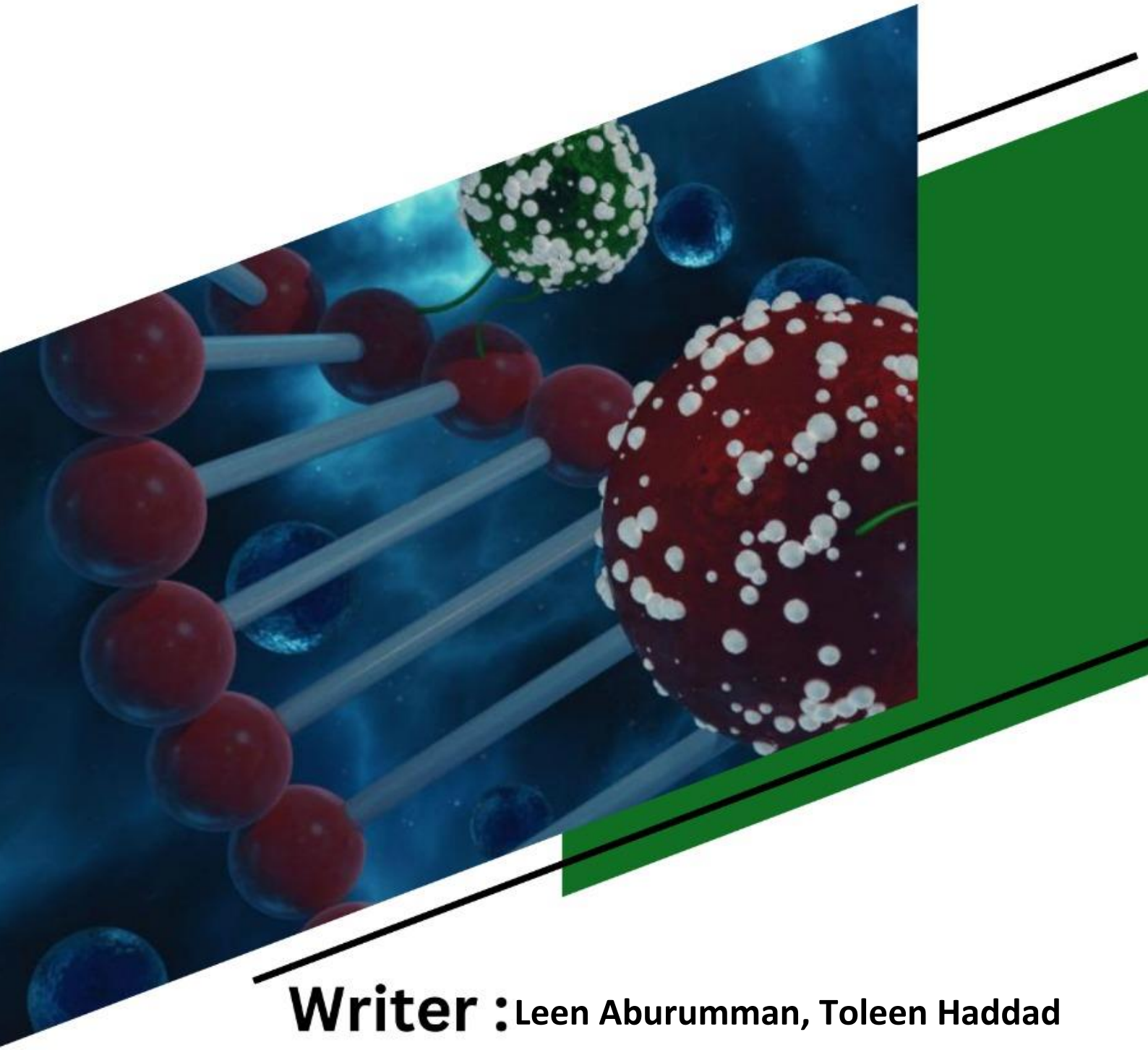
Doctor 021

METABOLISM

Sheet no. 31



Writer : Leen Aburumman, Toleen Haddad

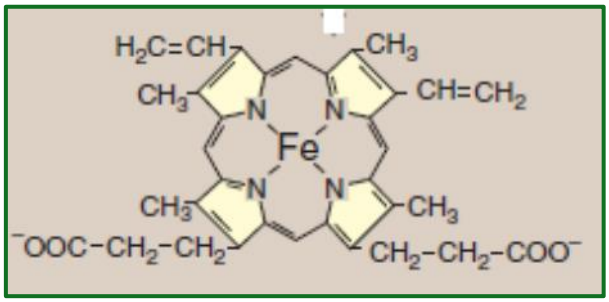
Corrector : Noor Abu Hantash

Doctor : Diala Abu-Hassan

AMINO ACID METABOLISM: CONVERSION OF AMINO ACIDS TO SPECIALIZED PRODUCTS

One of the depletion routes of amino acids is using them for the synthesis of other nitrogen containing compounds.

PORPHYRIN



- **Porphyrins are cyclic compounds that readily bind metal ions (Fe2+ or Fe3+).**
- **The most prevalent metalloporphyrin in humans is heme.**
- **Heme is found in hemoglobin, myoglobin, the cytochromes, catalase, nitric oxide synthase, peroxidase,** and many other proteins**.**
- **Hemeproteins are rapidly synthesized and degraded.**
- **6–7g of hemoglobin are synthesized each day to replace heme lost through the normal turnover of erythrocytes.**
- The name Porphyrin is related to the color purple (in Latin), because a lot of heme and porphyrin related diseases cause purple pigmentation, however, these diseases are rare.
- Ponder the figure: the general structure of porphyrins is one ring which contains four small five membered rings (pyrroles), an iron atom in the middle and eight side chains (two on each ring).
- In heme, the iron atom should be ferrous (+2) in order for it to be able to carry O_2. If it was ferric (+3), it wouldn't be active and it is called hemin.
