HEMATOLOGY
&LYMPH SYSTEM

Biochemistry sheet

Number	5
Done BY	Abdel-muez siyam
Correction	Abdullah nimer
Doctor	Dr. nayef

derivatives (mostly outside the book) that are of importance:

1. **Normal derivatives**:
   OxyHemoglobin
   DeoxyHemoglobin
   Carbaminohemoglobin
2. **Abnormal derivatives**:
   Methemoglobin (HbM)
   Sulphahemoglobin
   Carboxyhemoglobin (HbCO)

-Normally, **Methemoglobin** is present in blood, but its percentage is less than **1%**. When HbM level increases, it causes **methemoglobinemia**, which has two types:

I. **Inherited** (genetic): previously mentioned.

II. **Acquired**: caused by certain oxidizing *drugs* (drugs that oxidize chemical molecules, one of which can be **Fe^{+2}**, which can be oxidized to **Fe^{+3}**, forming **HbM**), certain oxidizing *chemicals* in the environment, or oxidizing agents in our *food*.

1. Inherited methemoglobinemia:

-Caused by a deficiency in **NADH-cytochrome b5 reductase** (Methemoglobin reductase), or abnormal hemoglobin forms (e.g. HbM in which the proximal histidine is replaced by tyrosine).

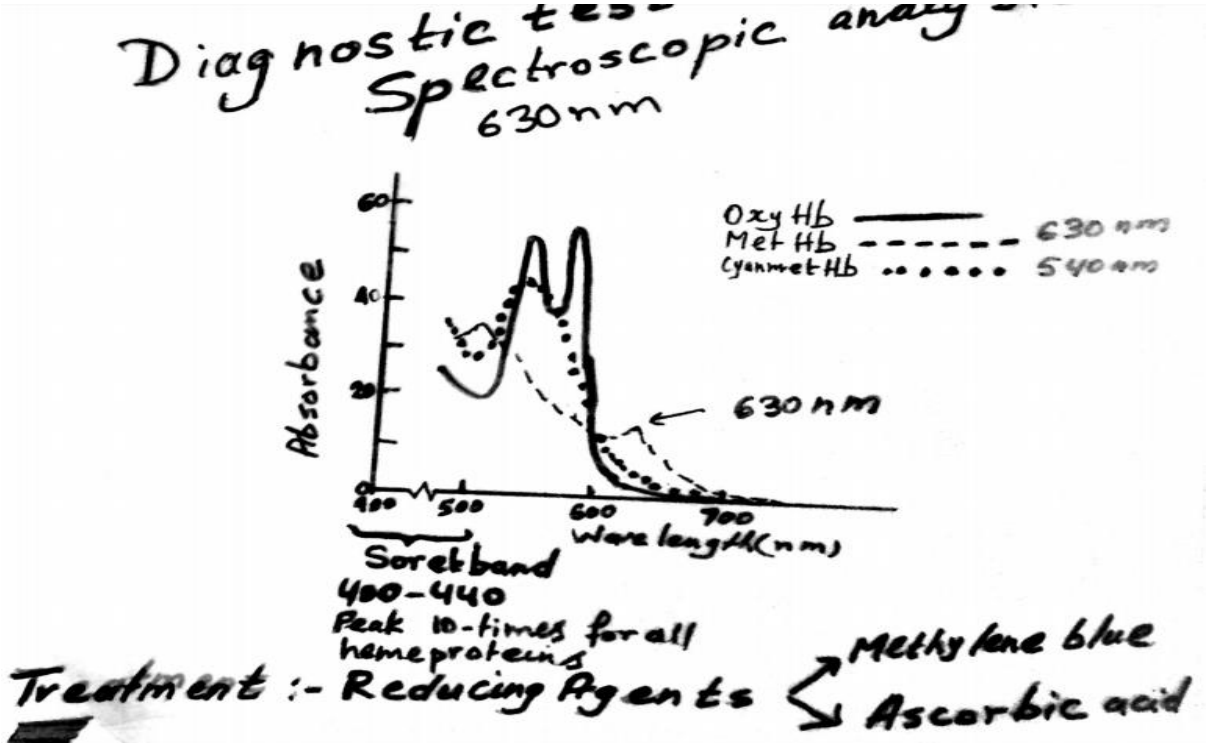
-**Symptoms**: cyanosis (bluish discoloration of skin and mucous membranes)

-**Diagnostic tests**: we perform a ***spectral (spectroscopic) analysis,*** by observing the absorption of light by the molecule at various wave lengths. We look for the absorption pattern above **500nm** wave length, and we will get:

1- **Oxyhemoglobin**: we'll see two peaks

2-**Methemoglobin**: small peak at first, then (***important***) It peaks at **630nm** (this peak ,when found, tells us that methemoglobin is detected).

