34

◉ Sheet ○ Slides

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Doctor

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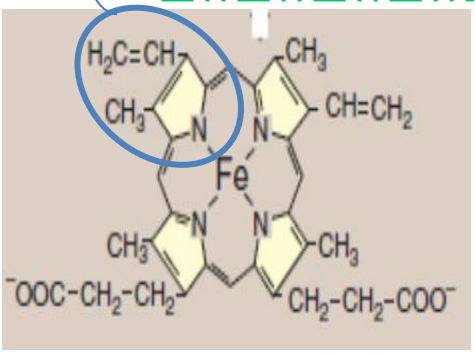
Hello there, in this lecture we'll talk about Heme, the irony unit in the heart of a number of proteins, stay tuned! It's going to get colorful towards the end.

Amino acids are used to synthesize a variety of other molecules with specialized functions. And today we'll talk about **Porphyrins.**

◆ Porphyrins' structure:

It's a **cyclic structure** composed of **four pyrrole rings**, connected together. In each ring there's a **nitrogen towards the center** of porphyrin structure and there are **two side chains coming out from each ring.**

This is a pyrrole ring with 2 side chains



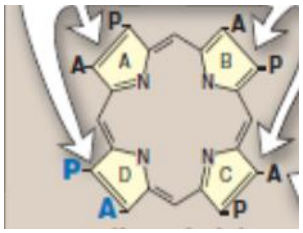
Heme: Is a **porphyrin with a ferrous atom** (Fe^{2+}) in the middle. It's the most prevalent metalloporphyrin in humans.

Heme is found in hemoglobin, myoglobin, the cytochromes, catalase, nitric oxide synthase, and peroxidase.

Hemeproteins are **rapidly synthesized and degraded**. 6–7g of hemoglobin are synthesized each day to replace heme lost through the normal turnover of erythrocytes

Porphyrins differ from each other in the side chains that come out of the pyrrole ring and the order or distribution of these side chains.

Example:

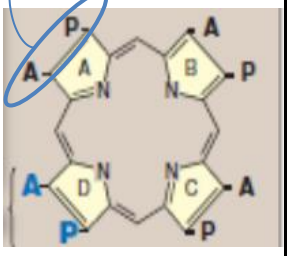


Uroporphyrin I (1)

On the sides of this box, we have the structures of two porphyrins: **uroporphyrin I & uroporphyrin III**.

There are **two types of side chains** that are **repeated on each pyrrole ring**, designated **A and P which stand for acetate and propionate**. How to differentiate between these two porphyrins? we look at the order of these side chains. On A, B & C pyrroles of both porphyrins, A and P are in the order A-P/A-P/A-P. However, **on the D pyrrole**: in **por. 1 the order is A-P** but in **por. 2 it's P-A**. this mild difference in order gave rise to a new different porphyrin.

Side chains



Uroporphyrin III (2)

Types of side chains that can be present in some medically important porphyrins:

- **Uroporphyrin** contains acetate (–CH2–COO–) and propionate (–CH2–CH2–COO–).
- **Coproporphyrin** contains 4 methyl (–CH3) and 4 propionate groups(–CH2–CH2–COO–).
- **Protoporphyrin IX (and heme)** contains 2 vinyl (–CH=CH2) groups, 4 methyl groups, and 2 propionate groups.
- **The nature** and the **distribution** of the **side chains** makes **different porphyrins**. There are Four different types of distribution (I to IV). Only **Type III porphyrins** (asymmetric substitution on ring D) are physiologically important in humans.

Porphyrins are first synthesized as **Porphyrinogens** (porphyrin precursors) which exist in a chemically reduced, colorless form, and serve **as intermediates between porphobilinogen and the oxidized, colored protoporphyrins** in heme biosynthesis.

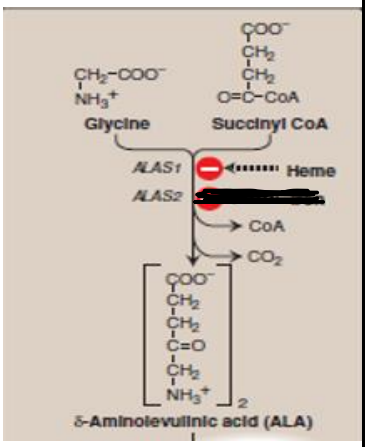
◆ Biosynthesis of heme:

1. **Liver. 15%** of heme production occurs in the liver, but it's mainly used for production of **cytochrome P450**, variable rate depending on demands for heme protein.
2. **Erythrocyte-producing cells of the bone marrow**. Are to account for more than **85%** of all heme synthesis. Heme produced here is used in **hemoglobin**.