- Heme is flat so it is considered rigid, but the iron atom in the middle can move when it binds O_2; so, the heme group can alternate between two forms: oxygenated and non-oxygenated.
- Heme is mostly hydrophobic except the iron atom, which has a charge of (+2)(even the carboxyl groups at the side chains will be interacting with other charged molecules so the overall structure of heme is hydrophobic).
- The ferrous atom can make 6 bonds, four of which are with the nitrogen atoms of the four pyrrole rings.

- The four pyrrole rings are identical except their side chains, (again, ponder the figure, there are methyl groups, vinyl groups and propionate groups).
- Porphyrins are usually synthesized as precursors (inactive form) and they are called Porphyrinogens (gen = generator of).

STRUCTURE OF PORPHYRINS

The medical significance of porphyrins is related to the following structural features of these molecules:

1. Nature of the side chains that are attached to each of the four pyrrole rings. The type and order of these side chains are used to distinguish between different porphyrins.

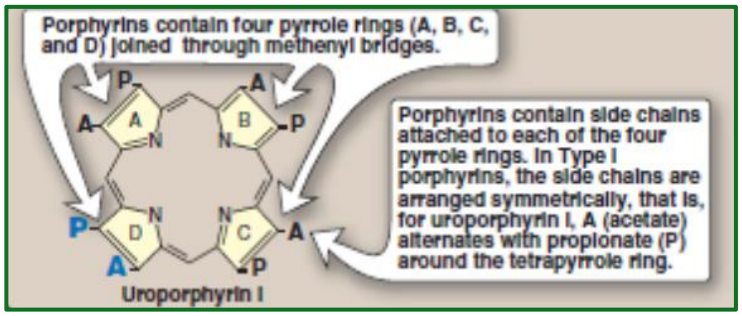
- **Uroporphyrin contains acetate (–CH2–COO–) and propionate (–CH2–CH2–COO–)**
- **Coproporphyrin contains methyl (–CH3) and propionate groups**
- **Protoporphyrin IX (and heme) contains vinyl (–CH=CH2), methyl, and propionate groups.**

