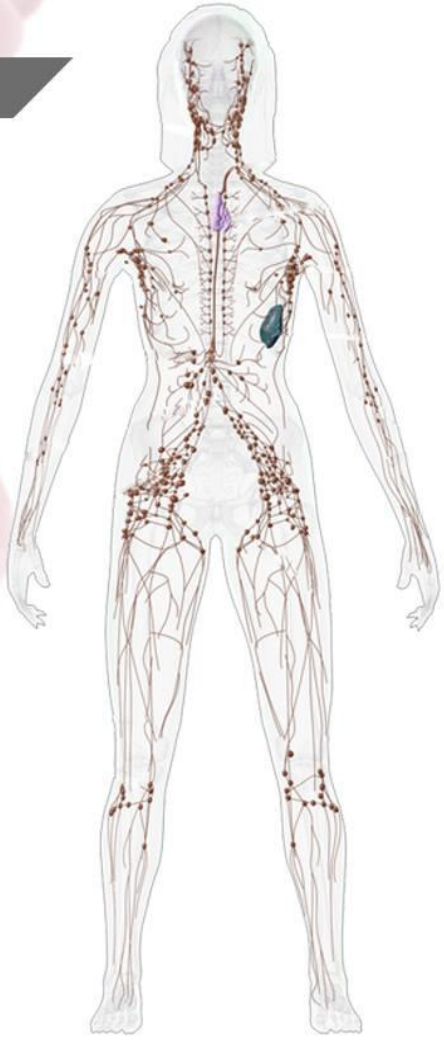
Sheet 7

Hematology and Lymphatic system

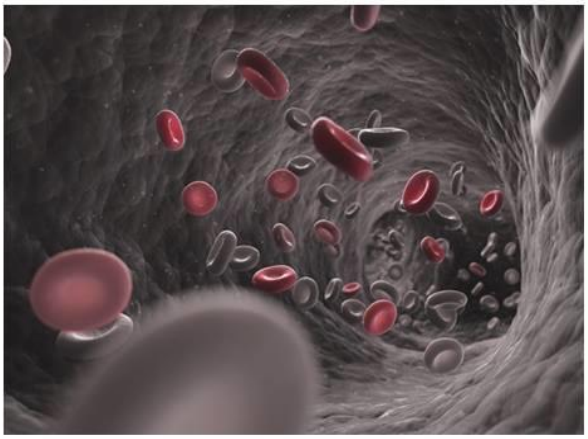
Subject | Biochemistry



Done by | Tala Saleh

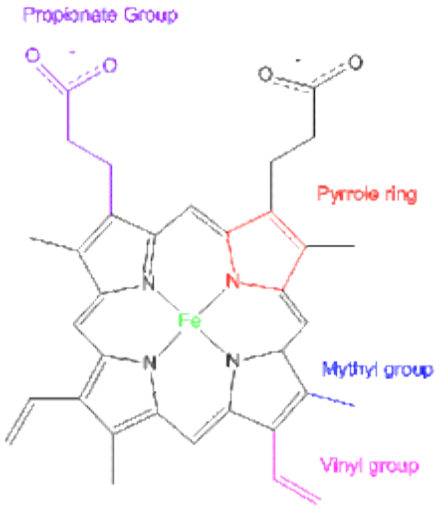
Corrected by | ...

Doctor | Ma'moun



Heme synthesis

Heme structure



- It is a complex of protoporphyrin IX + Iron (Fe^{+2}).
- The porphyrin is **planar** and consists of **four pyrrole** rings.
- Each pyrrole ring can bind with **two** substituents.
- **Two** rings have a **propionate** group each.
- The molecule is **hydrophobic**.
- Fe has **six** coordinates of binding.

Sites of synthesis

- The major sites of heme biosynthesis are in the:

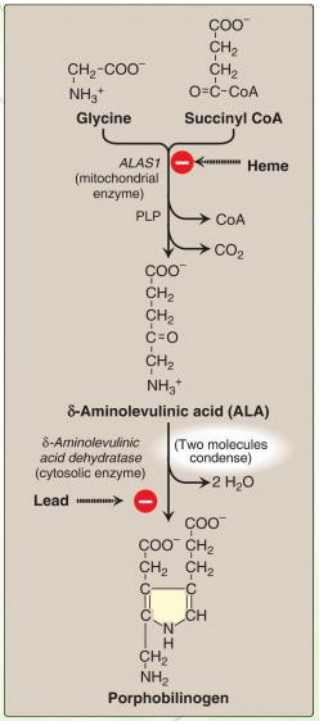
  1- Liver, which synthesizes several heme proteins (particularly the CYP proteins).

