# Hematology

## BIOCHEMISTRY

☑ Sheet ☐ Slide ☐ Handout

Number: **5**

Subject: **Derivatives of hemoglobin**

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Price:

### *Before we start*

●This sheet was written according to the recording that belongs to section 3.

●Please pay attention that the order of ideas in this sheet is a little bit different from that in the recording.

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## 5.1 derivatives of hemoglobins

●In the last lecture we discussed the types of hemoglobin *(classified according their chains components)*. But here we are going to talk about hemoglobin derivatives, either physiological or pathological.

- Hemoglobin derivatives could be normal *(occur as a part of hemoglobin's normal function)*, like: oxyhemoglobin, deoxyhemoglobin and *carbaminohemoglobin*.
- Or they may be abnormal (occur as a result of inherited or acquired defect(s) in the normal structure of hemoglobin), examples are: *Methemoglobin, sulphahemoglobin and carboxyhemoglobin*.

### *5.1. A Methemoglobin*

●***Methemoglobin*** (Met Hb) is a form of hemoglobin where its heme iron atom is oxidized from ferrous (Fe +2) into ferric (Fe +3).

- In normal individual Met *Hb* is less than 1% of total body Hb.
- In certain inherited or acquired conditions, it exceeds 1%, this is called *methemoglobinemia*.

●Inherited Methemoglobinemia:

- **Causes**: Can be due to a deficiency of the enzyme NADH-Cyt b5 reductase (or Met Hb reductase). Other causes may be due to a mutation in the heme binding pocket, *that disturb its normal hydrophobicity, proving a hydrophilic environment that favors separation of charges between oxygen and iron, and therefore oxidizing iron. An example of such mutations is the replacement of the proximal histidine by a tyrosine residue*.
- **Symptoms:** the major symptom of methemoglobinemia is **cyanosis**, *which is a blue discoloration of the skin and mucus membranes because of the reduced oxygen carrying capacity of Hb*.

- **Diagnosis** is done by spectroscopic analysis at 630 nm, as seen in figure 1.