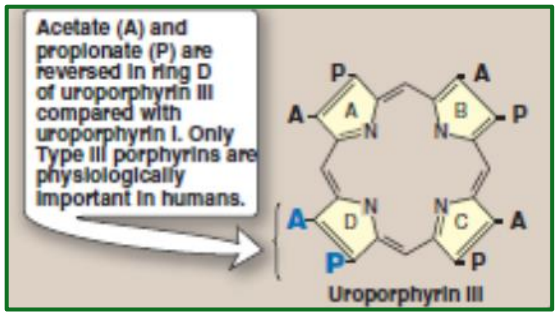
2. Distribution of side chains around the tetrapyrrole nucleus.

- **Four different ways (I to IV)**
- **Only Type III porphyrins (asymmetric substitution on ring D) are physiologically important in humans.**

3. Porphyrinogens (porphyrin precursors) exist in a chemically reduced, colorless form, and serve as intermediates between porphobilinogen and the oxidized, colored protoporphyrins in heme biosynthesis. (The doctor mentioned that Uroporphyrin I is an intermediate of heme biosynthesis)

ponder the figures!!





BIOSYNTHESIS OF HEME

The major sites of heme biosynthesis are:

1. Liver (cytochrome P450, myoglobin, catalase and peroxidase**), variable rate depending on demands for heme proteins,** but it constitutes almost 15% of all heme synthesis.

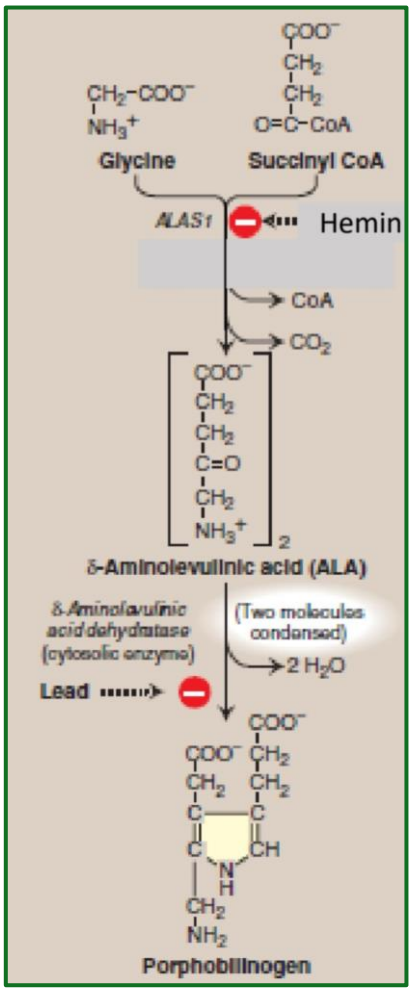
2. Erythrocyte-producing cells of the bone marrow (mostly used to synthesize **hemoglobin), more than 85% of all heme synthesis.**

- The bone marrow is the major site of synthesis because it has the precursor cells (erythrocyte-producing cells).
- **The initial and last steps in porphyrins formation occur in mitochondria**
- **The intermediate steps occur in the cytosol**
- **Mature RBCs lack mitochondria and are unable to synthesize heme.**

THE REACTIONS:

1.Formation of δ-aminolevulinic acid (ALA)

The rate-limiting step in porphyrin synthesis: the slowest step, highly regulated, inhibited by hemin and heme.