Notice that **heme cannot** be synthesized in erythrocytes because they lack nuclei and mitochondria.

*The **initial and last** steps in porphyrins formation occur in **mitochondria.***
*The **intermediate steps** occur in the **cytosol.***

◆ Biosynthesis of porphyrin-heme in steps:



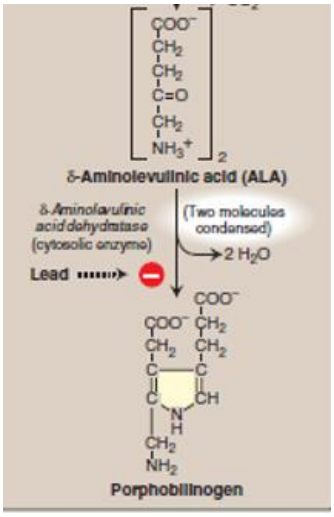
The journey starts with a **Glycine** (the star AA) and succinyl CoA (remember! It's also a final product of the carbon skeleton metabolism of several AAs like threonine).

Step 1: **we combine Gly with succinyl CoA and remove the CoA** to produce a molecule called **δ- aminolevuulinic acid (ALA)**. Catalyzed by $ALAS_{ynthase}$ with help of the coenzyme pyridoxal phosphate (PLP) (active vitamin B6).

Rate-limiting step in porphyrin synthesis
It occurs in the mitochondria.

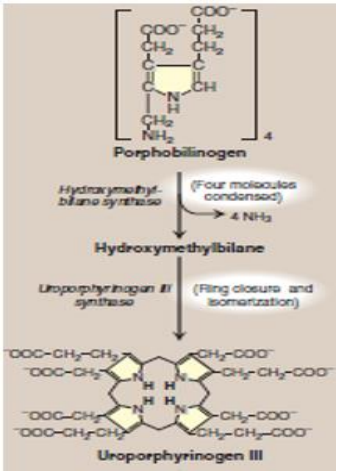
ALAS 1 → liver
ALAS2 → bone marrow

Step 2: **two ALAs are joined together** to form **porphobilinogen.**



Two ALA molecules are approximated, their functional groups interact with each other. By a **dehydration reaction** a **pyrrole ring structure forms,** ~~losing~~ 2 H_2O molecules. The resulting molecule is a porphyrin precursor called **porphobilinogen**. Catalyzed by Zn-containing ALA dehydratase (porphobilinogen synthase).

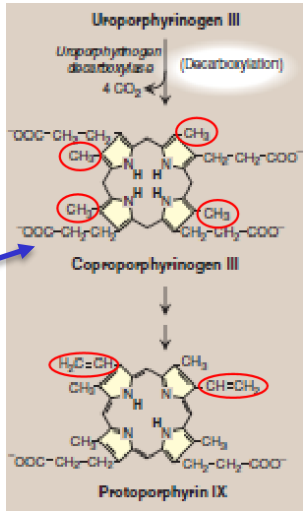
Occur in the cytosol



Step 3: **four porphobilinogens are condensed together** to eventually form **uroporphyrinogen III**.

First, the **4 molecules** are **condensed** by the **loss of 4 NH_2** to form the linear tetrapyrrole **hydroxymethylbilane** by the enzyme hydroxymethylbilane synthase

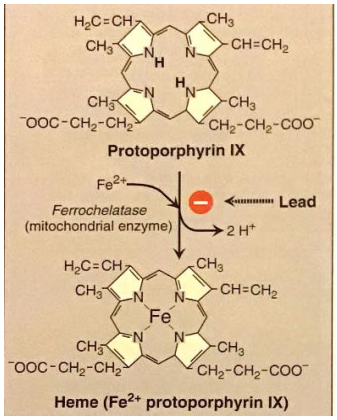
Then, **Hydroxymethylbilane** is **isomerized** and **cyclized** for ring formation and closure by uroporphyrinogen III synthase to produce the **asymmetric uroporphyrinogen III.** *Occur in the cytosol*



Step 4: **modifying the side chains to form heme.**

Heme contains propionate, vinyl and methyl, it **doesn't** contain acetate. So, we need to change things.