  The rate of heme synthesis in the liver is highly **variable**; it depends on the body and its conditions.

  2- Erythrocyte-producing cells, which participate mainly in Hb synthesis.

  Relatively **constant** production that matches the rate of **globin** synthesis. It is regulated at **multiple** points.

Steps of Heme Biosynthesis



- The reactions of heme biosynthesis **first** occur in the **mitochondria**, it continues in the **cytosol**. Finally, it returns to the **mitochondria**.

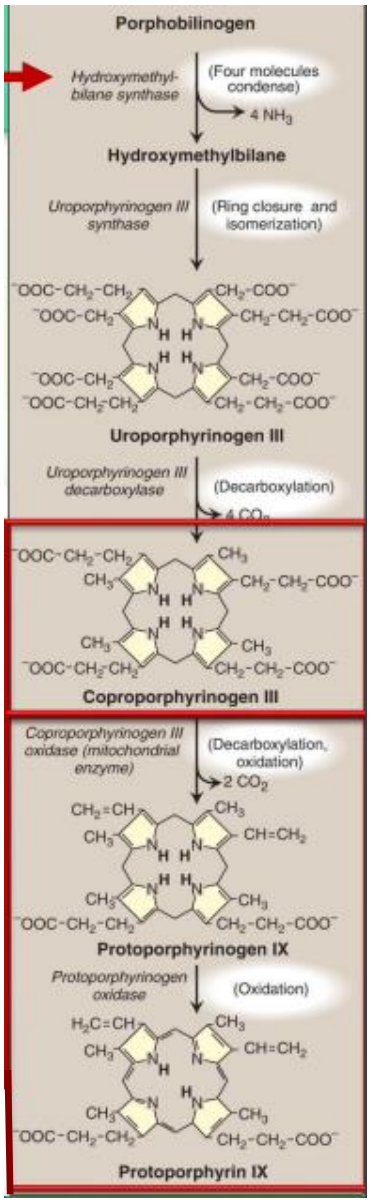
  1- The **first** reaction is catalyzed by **ALAS1** *(liver-specific)* or **ALAS2** *(erythrocytes-specific)*. It is the **rate-limiting** and committed step which requires **vitamin B6** *(pyridoxal phosphate)* and it takes place in the **mitochondria**.

  2- The first reaction takes place in the mitochondria, and then ALA moves out of it. **Two** ALA molecules **condense** to form **Porphobilinogen** in the **cytosol**.

3- **Four** molecules of **Porphobilinogen** form **uroporphobilinogen** III eventually, catalyzed by a **deaminase**.

4- Further reactions take place in the **cytosol** until **Coproporphyrnogen** III is formed which is then moved back into mitochondria.

5- The last reaction is **spontaneous** but can be catalyzed by **ferrochelatase**.



- **Note:** Protoporphorin is what gives the blood its red color.