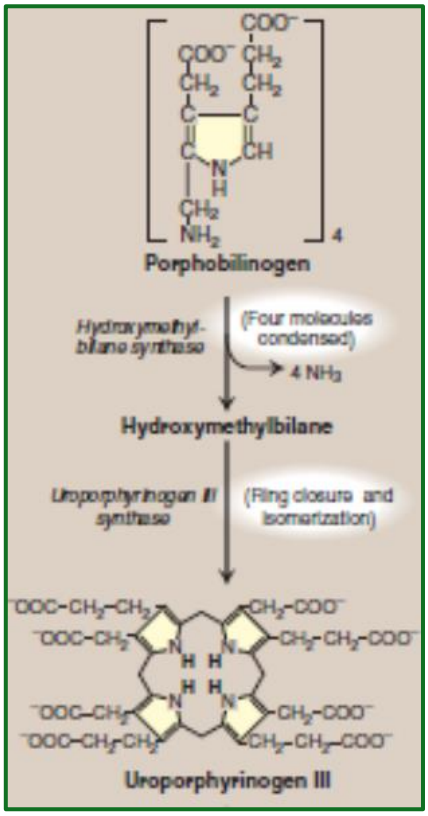
- The substrates are Glycine (the simplest amino acid) and succinyl CoA (produced from the metabolism of some amino acids, such as: threonine)
- The enzyme name is: δ-aminolevulinic acid synthase (ALA synthase).
- ALA synthase I: mainly found in the liver (can be produced by other cells).
- ALA synthase II: specifically found in erythrocyte-producing cells
- This is a building step, and this makes sense because we are building heme (very large) from simple molecules.
- The products of the reaction: δ-aminolevulinic acid (ALA), CoA and CO2. This step occurs in the mitochondria.

2. Formation of porphobilinogen

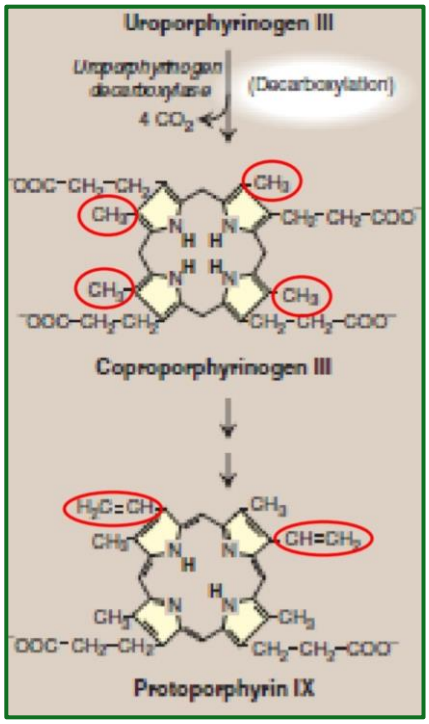
- Step #1 is repeated 8 times forming 8 molecules of ALA. Each two molecules will condense with each other forming one ring, called porphobilinogen (later it will form a pyrrole ring and its branches will be the side chains).
- The enzyme name is: δ-aminolevulinic acid dehydratase, it works by removing two water molecules to form the ring, and it is inhibited by lead. Lead poisoning stops heme synthesis which will affect oxygen transport inside the body; this will cause damage to the brain as it's the most sensitive to oxygen.
- **ALA is elevated in the anemia seen in lead poisoning.**
- This step occurs in the cytosol.
- *Side note*: The brain has a very small ability to regenerate; however, it can never go back to its original state. Children are usually the most susceptible to lead poisoning as lead is used in their toys (especially recycled ones).

3. Formation of uroporphyrinogen: The condensation of four porphobilinogens produces the linear tetrapyrrole, hydroxymethyl bilane.