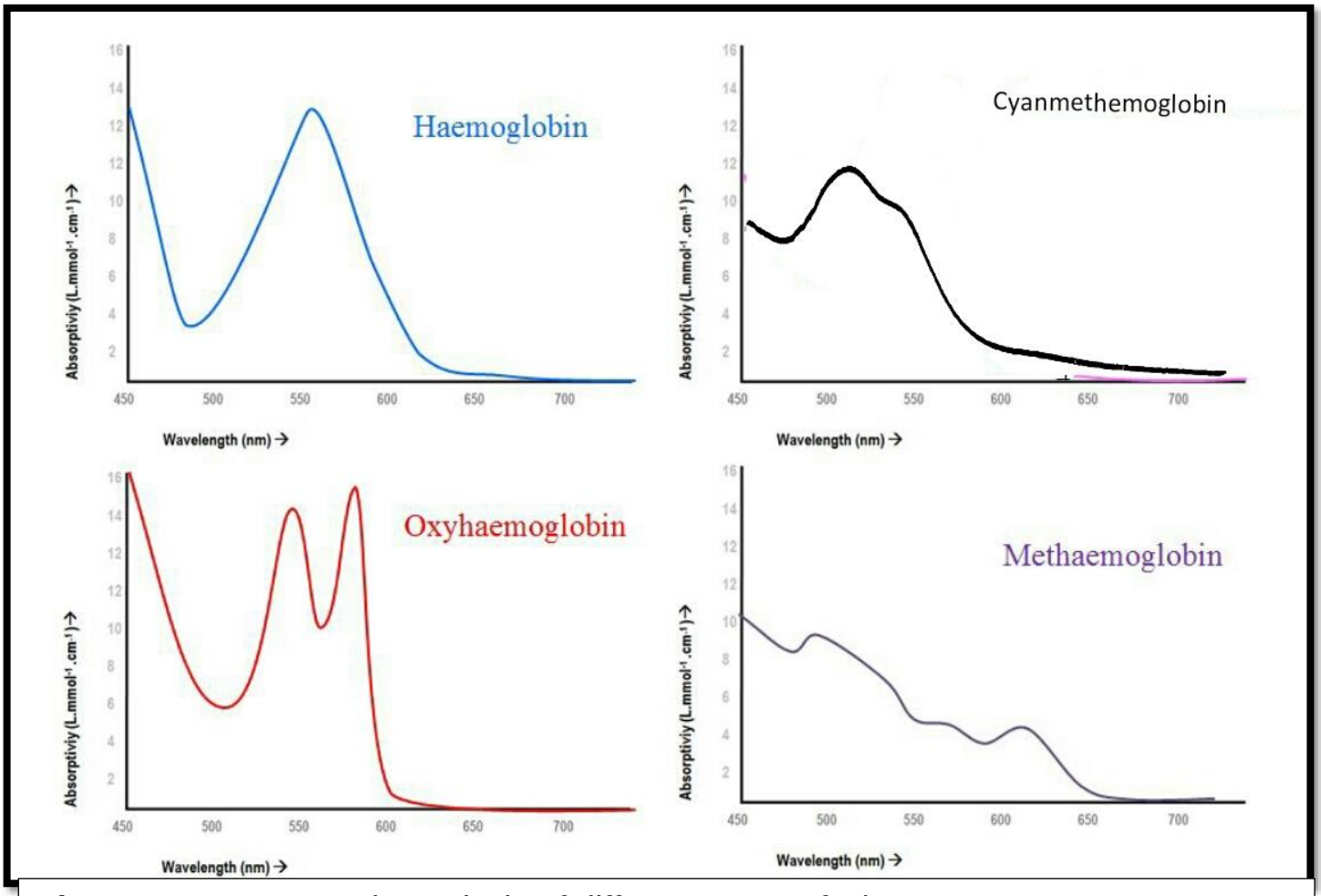


**Figure 1**: spectroscopic analysis of different types of Hb.

●Below WL of 500 nm we have a common region peaks 10 times in all hemoglobins, called *soret band*. Therefore it's not characteristic

● Oxyhemoglobin has two peaks in the region between 500 to 600 nm.

●in labs, Hb is oxidized and then mixed with cyanide, resulting in **Cyanmethemoglobin** that has one peak at 540 nm.

● Methemoglobin alone has one peak at WL around 630, which is a characteristic for metHb.

- **Treatment**: IV injections of methylene blue, but in causes of G6PD deficiency we have to use another agent that's not G6PD dependent, like ascorbic acid.

●Our defense mechanism against MetHb:

- Remember that oxidants accelerate the formation of MetHb.
- However, antioxidants protect us from reaching the state of oxidative stress.
- Important antioxidant enzymes are: *superoxide dismutase, Catalase, glutathione peroxidase and glutathione reductase*.
- Moreover, if some oxidants managed to oxidase Hb, we have an enzyme, NADH-Cyt b5 reductase that reduces MetHb back into Hb.
- If the oxidative stress exceeds our defense mechanisms, Methemoglobinemia will occur.
- Some oxidant drugs are: *phenacetin, sulphonamides, aniline and nitrites*.

## *5.1. B sulphaemoglobin*

● Sulphaemoglobin is formed usually along with MetHb, especially in the presence of sulfhydryl agents (Like $H_2S$).

● Sulphaemoglobin is worse than MetHb since its irreversible form.

## *5.1. C Carboxyhemoglobin*

●Hemoglobin has much higher affinity for CO than its affinity for Oxygen:

- Hb affinity for Co is 200 times higher than its affinity for oxygen.
- And if we extracted the heme group alone, its affinity for CO is 20000 times higher than oxygen.

●this high affinity for CO makes it lethal. If its partial pressure raises above 1% it will be fatal.

- CO bind Hb, converting it to CarboxyHb, that's very bad for your health.
- In normal non-smoker individual, CarboxyHb doesn't exceed 1% of all Hbs in the blood.
- In the smokers, it may reach 10% of all Hbs.
- If it reached 40% or more, it will lead to unconscious and death.
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●But why does CO has higher affinity than oxygen for heme? (Next page will tell you: p)