3- **Cyanohemoglobin**: it is mentioned because in the lab when we measure the hemoglobin, it is first converted to methemoglobin, then we add **cyanide** producing cyanohemoglobin, and this is what we measure. Cyanohemoglobin peaks at **540nm**

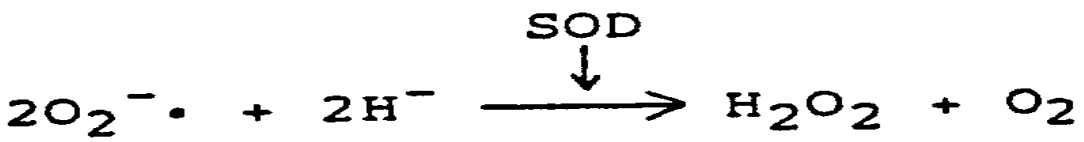


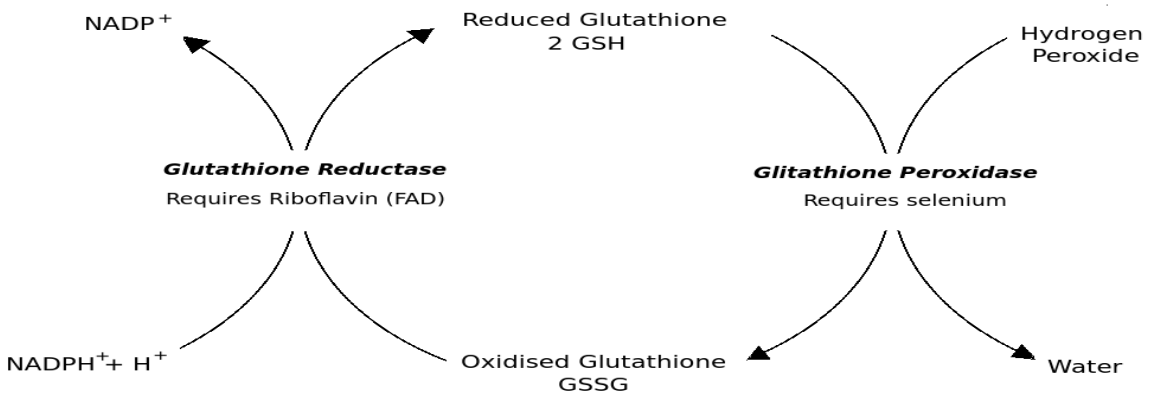
*Note: we observe the absorption at wave lengths starting from 500nm and above, because below that, **all heme containing proteins will have a very high peak**, this is called Soret band (very high peak at 400-440 nm) –not specific-.

-Methemoglobinemia treatment is using a reducing agent; as a first choice we use **methylene blue**, but if the patient has G6PD (glucose-6-phosphate dehydrogenase) deficiency we give **Ascorbic acid**.

→Antioxidant enzymes:

They reduce free radicals and reactive oxygen species.





Note that glutathione reductase is needed to re-reduce oxidized glutathione.

The presence of these enzymes is very important to remove the harmful effects of oxidants. One of these effects is the conversion of ferrous iron to ferric iron.

*SOD: superoxide dismutase.

Examples of oxidants:

1. Certain drugs like phenacitin and sulphonamides.
2. Chemicals like aniline, and excess nitrite
3. Oxidizing agents in diet.
   The produced ferric iron is reduced to ferrous iron by **methemoglobin reductase**, which is usually sufficient, except if there is an overload (excess production of methemoglobin) that exceeds the enzyme's capacity.

2. Sulphahemoglobin:

-Produced when methemoglobin is produced (it is produced by the same substances that produce methemoglobin) but only **in the presence of sulfide ions/ sulfur containing compounds**, like **H2S** gas. So usually methemoglobin and sulphahemoglobin are present together.

-Both methemoglobin and sulphahemoglobin **cannot carry oxygen**.

