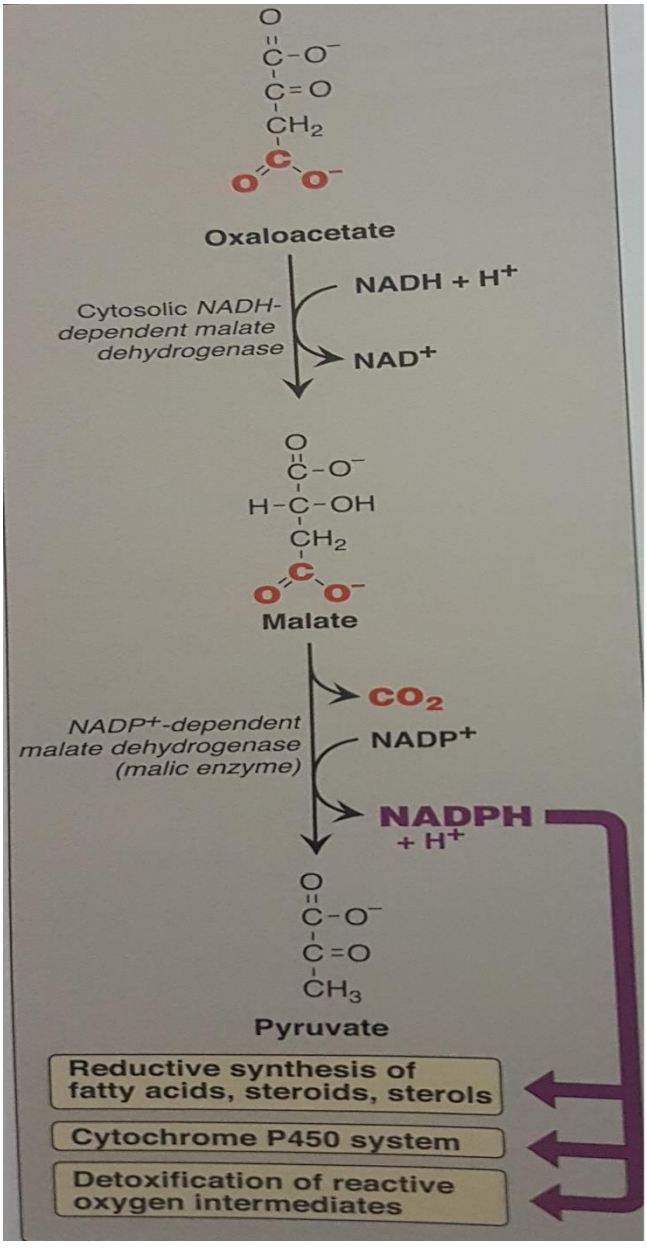
1- Prevention and repair ROS damage.

2- Generation of energy (ATP), which is needed for ion transport (Na/K/Ca), phosphorylation of certain membrane proteins, and priming of glycolysis. The glycolytic pathway needs priming (activation) via ATP molecules. (Remember that it ***originally consumes*** 2 ATPs to produce 4 ATP).

- The enzymes present in mature RBCs include enzymes of the glycolytic pathway (all the way from glucose to pyruvate, and also pyruvate to lactate), which produce ATP, as well as NADH, which is needed for the enzyme cytochrome b5 reductase (MetHb reductase) in order to reduce cytochrome b5, which in turn reduces MetHb to Hb. In newborns, cytochrome b5 reductase has half the capacity of the adults' enzyme. That's

why we are careful about exposing a newborn to oxidizing drugs that form more MetHb.
Moreover, this pathway is important for the production of 2,3-BPG (which is present in minute amounts in other cells but in high amount in RBCs; ~ 4-5 mM). 2,3-BPG is important in RBCs as it controls oxygen delivery to tissues.
So, glycolysis yields vital molecules for the RBC→ ATP, NADH, and 2,3-BPG.

- Almost all the glucose in RBC goes into the glycolytic pathway. Around 5-10% only is diverted towards the pentose phosphate pathway (**REVISION**: This pathway –also called hexose monophosphate shunt- is important to produce ribose-5-phosphate (for nucleotide synthesis), as well as **NADPH** (Each glucose consumed yields 2 NADPH molecules). **NADPH is a biochemical reductant important for keeping glutathione reduced, thereby reducing and neutralizing the effects of oxidants and ROS.** *Cells other than RBCs* have another pathway by which they can produce NADPH if this pathway is deficient, by using the malic enzyme (see figure)



-**REMEMBER** that RBCs can **only** produce NADPH via the pentose phosphate pathway (no malic enzyme in RBCs), so a deficiency in this pathway's enzymes (particularly glucose-6-phosphate dehydrogenase – G6PD) will greatly affect RBCs, making them more susceptible to oxidant-induced damage.