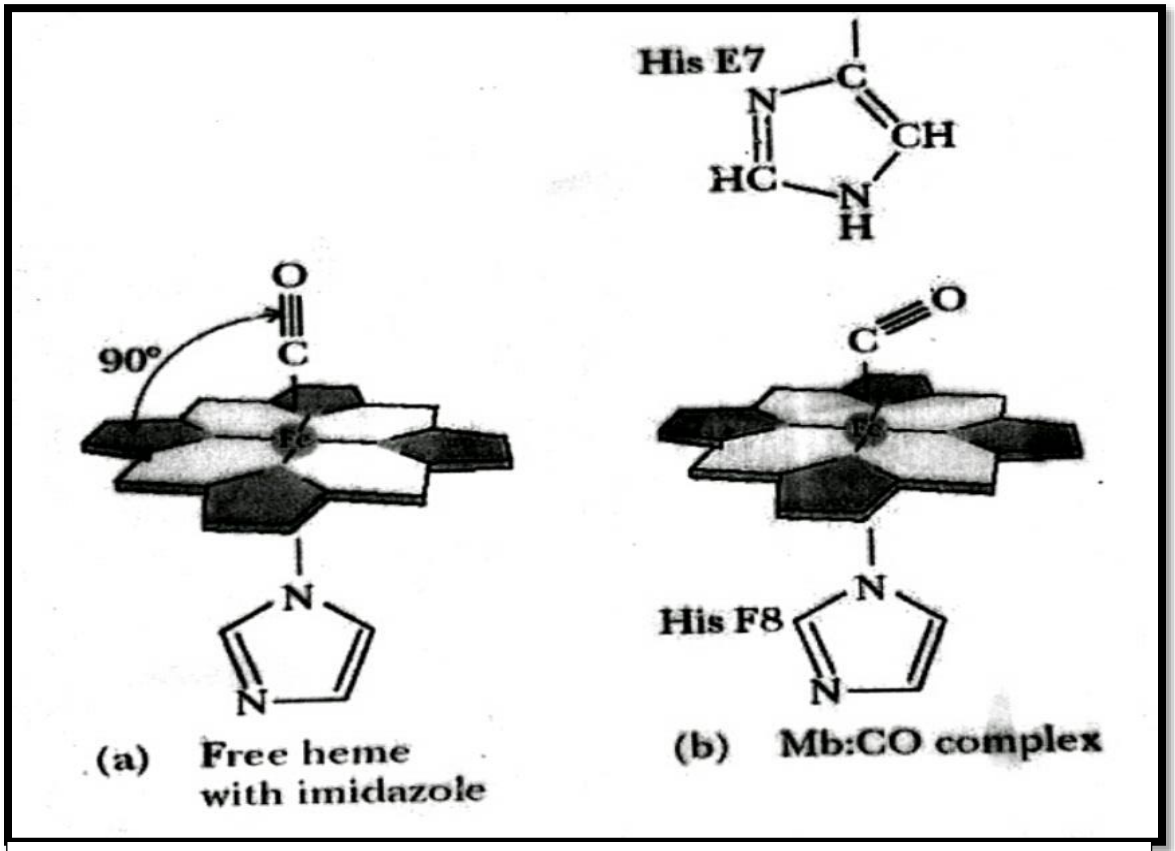


**Figure 2: CO binding to heme, the old theory.**

- In free heme, *CO bind to the same binding site for O2*, but the bond is much linear than O2-Heme bond, so it's more thermodynamically favored, that's why CO binds 20000 stronger than oxygen. (Figure2, a)

●But why does this ratio is decreased when heme is bound to hemoglobin?

- **Old theory**: heme binding pocket bends CO – iron bond due to steric repulsion, making it harder for CO to bind heme and therefore reducing its affinity.
- Recent studies has shown that CO-Iron bond angle doesn't change that much.
- They have noticed that O2 binding affinity is increased instead in hemoglobin.