-Methemoglobin can be reversed back to normal hemoglobin, but Sulphahemoglobin can't.

3. Carboxyhemoglobin: (DANGEROUS)

-It is Hb bound to carbon monoxide (CO), which has bad effects on our body, one of which is the inhibition of complex IV in the electron transport chain, reducing ATP production.

-CO affinity to bind hemoglobin is **210 times more than that of oxygen**, meaning that if our air contains only 1% CO, it will be fatal to humans in minutes.

-The exhaust of cars contains CO. Sometimes –especially in cold places- when one wants to heat up his car (old one) before riding it, if it is done in a closed garage, CO level increases in the garage air, and it could be fatal on that person.

-Normally, around **1%** of hemoglobin in the circulation is bound to CO (for non-smokers), but for smokers, the percent rises to reach **10%** or more. If this percent reaches **40%**, it causes unconsciousness and **death**.

-CO binds heme in the oxygen binding site. If we take **free heme** (without the globin part), **its affinity for CO is 20,000 more than that of oxygen**. The presence of the globin part (**especially the distal histidine**) reduces it to **210**. In the past, it was believed that this histidine, due to its large size, makes CO binds at an angle less than 180^{o}, so by its steric hindrance it weakens the CO-heme bond, and this is the explanation in the drop-in affinity. However, it was recently discovered that this is not the case, because the final CO-heme bond angle equals 172^{o} (not that far from 180^{o}, so not that week), so it must be something else, but what?

It was found that the oxygen molecule bound to heme (which consists of 2 oxygen atoms like this: heme—O—O) will concentrate its negative charge on the oxygen atom not directly bound to heme. This will make this **oxygen make a hydrogen bond with the distal histidine**, **stabilizing the oxygen binding to heme**. The proof of this finding is that if we make mutant hemoglobin containing a non-polar atom instead of the distal histidine, **CO affinity will increase by 100 folds** compared to the normal one.

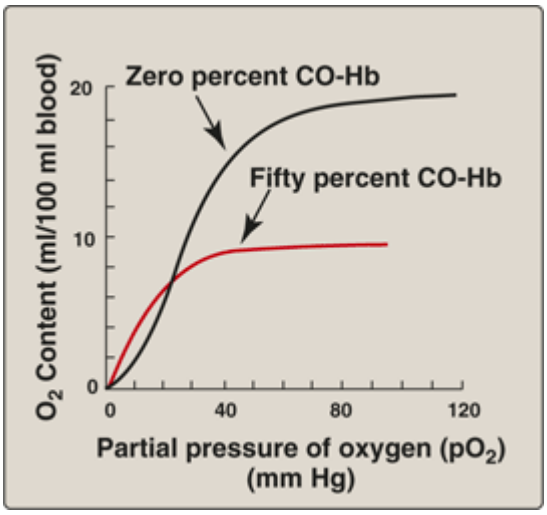
HEME——O——O^{-}……$^{+}$H(Histidine)

-Fortunately, CO binds hemoglobin tightly but ***REVERSIBLY***. So, in CO poisoning, we give **100% oxygen at high pressure** (**hyperbaric oxygen**) to the patient, so that **oxygen can replace CO from heme**.

-What makes CO even worse is that when hemoglobin is bound to both CO and oxygen (some subunits are bound to CO and some to oxygen in the same hemoglobin molecule), **this molecule binds oxygen with higher affinity**, so it's **hard to release oxygen into tissues (low p50)**. So not only did we lose an oxygen-binding site for CO, even those binding oxygen are tightly bound, making hemoglobin ineffective in passing oxygen to tissues. When CO binds one or more sites in the hemoglobin molecule, it shifts it to the R conformation (high oxygen

affinity conformation), so it acts as a trap where it binds to oxygen and makes it hard to realise it.

-In the below figure, when 50% CO bound to hemoglobin, the curve became **hyperbolic** (having a higher oxygen affinity).



*Note: hemoglobin has a role in the transport of nitric oxide (NO), binding and releasing it. NO is a vasodilator.

****

Thalassemia:

-A globin gene contains **3 exons** (whether α or β) separated by introns. Transcribed mRNA undergoes processing, splicing to release the introns to yield the final mature RNA, which goes to the cytoplasm for protein synthesis.