- Four ammonia molecules are produced as by-products.
- **Hydroxymethyl bilane is isomerized and cyclized by uroporphyrinogen III synthase to produce the asymmetric uroporphyrinogen III.**
- Acetate and propionate will protrude as side chains from each pyrrole ring.
- **These reactions occur in the cytosol.**
- **The cyclic hydroxymethyl bilane** (uroporphyrinogen III) **is decarboxylated** by uroporphyrinogen III decarboxylase **(of its acetate groups** producing methyl groups**) generating coproporphyrinogen III**

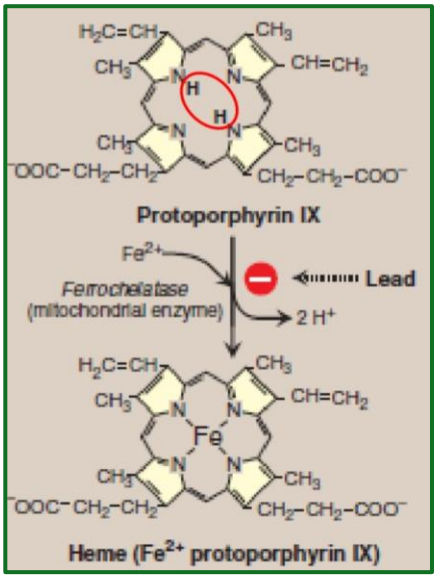


- Note that heme does not contain acetate! It has four methyl groups, two propionate groups and two vinyl groups.



- **These reactions occur in the cytosol.**
- **Coproporphyrinogen III enters the mitochondrion**
- **Two propionate side chains are decarboxylated** (and oxidated by removing hydrogens) **to vinyl groups generating protoporphyrin IX.**
- protoporphyrin IX is the first molecule to be produced in the mature form (it is not a gen!).
- Note that this molecule has the same side chains as heme, and we only need to remove the hydrogens in the middle and replace them with an iron atom.

4. Formation of heme:



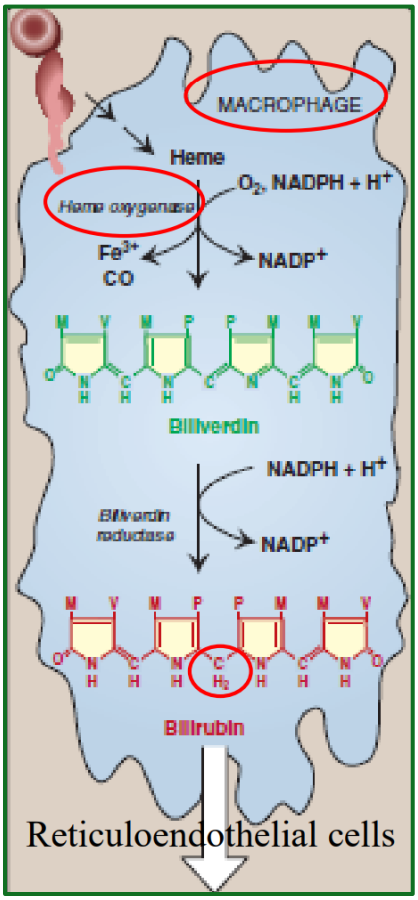
- **Protoporphyrinogen IX is oxidized to protoporphyrin IX.**
- **The introduction of iron (as Fe2+) into protoporphyrin IX.**
- **occurs spontaneously.**
- **The rate of Fe addition is enhanced by ferrochelatase (an enzyme that is inhibited by lead).**
- This reaction occurs in the mitochondria.
- Heme is also called Fe $^{+2}$ protoporphyrin IX.
- <u>To clear things out: 1st step + last 2 steps occur in the mitochondria (rest in cytosol).</u>

HEME DEGRADATION

RBCs are degraded by the reticuloendothelial system (liver and spleen)

- RBCs are renewed every 120 days. (4 months)
- We don't recycle RBCs in our bodies; groups of heme in hemoglobin that become senescent aren't reused but rather degraded and new heme is made instead.
- The same thing applies to the protein part (globulin); we need to make new ones.
- Why so though?
  - Because the cell itself is senescent; therefore, all of its components are senescent as well and the overall functionality is not as high. You need brand-new functional molecules!!
- Degradation usually begins in different types of macrophages that form a system called the reticuloendothelial system. The reticuloendothelial system is basically made of groups of macrophages present in the whole body; they're not necessarily beside each other. Eg: blood, liver (Kupffer), spleen, etc.
- RBCs burst and release all their contents. The protein part is degraded by the pathways specific to it and the heme group will be dealt with by the macrophages.
- **85% of degraded heme comes from senescent RBCs**
- **15% of degraded heme comes from immature RBCs turnover and cytochromes of nonerythroid tissues.**
  - Myoglobin and cytochromes are involved as well. Cytochromes deal with detoxification so they're prone to get affected by these toxins; therefore, we need to degrade the protein and the heme group attached to it.