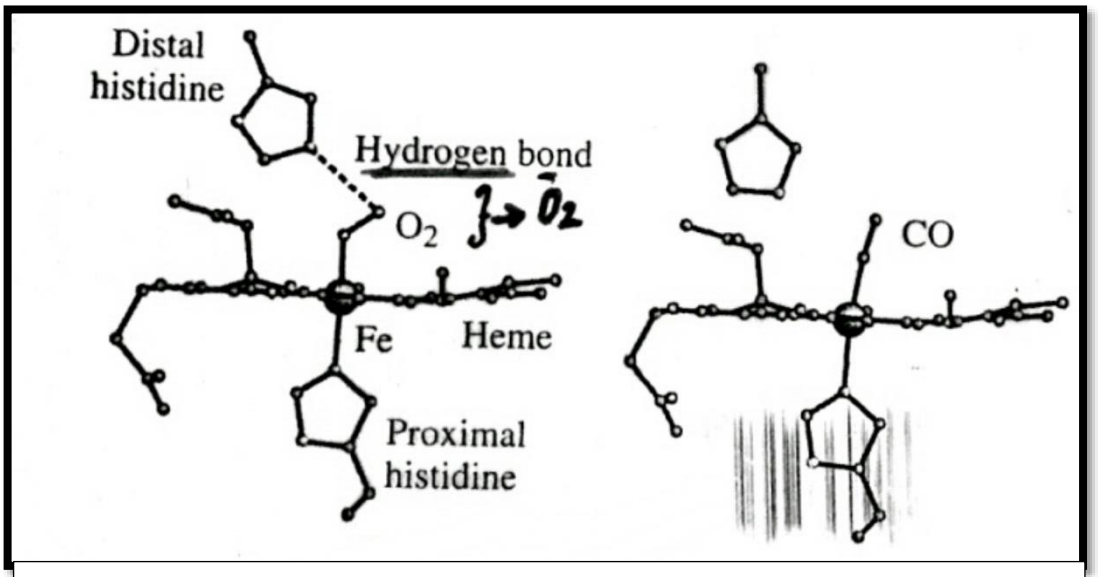


**Figure 3: CO and O2 binding to heme, the new theory.**

- When oxygen binds heme, Oxygen molecule will be polarized (the negative charge will be concentrated in oxygen atom that's near distal HIS. So a hydrogen bond will be formed between oxygen and HIS nitrogen stabilizing oxygen binding to heme in hemoglobin, and therefore increasing its affinity.
- That's way CO binds only 200 times stronger than oxygen with hemoglobin.

●Partial binding of CO to Hb will change oxygen dissociation curve: (see figure 4)

- It will be more hyperbolic and shifted to the left.
- So in the presence of CO, Oxygen will bind with higher affinity to Hb, converting Hb to a "store" for oxygen.
- And therefore decreasing oxygen delivery to distant tissues, worsening the problem more and more.

●So to sum up, the problem with CO binding to hemoglobin is that it occupies oxygen binding sites, and even if some oxygen molecules have managed to bind heme, it will be difficult for oxygen to disassociate from heme.

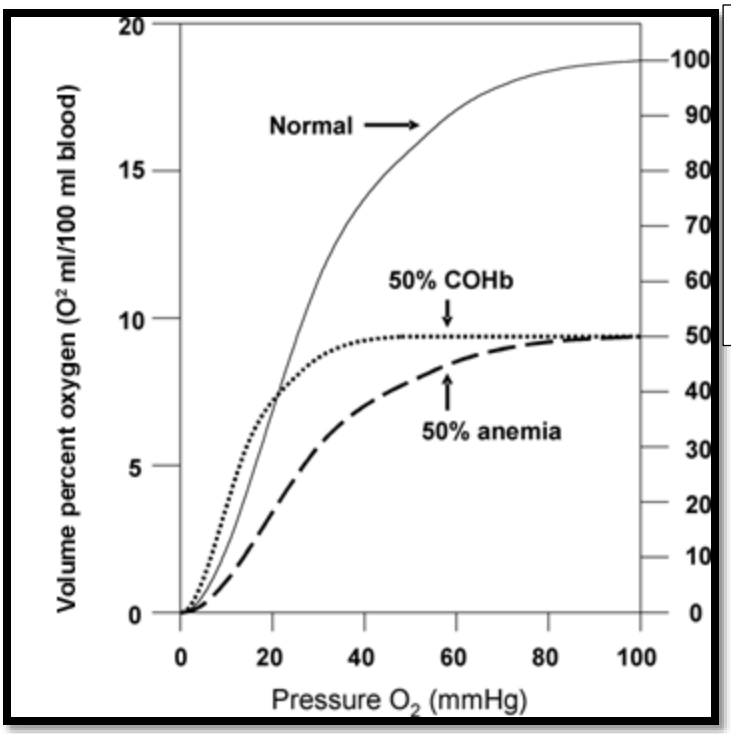


**Figure 4: oxygen-hemoglobin dissociation curve in the presence of CO, notice that the curve is shifted to the left. Moreover it's more hyperbolic**

## 5.2 Thalassemia

●globin genes are composed of exons and introns, introns will be removed from pre-mRNA and it will leave the nucleus, it's then transcripted to form certain globin chains.