- **Decarboxylation** of the **acetates** of uroporphyrinogen to **form methyl** groups in their place by uroporphyrinogen decarboxylase forming a molecule called **coproporphyrinogen III.** *cytosol*
- Coproporphyrinogen III enters the mitochondria and **Two propionate** side chains are **decarboxylated to vinyl groups** (accompanied by oxidation of their bearing pyrrole ring) generating **protoporphyrinogen IX.** *mitochondria*



Step 5: **oxidation** of **protoporphyrinogen IX** to form **protoporphyrin IX**.

Notice: during the last step two hydrogens were removed from the center of the porphyrin. In this reaction we'll remove the remaining two hydrogens.

Fe^{2+} will spontaneously be introduced in the center.

However, the rate of Fe addition is **enhanced** by the enzyme **ferrochelatase.**

Now we have the final structure of heme **(Fe2+ protoporphyrin IX).**

- ## Regulation of heme biosynthesis:

  - **End-product inhibition of ALAS1 by hemin:**

When porphyrin production exceeds the availability of the apoproteins that require it, **heme** accumulates and **is converted to hemin** by the **oxidation of Fe^{2+} to Fe^{3+}**.

Hemin decreases the activity of hepatic ALAS 1 by reducing its synthesis (mRNA synthesis and mitochondrial import).

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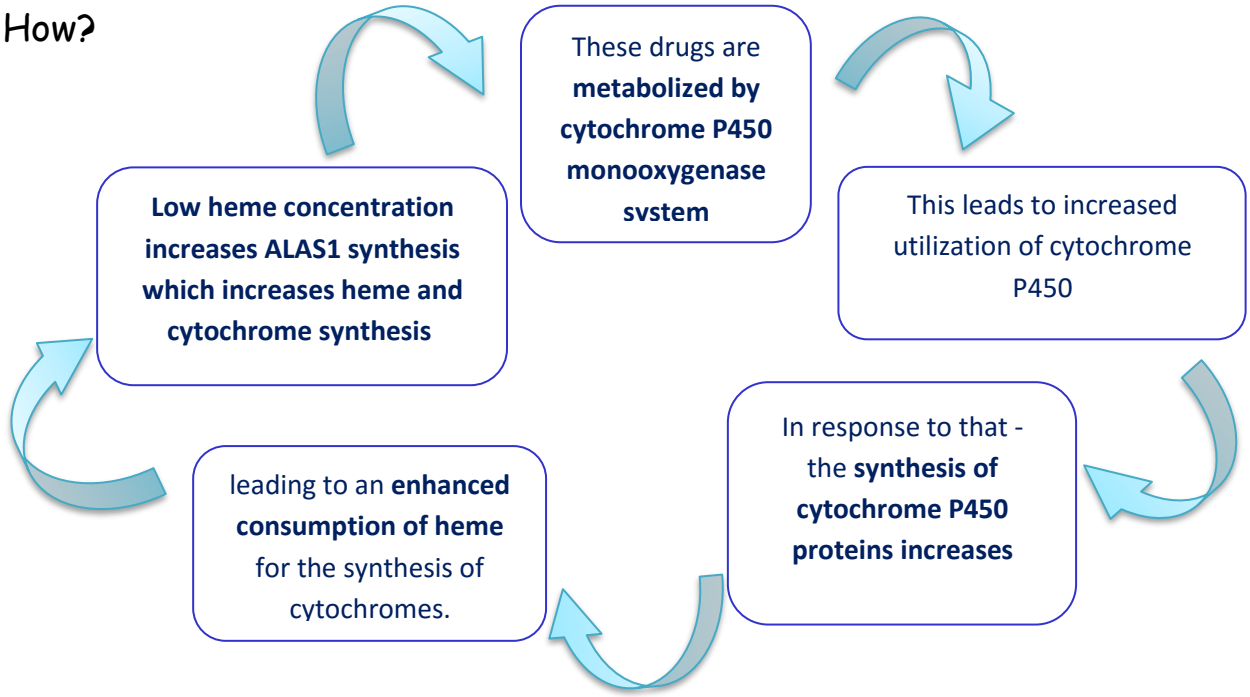
*An unnatural inhibitor of this pathway is **lead,** it's present in children's toys for example.*
*Lead acts on **the second step** (ALA dehydratase) and **the last step** (ferrochelatase). It inhibits the formation of heme, so it gives effects like anemia. This could lead to mental retardation in children.*

Drug interference: **Many drugs increase hepatic ALAS1 activity**.