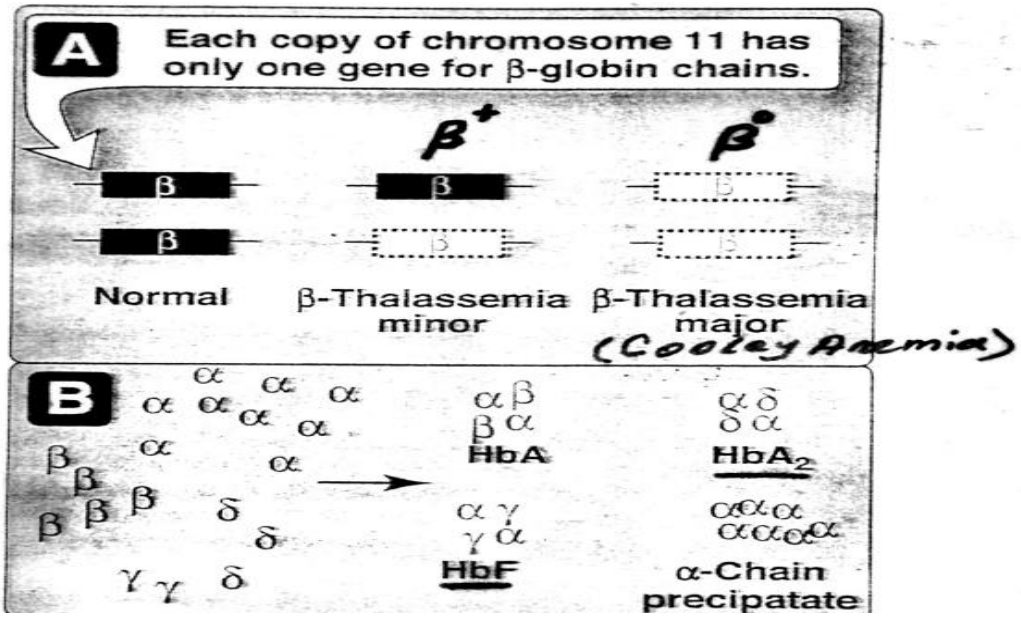
-Thalassemia refers to **decreased synthesis of either α or β chain**. If **↓α**, it's called α thalassemia, while β thalassemia results from **↓β** synthesis.

-The rule is: when α synthesis is reduced, we'll have excess β chains and vice versa. Those excess chains (especially α chains) tend to precipitate, causing hemolysis and hypochromic anemia.

I. β thalassemia:

-For β chains, we only have a pair of genes (one gene on each chromosome 11).
When both are normal (expression level is normal)→ no thalassemia
If one copy (one gene of the two) is defective→ **β thalassemia minor**
If both genes are defective→ **β thalassemia major**.

*Note: when one active gene and one defective gene, we donate it as **β^+ (minor)**. If both genes are defective, we donate it as **β^o (major).**



-In β thalassemia, there **is lack of β and excess of α**. As a result, the body compensates the lack of β by **activating ɣ (gamma) and δ (delta) genes**. These globins are β-like globins.

-This is important for the diagnosis of β thalassemia: we will find elevated **HbF (α2ɣ2)**, because of gamma gene activation and **HbA2 (α2δ2),** because of delta gene activation.

-Excess α will make **tetramer** chains by itself (a tetramer protein composed of **four α chains**) which is called **Cooley's hemoglobin** (**α4**), and the developing anemia –in β thalassemia major- is called **Cooley's anemia**. These **α4** precipitate forming ***Heinz bodies,*** which damage the cell membrane and cause premature death of erythrocytes.

-The manifestations of β thalassemia are not that quick, meaning that when the child is born, he/she will look normal, due to the presence of **HbF (α2ɣ2).** When adult Hb replaces fetal Hb the problem manifests. So, severe anemia usually develops at the end of the 1st year, or between the 1st and 2nd year of life.

-**Causes of β thalassemia**: can result from several defects; point mutation in the promoter, or mutation in the translation initiation codon, or a point mutation in the polyadenylation signal, or mutations causing splicing abnormalities.

II. α Thalassemia:

-There are two pairs of genes for the α chains (two copies of α gene on each chromosome 16) so in total there are 4 genes.

If 3 normal, 1 defective → silent carrier, no clincal manifistations.