1. Formation of bilirubin:



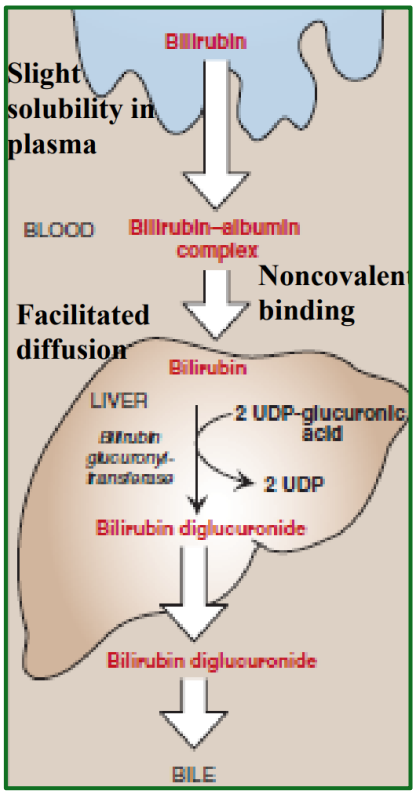
A. Biliverdin formation by the addition of an OH to the methenyl bridge between two pyrrole rings, and then a second oxidation by the same enzyme system to cleave the porphyrin ring.

- First, I need to open the big porphyrin ring structure to get a linear structure instead.
- Enzyme: heme oxygenase. Obviously, I need oxygen.
- We will also consume NADPH.
- **Products: the green pigment biliverdin, ferric iron (Fe3+) and CO**

B. Biliverdin reduction to bilirubin (red-orange)

- **Bilirubin and its derivatives are called bile pigments.**
- **Bilirubin functions as an antioxidant (oxidized to biliverdin)**
- This happens by biliverdin reductase; we also need NADPH. Notice that RBCs renewal needs NADPH (apart from the oxidative stress mechanisms that use NADPH as well).
- The overall structure of bilirubin is highly hydrophobic and insoluble.
- As you know, water-soluble structures can easily move around and get excreted in urine. We need to find a way for the hydrophobic ones though. The metabolites of heme are colored; luckily, this is something you can make use of.
- We need to transfer bilirubin to hepatocytes to transform it to a soluble format.

2. Uptake of bilirubin by the liver:



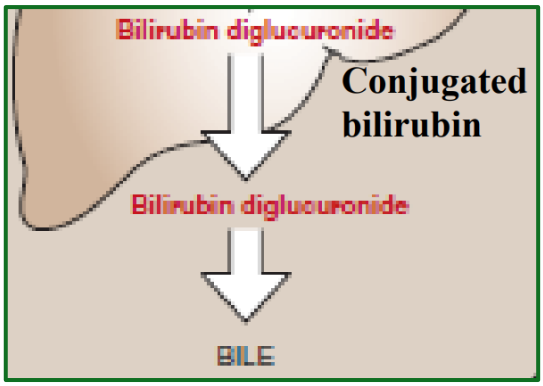
- Bilirubin binds to albumin by noncovalent bonds and moves through blood. Albumin is released into the bloodstream and bilirubin enters hepatocytes by facilitated diffusion.
- **In hepatocytes, bilirubin binds to intracellular proteins, such as, ligandin.**

3. Formation of bilirubin diglucuronide

(Also called conjugated bilirubin which is bilirubin's soluble format):

- **Two molecules of glucuronic acid are added to increase solubility (conjugation) by bilirubin glucuronyl-transferase.**
- UDPs are released.
- **Deficiency of this enzyme results in Crigler-Najjar I and II (more severe) and Gilbert syndrome.**
  - Gilbert syndrome is less severe; it is very common and it's not easy to diagnose. People with this syndrome find fasting exhausting and their faces appear yellowish (not as much as jaundice though). Nevertheless, they live a normal life.