How?



***cytochrome P450 monooxygenase system** a heme protein oxidase system found in the liver.

- ## Porphyrias:

"Porphyria" refers to the **purple color caused by pigment-like porphyrins in the urine of patients.**

- Each porphyria results from a different **enzyme deficiency** and results in the accumulation of different intermediates.

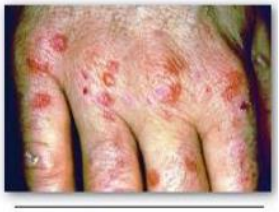


Figure 21.6
Skin eruptions in a patient with porphyria cutanea tarda.

Figure 21.7
Urine from a patient with porphyria cutanea tarda (right) and from a patient with normal porphyrin excretion (left).

They're Rare, inherited (or occasionally acquired) **defects in heme synthesis.**

→The result is Accumulation and increased excretion of porphyrins or porphyrin precursors.

Mutations are heterogenous (not all are at the same DNA locus), and nearly every affected family has its own mutation.

Biochemical changes: Increased ALA synthase activity and decreased synthesis of heme. Due to a stop **in the reaction at some point**, we **don't end up with the production of heme.**

In the liver, heme acts as a repressor of the gene for ALAS1.

Less heme results in an **increased synthesis of ALAS1** and **synthesis of intermediates upstream to ALA .**

Treatment: Treatment is **symptomatic** for pain and vomiting during acute attacks.

IV injection of **hemin and glucose**, which decreases the synthesis of ALAS1, which will stop exhausting the liver with producing an enzyme it actually has no use for, to **reduce symptoms and pain of the patients.**

Ingestion of **β-carotene** (a precursor of vitamin A that acts as a free-radical scavenger), because low synthesis of heme causes the oxidative stress to go up.

- ## Degradation of heme:

Every 120 days RBCs will get renewed, this will mean degradation of cells and release of contents including **hemoglobin.** The Globin part is going to be degraded through the ubiquitin-proteasome system because it's an **intracellular protein** and heme will be metabolized. (heme re not recycled nor reused in new protein nor RBCs).

❖ **Formation of** bilirubin:

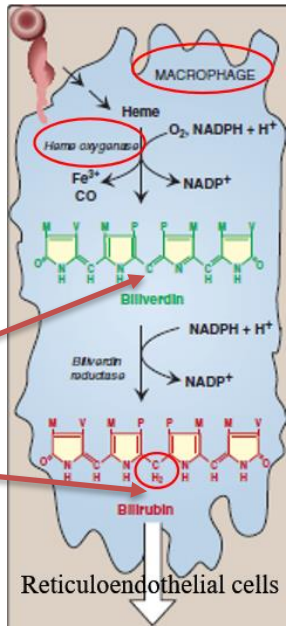
Degradation of heme **starts in macrophages**, it mainly occurs in the spleen, but it may use any cell in the reticuloendothelial system (group of macrophages distributed all over the body).

Degradation starts by opening the cyclization of the heme, to produce a structure called **biliverdin** by the enzyme heme oxygenase.

Biliverdin is reduced to **bilirubin** through **NADPH** by biliverdin reductase.

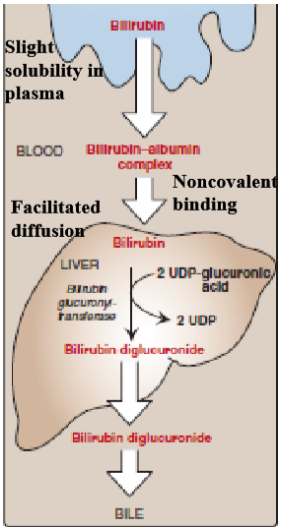
Bilirubin and its derivatives are called bile pigments.

Bilirubin functions as an antioxidant (gets oxidized to biliverdin).



So far, bilirubin's structure is insoluble in water. In order to make use of it we need to solubilize it in order to move it around freely. **How?** this happens by **bilirubin conjugation**. This **conjugation occurs in the liver**. We **transport bilirubin to the liver through noncovalent binding to albumin**.