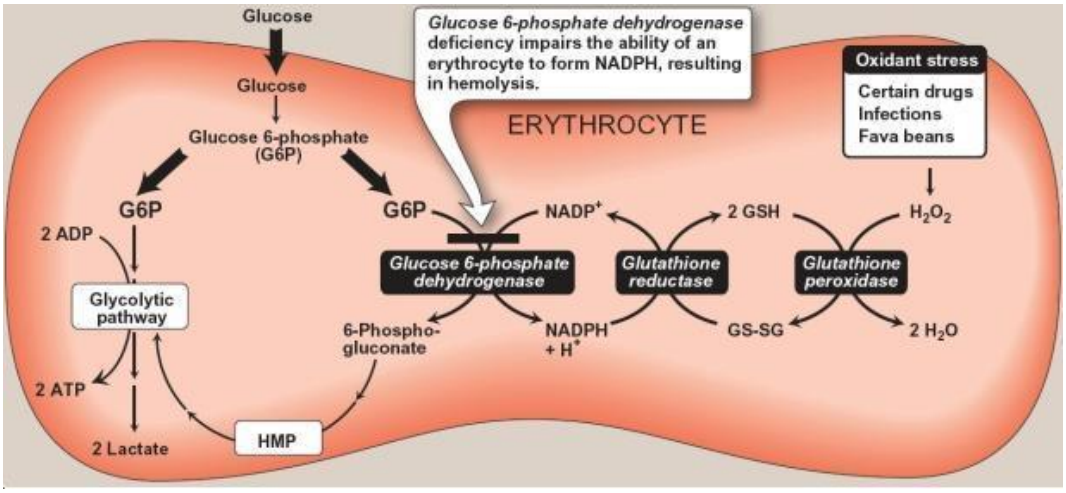
- This damage usually causes hemolysis of RBCs.

-NADPH gets oxidized to NADP+, while oxidized glutathione (GSSG) is reduced back to (2 GSH) via the enzyme Glutathione reductase. GSH (reduced glutathione) is important to deal with inorganic and organic peroxides. It neutralizes their effect by being oxidized to GSSG (the enzyme: Glutathione peroxidase), which is later reduced back to GSH.

-Genetic deficiencies in erythrocytic enzymes:

[1] Glucose-6-phosphate dehydrogenase (G6PD) deficiency:

-The reaction is Glucose-6-phosphate + $NADP^+$ → 6-phosphogluconate + **NADPH**



-As mentioned above, NADPH is used to keep glutathione reduced. Glutathione is used to reduce oxidants and peroxides, the source of which can be: certain drugs, certain foods like fava beans, and infection. Moreover, GSH oxidation spares –SH groups of intracellular proteins, so if G6PD is deficient→ no NADPH→ no GSH→ oxidation of –SH groups within proteins, forming denatured proteins as insoluble masses (***Heinz bodies***). Additional oxidation of membrane proteins causes RBCs to be rigid (less deformable), and they are removed from the circulation by macrophages in the liver and spleen.

-G6PD deficiency is a common issue in the area. Around 5-10% of Amman's population has it, and in Aghwar region, it reaches 10%.

8:00-18:00

-It is the most common disease causing enzyme abnormality in humans. Around 200-400 million humans are estimated to have it. The prevalence is highest in the Middle East region, tropical areas in Asia and Africa, and parts of the Mediterranean region (including Greece, south Italy, and southern regions of Spain).

- G6PD gene is on the X chromosome, so males are more affected by the deficiency than females; who need to inherit both defective copies to have the disease.

-The mutant gene survived because this deficiency provides some resistance against malaria infection (not as much as HbS but it provides some resistance).

-There are many variants of the enzyme (more than 140 described). The wild type (normal, most common) is designated as G6PD B. The Mediterranean variant is **G6PD B^-**; because it migrate same as the wild type in electrophoresis (but it has less than

10% of the activity). Moreover, there is the African variant, **G6PD A⁻**, whose mutations include an Aspartic acid residue (instead of Asparagine), and this negative charge of Asp makes it migrate faster on electrophoresis (that's why it's A⁻). In addition, an African variant, **G6PD A (or A⁺)**, has 80% of residual activity- so it's not considered deficient but it's a variant.