If 2 normal, 2 defective → **α thalassemia trait** (heterozygous form), show mild symptoms, the 2 defective genes can be from the same chromosome or each from each homologous chromosome.

If 1 normal, 3 defective → **Hemoglobin H disease** (variable severity, but generally more severe than the heterozygous form).
If 4 defective→ **fatal at birth or before** due to total loss of α chains.

- Low α, excess β which binds as tetramers (**β4**) that are called **HbH**. B4 doesn't precipitate as readily as α4 **because it's more soluble**, but the β4 problem is that it binds oxygen **with high affinity** (not sigmoidal plot), rendering it a useless deliverer of oxygen (oxygen will be harder to release, low p50).
- Due to α chain deficiency**, ɣ chains also make tetramers (ɣ4)** which are called **Hb Bart** (results when all four α genes are defective→no HbF is made→ ***Hb Bart*** formation and **hydrops fetalis** causing fetal ***death*** or at birth).
- Note: **Cooley's Hb**: α4. // **HbH**: β4./// **Hb Bart**: ɣ4.
- **Causes of α thalassemia**: gene deletion.

Other abnormalities:

***Very Rare**: the combination of thalassemia as well as structural abnormalities in Hemoglobin. It is called **HbE.** In HbE, there is a **quality/structure** defect (the 26th amino acid in the β chain glutamate has been replaced by **Lysine**), and a **quantitative defect** (only 60% of the **β chain** is synthesized)

*HbHF: the hereditary persistence of HbF. The adult body continues to make HbF but it's benign and with no symptoms.

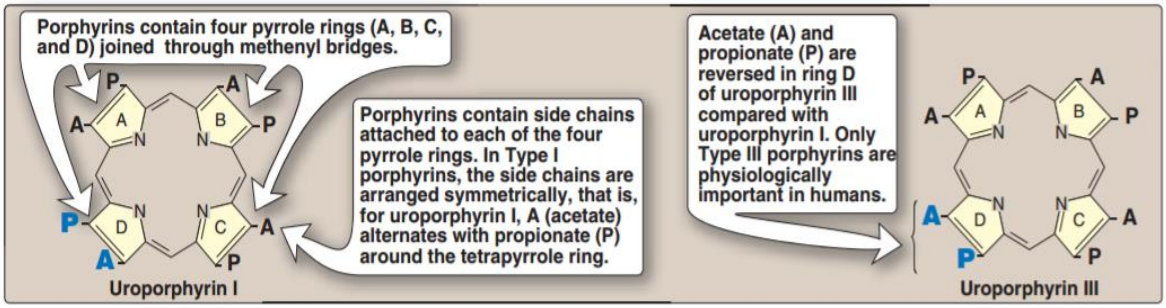
****

Heme synthesis:

-**Porphyrins**: cyclic molecules formed by the linkage of **4 pyrrole rings**. The pyrrole rings are usually attached to side chains, which specify different porphyrins.

-Heme is composed of **iron molecule as well as 4 pyrrole rings** which are called **protoporphyrin IX** (a type of porphyrins). There are two isomers of interest of porphyrins, which differ in their distribution of chemical groups on each ring. **Isomer I** has symmetry (can be divided into two identical halves, so groups are alternating), while **isomer III** doesn't have it (this is the physiologically important).

To understand, take this porphyrin (called uroporphyrin) as an example:



-Porphyrins are **colored**, but when they are reduced they are called porphyrinogen, which are **colorless**.

-This heme (isomer III + Fe) is the most prevalent in humans, and it is the prosthetic group of many proteins: hemoglobin, myoglobin, catalase, cytochrome, nitric oxide synthase…etc.

-Heme biosynthesis takes place in all mammalian tissues, but it is a major process particularly in **the liver** (where many hemeproteins are produced like cytochrome) and **erythrocyte producing cells of the bone marrow**. Around **85% of the heme made is made in the bone marrow**. Moreover, during the turnover of RBCs, every day we make 6-7 grams of hemoglobin.

-Heme synthesis takes place partly in the **mitochondria**, and partly in the **cytoplasm**.

-In order to make the 4 pyrrole rings, we need **8 glycines and 8 succinyl-CoA** molecules (TCA cycle intermediate).