Note: certain anionic drugs, such as salicylates and sulfonamides, can displace bilirubin from albumin, allowing bilirubin to enter the CNS causing a neural damage in infants.



It enters the liver through **facilitated diffusion**, by the force of the concentration gradient . **Hepatocytes** are where conjugation occurs.

Bilirubin enters

Two glucuronates will be carried by UDP and donated to the bilirubin
Diglucuronate bilirubin (**conjugated bilirubin**) will be formed. Enzyme:
Bilirubin glucuronyl transferase.

It's actively transported from hepatocytes to the canals of biliary system of **gall bladder**. *Rate limiting step in heme degradation because it needs energy* bilirubin is the colorant of bile, it gives it a **yellow color**.

Bile emulsifies lipids for digestion.

Bile salts (cholesterol derivatives) with the bilirubin get secreted **into the duodenum of the small intestines.**

By the normal flora bilirubin gets converted to **urobilinogen** (urobilin precursor) (colorless)

Part of it **oxidized to stercobilin**, it has a brown color, it gives the feces it's brown color and gets excreted with it.

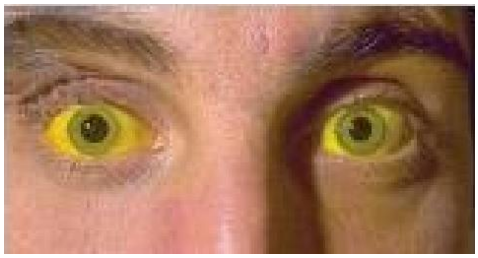
Reabsorbed to the liver

Some **urobilinogen** participates in the enterohepatic **urobilinogen cycle** where it is **taken up by the liver, and then resecreted into the bile**

And some **moves to systemic circulation** reaching the **kidney** where it gets converted to **urobilin** (yellow)

APPLICATIONS:

- Mutation in **bilirubin glucuronate transferase** → Deficiency of this enzyme results in **Crigler-Najjar I and II** (more severe) and **Gilbert syndrome** (less severe deficiency)
- **Dubin-Johnson syndrome** results from a **deficiency in the transport protein** of **conjugated bilirubin** (which **transports it from hepatocytes to bile**).



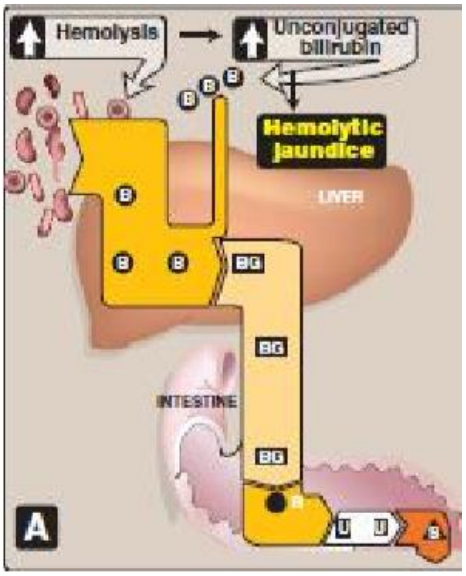
Jaundice: Jaundice (or icterus) is the **yellow color** of **skin, nail beds, and sclera** due to **bilirubin deposition** secondary to **hyperbilirubinemia.**

Jaundice is a symptom not a disease

There are different **types of jaundice**:

a. **Hemolytic jaundice**

When there's **excess degradation of RBCs** like in <u>Sickle cell anemia, pyruvate kinase or glucose-6-phosphate dehydrogenase deficiency</u>



BG = bilirubin glucuronide; B = bilirubin
U = urobilinogen; S = stercobilin.

There's a **release of heme mounts beyond the ability of hepatocytes to perform heme degradation**.