●Thalassemia refers to a group of genetic desiases that shares one problem, which id decreased production of one or more chanies in hemeoglonin due to a problem in its gene expression.

●Thalassemia is two major groups, alpha and beta Thalassemia

### *5.2. A Beta thalassemia (Cooley's anemia)*

●as you know, we have two homologues genes that control the synthesis of beta chains:

- Loss of one gene will cause beta thalassemia with mild symptoms, *beta thalassemia minor*.
- And loss of both alleles will give more severe form, namely *beta thalassemia major or Cooley's anemia*.

- There are types of ***point mutations*** that may lead to beta thalassemia (*in the promoter, translation initiation codon, poly A signal, and splicing abnormalities*)

●because of low amounts of produced beta chains, alpha chains will form a tetramer, called Cooley's Hb.

- Alpha chains are not water soluble.
- Therefore, Alpha chains will precipitate, forming *Heinz bodies* that cause membrane rapture and premature hemolysis of erythrocytes.

●Nearby genes will be activated to compensate for the loss in beta chains, producing beta-like chains.

- Thus HB f (**Fetal hemoglobin**) and HbA2 will increase.
- Elevation in these hemoglobins has a diagnostic value for beta thalassemia

●**Symptom**: doesn't appear until second year of life, because some HbF remains in the blood of the newborn until he end of the first year

●**Treatment** is blood transfusion, multiple transfusion may lead to iron overload is these patients, so it's better to give these patients iron chelating agent with.

## *5.2. B Alpha thalassemia*

●Unlike beta chains, we have two pairs of alleles for alpha

- So we have 4 genes that control alpha chain production.
- If one gene is affected, the individual is a silent carrier.
- If two genes are affected, the individual in heterozygote, with mild symptoms.
- if three genes are affected, the patient will develop severe form of alpha thalassemia:
  - This will cause excess of beta chains that will form tetramers.
  - Beta tetramers are called HbH.
  - Beta tetramers are more water soluble than alpha.
  - beta tetramers show hyperbolic curve with higher affinity for oxygen, making it useless
  - gamma chains will aggregate also forming Hb barts
- If four genes are affected, the individual will develop hydrops fetalis and die in uterine or after birth immediately.

### *5.2. C other rare forms of Hb abnormalities*

●If there's a substitution in amino acid number 26 in beta chain (glu → Lys), this will affect structure and quantity of Hb, resulting in Hb called HbE-beta

●HPFH (Hereditary persistence of fetal hemoglobin) is a benign asymptomatic condition characterized by elevated HbF in adulthood.

- It may beneficial for patients with sickle cell anemia, as HPFH decreases their requirements for beta chains.

## *5.3 RBCs metabolism*

●In biochemistry, RBCs are considered semi-dead cells, lacking nuclei, mitochondria and many organelles.

- However it survives 120 days in the circulation, and it has very important functions on O2, CO2 and H+ transport.
- But sadly, RBCs can't renew its own proteins, so it have to live with enzymes and proteins that have produced during maturation.
- Any problem in these enzymes will cause hemolytic anemia

●This topic is a very important topic, the source is some pages from Mark's Sources are marks and Lippincott's, page numbers are written in the slides.

●Figure 5 summarizes the major metabolic pathways in RBCs:

- **Glycolysis** is a very important metabolic pathway, we need it for:
  1- Production of ATP, that's important for phosphorylation, ion transport and priming reactions for Glycolysis.
  2- Production of 2, 3-BPG that's important for regulation of oxygen transport.
  3- Production for the substrates that's required in pentose-phosphate pathway.
- Pentose-phosphate pathway we need it for:

1. Production of NADPH that's important for protection against ROS and prevention of Methemoglobinemia...

●Final note: pentose phosphate pathway is very important in RBCs as it's the only source for NADPH in RBC.