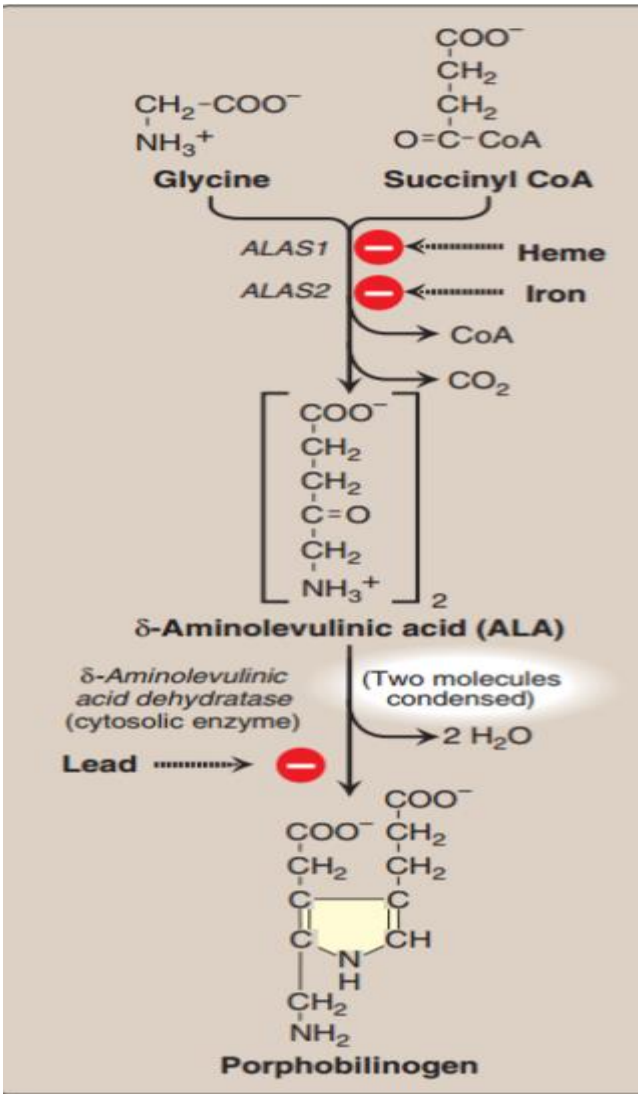
- One glycine and one Succinyl CoA condense in the mitochondria by the action of ***δ-Aminolevulinic acid Synthase (ALAS)***, and then by the loss of the CoA and the

CO2, **δ-Aminolevulinic acid (ALA)** is formed. **This condensation is the rate limiting step in the heme synthesis pathway.** ALAS is encoded by two different genes and each one's regulation is different from the other:

*__ALAS1:__ present in the liver, it is inhibited by the product of the pathway (**hemin**). When excess heme is formed, it is oxidized to hemin (contains ferric iron). This hemin **reduces heme synthesis by affecting ALAS1** in multiple ways:

1-It inhibits the transcription of ALAS1 gene ,2-it increases the degradation of ALAS1 mRNA. 3- it reduces the import of the enzyme from the cytosol to the mitochondria –where it will function-.

*__ALAS2__**:** present in erythroid cells. The 3 regulatory actions of hemin do not affect ALAS2. This enzyme is regulated by **the hormone erythropoietin** and **the presence or absence of iron (iron inhibits it).**



Notes:

1- ALAS requires **pyridoxal phosphate (PLP)** as a **cofactor**.

2- Heme embedded in globin is protected from oxidation. As soon as it gets out and becomes free, it is **fastly oxidized to hemin**.

3- ALAS2 mutations cause **X-linked sideroblastic anemia** (anemia caused by **low heme**).

-The produced ALA leaves the mitochondria to the **cytosol**, where ***ALA dehydratase*** (other name: porphobilinogen synthase) condenses **2 ALA** molecules and removes **2 H2O** molecules to yield **porphobilinogen** (the 1st pyrrole ring). This enzyme requires **zinc** to function, and therefore this enzyme is **very sensitive to inhibition by heavy metals,** especially **lead**- because **Pb** replaces **Zn** rendering the enzyme **non-functional.** This is partly responsible for the anemia seen in lead poisoning. ALA dehydratase enzyme is composed of 8 subunits.

-So, to make the 1st pyrrole ring, we needed 2ALAs, each needed 1 glycine and 1 Succinyl CoA. we'll need 8 glycines and 8 succinyl CoA in total.

The End