4. Secretion of bilirubin into bile:



- **Conjugated bilirubin is actively transported into the bile canaliculi and then into the bile.** (Canaliculi are small canals that coalesce to form a large canal)
- Bilirubin is now in the gall bladder.
- Gall bladder stores bile secretion. Bile secretion is yellow because of bilirubin.
- Having colors is much better than transparency as it provides clinical indications.
- Bile secretion along with bilirubin spills into the small intestine (duodenum in precise).
- Bile secretion's function is emulsification to facilitate the digestion of lipid molecules. In other words, to get the enzyme and fats closer to each other.

- **The rate-limiting step (energy-requiring step).**
  - The transport of bilirubin outside is the rate limiting step; again, it's transport, not a reaction here.
- **Dubin-Johnson syndrome results from a deficiency in the transport protein of conjugated bilirubin.**
- **Unconjugated bilirubin is normally not secreted.**

5. Formation of urobilins in the intestine:

- **Bilirubin diglucuronide is hydrolyzed and reduced by bacteria** (flora) **in the gut to yield urobilinogen (colorless).**
- **Urobilinogen fates:**

1- Oxidation by intestinal bacteria to stercobilin (gives feces the characteristic brown color).

2- Reabsorption from the gut and entrance to the portal blood.

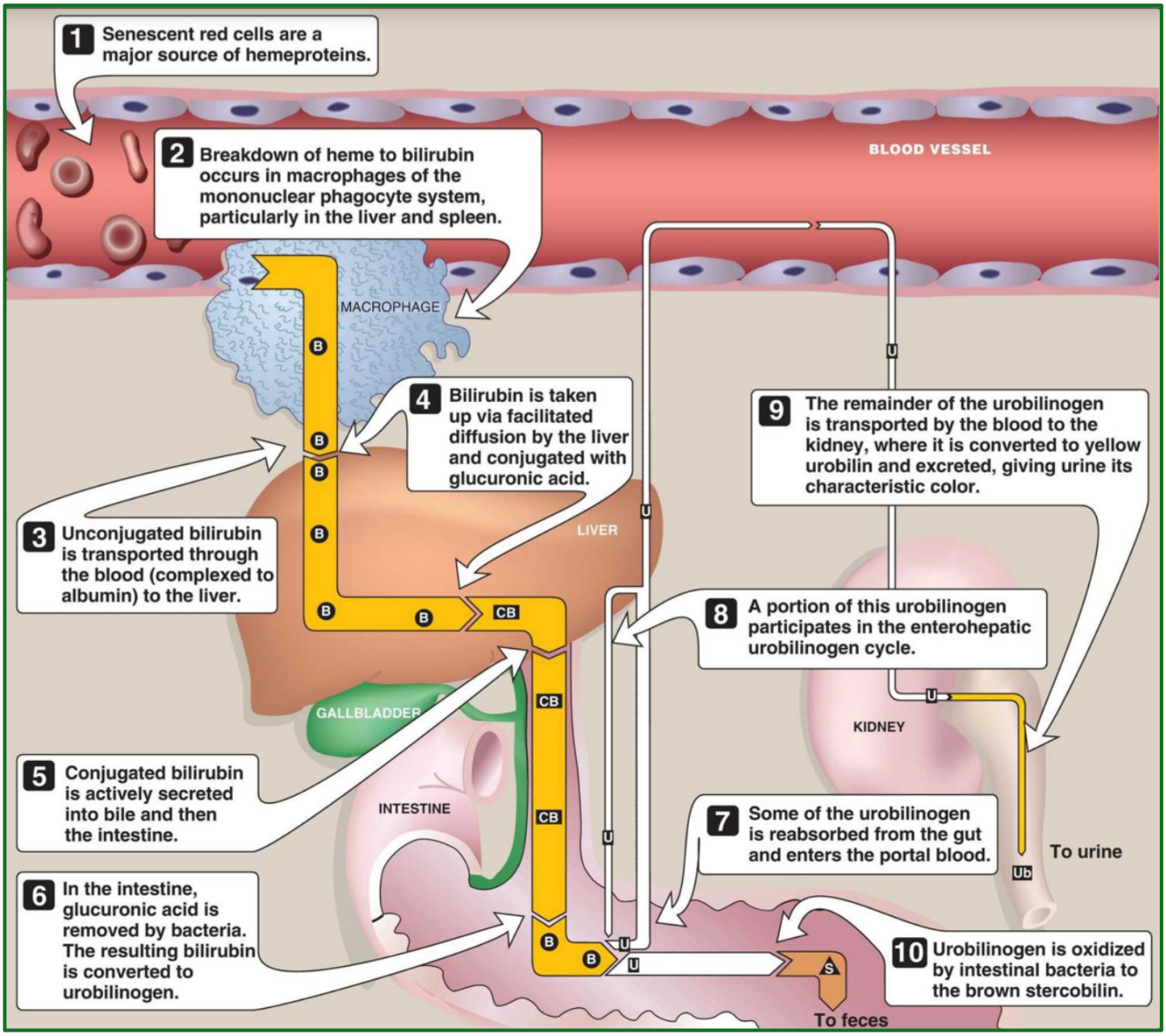
a. Some urobilinogen participates in the enterohepatic urobilinogen cycle where it is taken up by the liver, and then re-secreted into the bile.