- So G6PD deficiency Affects RBCs exclusively.

- Other Cells can produce NADPH by another pathway...

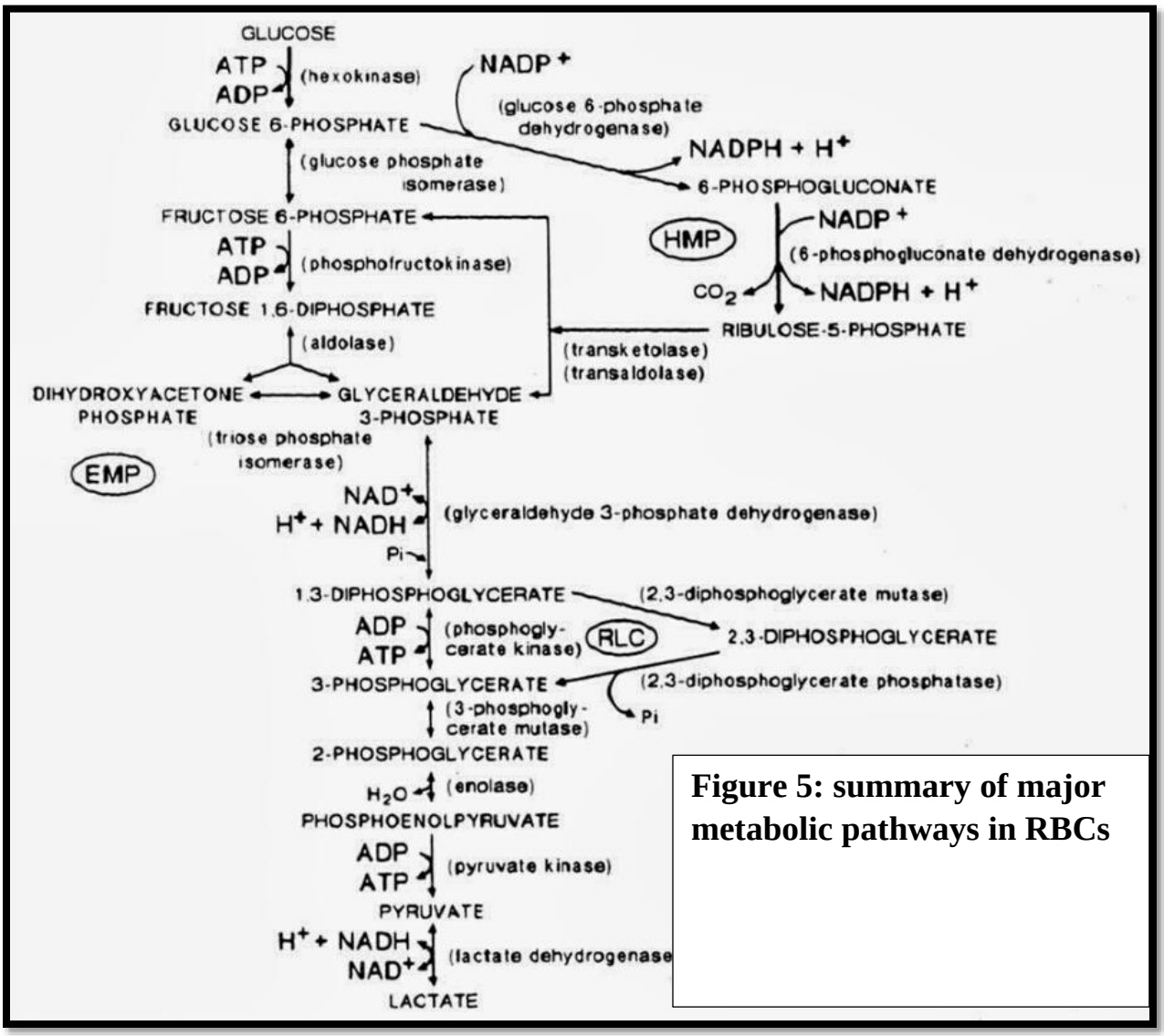


**Figure 5: summary of major metabolic pathways in RBCs**

●Good luck

●"Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning."

-Albert Einstein

●**THE END: D: D: D**