The mutations of each variant (***not for memorization***):
***G6PD B⁻** → point mutation (563 C→T) so (188 Ser→ Phe).
***G6PD A⁻** → two point mutations (376 A→G, so 126 Asn→Asp) and (202 G→A, so 68 Val→ Met). Usually, the first mutation occurs, and then the second is superimposed on the first.
***G6PD A** → point mutation **(**376 A →G), so (126 Asn→ Asp).

For your own knowledge: G6PD variants are detected via molecular analyses. In the past, it was done via observing the biochemical properties of the enzyme –due to lack of molecular techniques-. These methods were inaccurate and give different results from time to time. Dr.Nayef did a study on Jordan's variants of deficient G6PDs. Jordan's most common (abnormal) variant is the Mediterranean variant (around 70%), while the rest are G6PD A⁻ (around 5%) and some other minor variants.

-Precipitating factors in G6PD deficiency:

Most individuals with G6PD deficiency don't have clinical symptoms. However, some patients develop haemolytic anemia if exposed to:

1- ***Oxidant drugs*** (AAA):
   A: Antibiotics, such as sulfamethoxazole and chloramphenicol.
   A: Antimalarial agents, such as Premaquine
   A: Antipyretics: Acetanilide, but **not** acetaminophen.

2- ***Favism***: fava beans contain oxidants, such as Vice and Convicine, which are converted to ***devicine*** and ***isouramil***, respectively. These need to be reduced, so these two oxidants cause a rapid decline in GSH level.

3- ***Infection***, which is the most common precipitating factor in G6PD deficient people. The oxidative burst during inflammation causes the release of many ROS, which can diffuse into the RBC and cause oxidative damage.

4- ***Neonatal jaundice.*** Also of note; G6PD deficient infants develop neonatal jaundice more than non-G6PD deficient infants.

-Classes of G6PD deficiency:

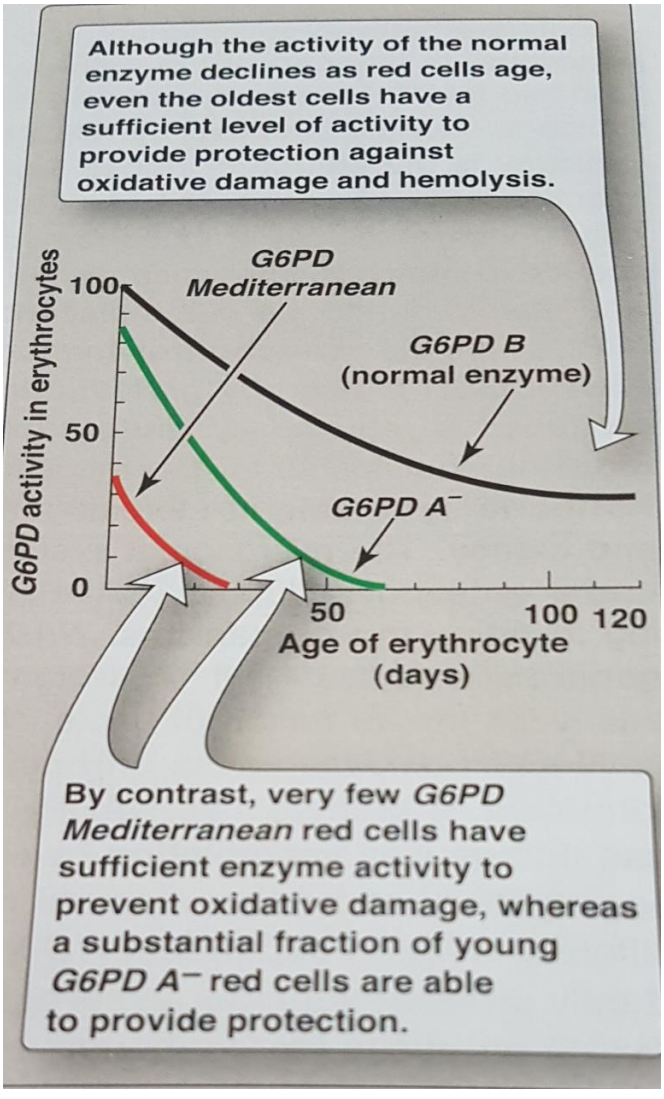
Class	Clinical symptoms	Residual enzyme activity
I	Very severe	<2%
II	Severe	<10%
III	Moderate	10–50%
IV	None	60–150%