Bilirubin conjugation and excretion capacity of the liver is >3g/day

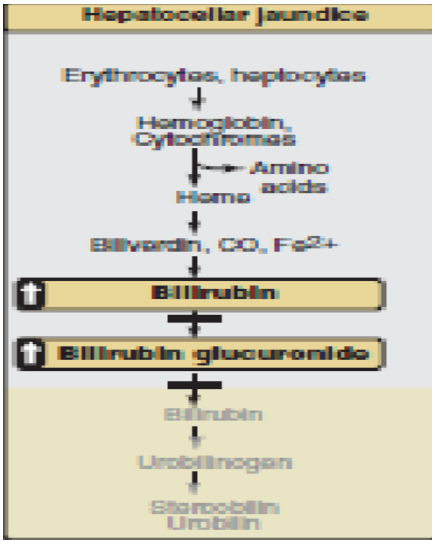
300 mg/day of bilirubin produced

(**accumulation of unconjugated bilirubin in this situation**)

b. **Hepatocellular jaundice**

Is due to **damage to liver cells**

Liver can't conjugate the regular three grams.

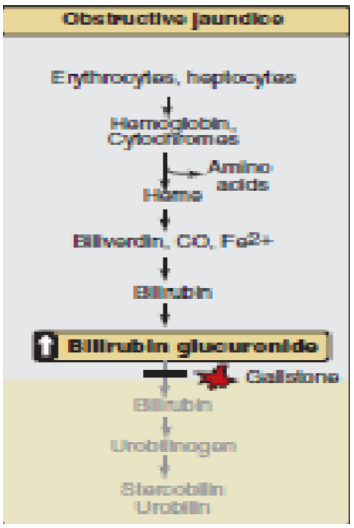


More unconjugated bilirubin levels in the blood

* remember liver is the conjugation site*

Urobilinogen is increased in the urine (the enterohepatic circulation is reduced) resulting in dark urine .

Stools may have a pale, clay color.



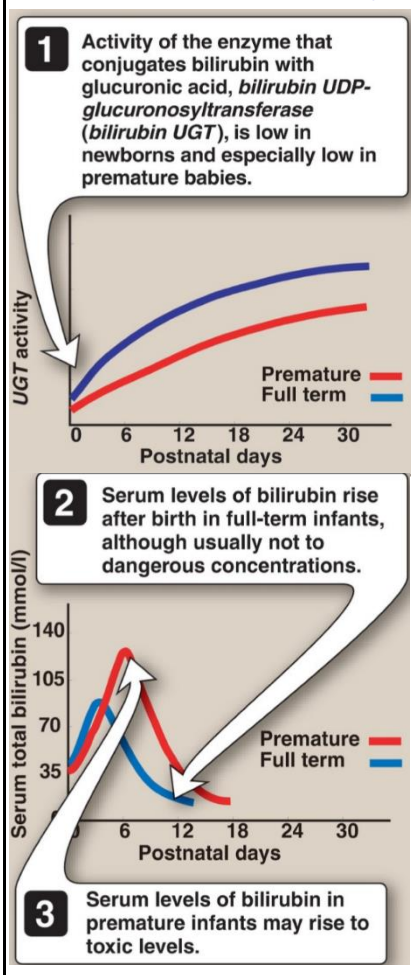
c. Obstructive jaundice.

Liver is normal, no excess heme to be degraded, no overproduction of bilirubin or decreased conjugation, but the **problem is with the secretion**.

Canals of the bile duct are blocked due to a tumor or bile stones, **preventing bilirubin passage into the intestine**.

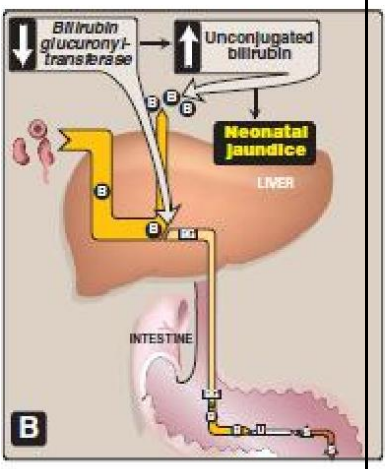
conjugated **bilirubin** (which is the form it's stored in) **accumulation increases concentration to over saturation** and starts the **formation of bile stones**.

Baby jaundice:



Newborn infants, particularly if premature, often **accumulate bilirubin**, because the activity of hepatic bilirubin glucuronyltransferase is low at birth.

Enzyme adult levels are reached in ~4 weeks (there's no genetic mutation, it is a developmental issue).



BG = bilirubin glucuronide; B = bilirubin; U = urobilinogen; S = stercobilin.

Risk: High bilirubin above the binding capacity of albumin, can diffuse into the basal ganglia and cause toxic encephalopathy (kernicterus).