b. The remainder is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.

- These colors give clinical indications.

CATABOLISM OF HEME

The following diagram is a summary of what we've discussed.



Notes on the figure:

2. The renewal process can start in any macrophage, but it mostly happens in the spleen. A person that has had his spleen removed can lead a normal life.

5. Liver has a specific capacity: 3g/day of bilirubin can be conjugated by hepatocytes. As a result, all bilirubin gets conjugated eventually.

Side note from the doctor:

The gall bladder is only a store; it doesn't produce anything. It secretes a great amount of bile at once for normal people. Therefore, people that have had it removed are not advised to eat very fatty meals. They obviously still have bile made by the liver, but it's released in small portions.

PAST PAPERS:

1. **Albumin binds all of the following except:**
   a. Free fatty acids
   b. Steroid hormones
   c. Conjugated billirubin
   d. Ca+2
   Answer: C

2. **Which of the following enzymes is inhibited by lead?**
   a. ALA dehydratase
   b. Ferroxidase
   c. ALA synthase
   d. Biliverdin reductase
   Answer: A

3. **Regarding heme synthesis in the liver, all statements are correct EXCEPT:**
   a. Uroporphyrinogen Ill is synthesized from porphobilinogen
   b. ALA synthase requires pyridoxal phosphate and located in mitochondria
   c. ALA synthase can be induced by many drugs
   d. Synthetic pathway involves carboxylation reactions at more than one step
   e. ALA synthase is suppressed by hemin
   Answer: D

4. **In the following table, which case mostly represents liver disease?**

a. I
b. II
c. III
d. IV
e. V

Answer: C

	I	II	III	IV	V
High blood levels of ALT and AST	No	No	Yes	Yes	Yes
High blood levels of bilirubin	No	Yes	Yes	Yes	No
High levels of plasma proteins	Yes	Yes	No	No	No
Edema	No	Yes	Yes	Yes	No
High levels of blood ammonia	No	Yes	Yes	No	Yes

BEST OF LUCK

V2

The changes that were made in this version are nothing major; they're only slight deviations between different sections. You'll find any corrections or additions underlined.