Chronic non-spherocytic hemolytic anemia CNSHA
e.g. Med. Variant B⁻
e.g. A⁻ (African)

*Note that most G6PD deficient patients are asymptomatic unless exposed to an oxidative stress. An exception to this is **class I patients** (Most severe, residual enzyme activity is <2%, rare), which is associated with **chronic non-spherocytic haemolytic anemia** of various degrees even in the absence of oxidative stress.
*Mediterranean variant B^- is class II (severe, residual enzyme activity < 10%)
*African A^- variant is class III (moderate severity, residual enzyme activity 10-50%)

-Molecular biology of G6PD:
*Majority of cases → Missense mutations (only one or two point mutations). The G6PD enzyme is a housekeeping enzyme (necessary for survival of the cell, thus expressed in all cells). So, any frame shift or non-sense mutations resulting in complete loss of the enzyme may not be compatible with life.
*Large deletions and frame shifts → not observed in patients.
*Variants important to us are A^- and B^-. There is the A^+ variant, which is more prevalent among black people and it doesn't produce any clinical symptoms.

18:00-28:00



-Decline in G6PD level as erythrocytes age:

*The stability and activity of the enzyme in the RBCs decline as the RBC ages.

*Although the activity declines, even in old **normal G6PD B** cells, the activity reaches 30-35%, which is sufficient for protection.

* **G6PD A⁻**: in young fresh cells, the activity is about 80%, but it declines quickly, so oldest cells have very low level.

* **G6PD B⁻:** the activity is already low from the beginning even in fresh RBCs, and it declines with age, and since the initial activity is low, you can do the G6PD activity test any time, but with G6PD A⁻, it's different (see below then return to this point).

WHY is this important?

Assume an African patient comes to you complaining about a haemolytic episode he had just after the ingestion of fava beans. You will suspect G6PD deficiency and order a test to know the level of G6PD activity. An important thing is that, since he is African →his variant is G6PD A⁻ (common in Africans), and the haemolytic episode preferentially removed old RBCs with low G6PD activity (meaning that the fava bean oxidative stress caused hemolysis in old unprotected cells a lot easier than fresh protected cells), so what you'll find in his blood after the haemolytic episode is young fresh cells with around 80% enzyme activity → you'll say it's not G6PD deficiency and you'll be wrong. What you really need to do is waiting for 2-3 weeks then test the enzyme activity.

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[2]: Pyruvate Kinase Deficiency:

-Reaction: Phosphoenolpyruvate + ADP → pyruvate + ATP

-Incidence is not as high as G6PD deficiency. Among glycolytic pathway enzyme deficiencies, PK deficiency is the most prevalent one; it constitutes 95% of them, and then comes phosphoglucose isomerase (4%), and the last 1% is distributed among the rest.

-The diagnosis of PK deficiency is difficult, because in the lab, we provide optimum conditions for enzyme function (high substrate concentration, activators…) so it might show normal activity there although it is not active in vivo. If the mutation affects the enzyme's activity or stability, it may be easier to diagnose, but if it alters the Km or Vmax, it may not be evident to us that something is wrong (because in the lab we put a lot of substrate, which usually overcomes the problem if it's the affinity) Also, if the problem is decreased responsiveness to the activator (Fructose 1,6-bisphosphate) it will not be evident as well. A specialized lab is required.

-Deficiency in this enzyme can sometimes cause severe hemolysis that requires regular *blood transfusion*.

- Biochemical consequences of the deficiency:

  ↓ATP (see below)

  ↑ in the substrate (due to no proceeding in the forward direction), which causes accumulation of the previous substrates, which will lead to formation of **more 2,3-BPG** (Don't forget that 1,3-BPG is a glycolytic intermediate, and when the pathway doesn't go in the forward direction, 1,3-BPG increases, allowing it to be converted to 2,3-BPG). This is good, because this deficiency causes anemia, so increased 2,3-BPG compensates that by enhancing oxygen delivery to tissues.

28:00-38:00

From Lippincott: '' Mature RBCs lack mitochondria and are, therefore, completely dependent on glycolysis for ATP production. ATP is required to meet the metabolic needs of RBCs and to fuel the ion pumps necessary for the maintenance of the flexible biconcave shape that allows them to squeeze through narrow capillaries. The anemia observed in glycolytic enzyme deficiencies is a consequence of the reduced rate of glycolysis, leading to decreased ATP production. The resulting alterations in the RBC membrane lead to changes in the cell shape and, ultimately, to phagocytosis by cells of the reticuloendothelial system, particularly macrophages of the spleen.''

The End