The treatment for it is **Blue fluorescent light** that converts bilirubin to more polar water-soluble isomers (I just need to do this until babies become normal) (we need to cover babies eyes because we expose them to blue **fluorescent** light).

- The resulting photoisomers can be excreted into the bile without conjugation to glucuronic acid.

Determination of bilirubin concentration:

The most common way to measure bilirubin uses **van den Bergh reaction** (part of liver function tests).

*Diazotized sulfanilic acid + bilirubin → **Red azodipyrroles***

* Diazotized sulfanilic acid can't distinguish between conjugated and non-conjugated bilirubin, so we change the media of the reaction to make it able to distinguish (aqueous for conjugated, methanol for unconjugated)

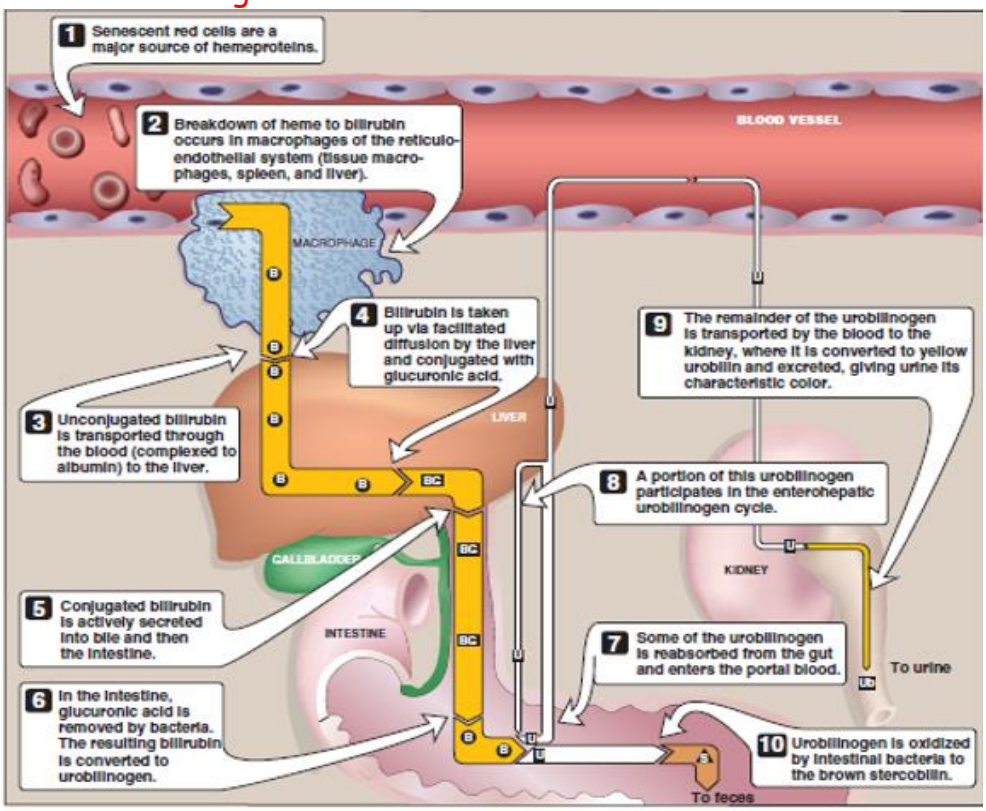
* Methanol has polar side (OH) = react with conjugated bilirubin, and it has nonpolar side (CH_3) = react with unconjugated one.

*The **color change is measured colorimetrically** *

There are two types of bilirubin measurement:
Direct-reacting measurement of **conjugated bilirubin** (aq/ conjugated bilirubin is naturally soluble in the blood) by **rapid reaction with the reactant** (within 1 min).
Indirect-reacting measurement of **total bilirubin** (methanol) by reaction with the reactant.

- **Unconjugated bilirubin= total bilirubin - conjugated bilirubin**
  In normal plasma, only about 4% of the total bilirubin is conjugated or direct-reacting, **because most is secreted into bile.**

This cartoon summarizes the whole thing:



B = bilirubin; BC = bilirubin diglucuronide; U = urobilinogen; Ub = urobilin; S = stercobilin

In the body, it starts with a couple hundred grams of food and ends in great energy production. Remember, everything could end up being great only if you add a little bit of effort!