Regulation

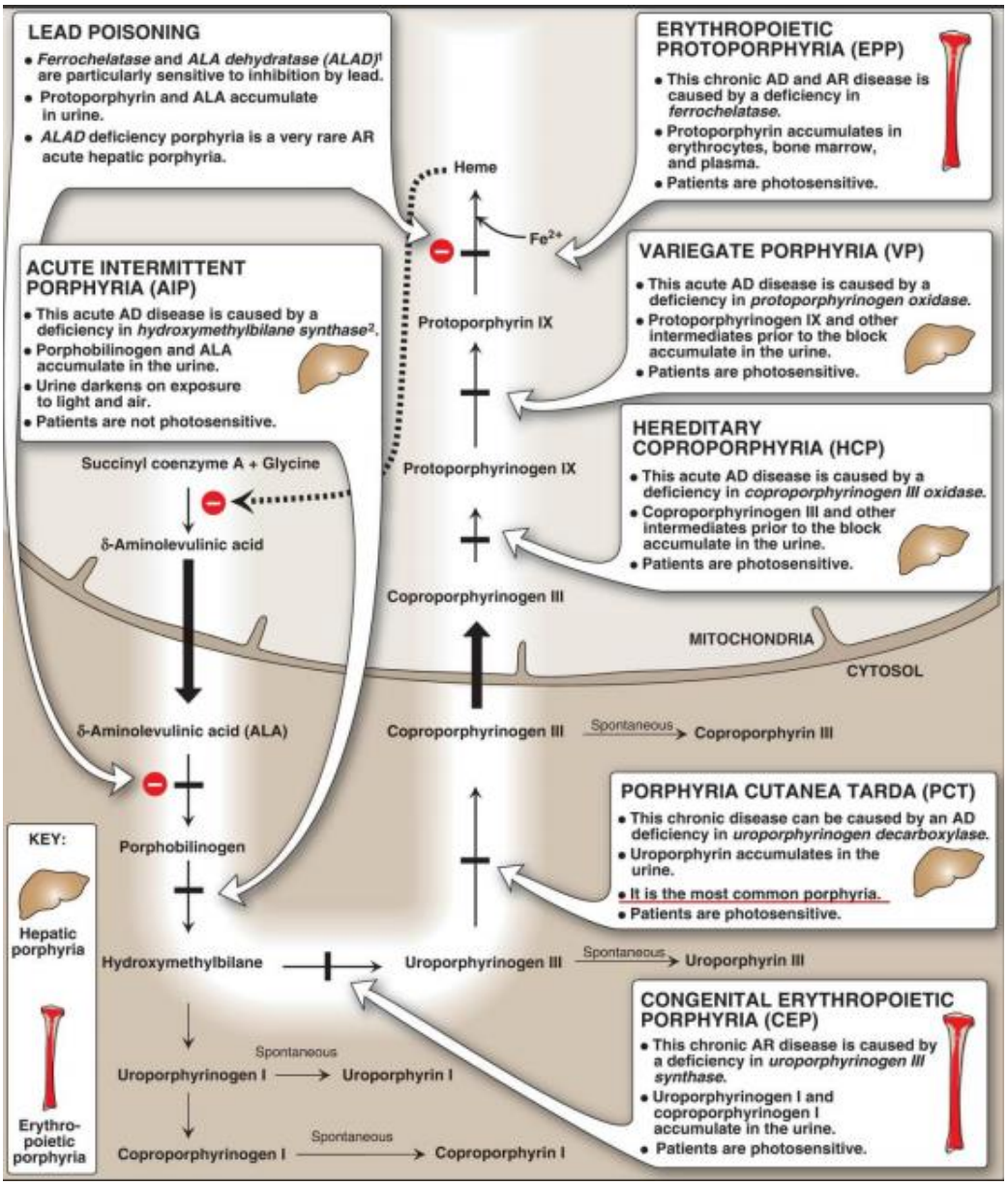
- ALAS1 (liver-specific) is **inhibited** by **hemin** through:
  - **a-** Degradation of mRNA.
  - **b-** Inhibition of mitochondrial transport to the cytosol.
- Some drugs may **induce** ALAS1 expression.
- ALAS2 (erythrocyte specific) is regulated by the **level of iron**.
- In the **liver**, the **first** reaction is what gets regulated. In **erythrocytes**, synthesis is regulated at **ferrochelatase** and **porphobilinogen deaminase**.

Porphyrias

- Porphyrias are inherited or acquired disorders caused by a **deficiency** of any **enzyme** in the heme biosynthetic pathway resulting in **elevations** in the **serum** and **urine** content of the **intermediates** in heme synthesis.
- Porphyria = purple.
- These disorders are classified as either **erythroid** or **hepatic** (acute or chronic); there's a lot of variabilities.
- Individuals with an enzyme defect **prior** to the production of Tetrapyrroles *(i.e. before **Hydroxymethylbilane** production)* manifest **abdominal** and **neuropsychiatric** signs. Whereas those with enzyme defects leading to the **accumulation** of **tetrapyrrole** intermediates *(i.e. after **Hydroxymethylbilane**)* show **photosensitivity**.

The doctor said to focus on the following things from the picture:

1- Porphyria Cutanea Tarda
2- Acute intermittent porphyria
3- Whether the disorder causes photosensitivity or not.
4- Deaminase, ferrochelatase, and ALA synthase enzymes from the previous page.



Treatment of Porphyrias

1- **Hemin or hematin**: they strongly **inhibit** the activity of **ALAS**, thus reducing the accumulation of the heme biosynthesis intermediates.

2- **Glucose**: fasting (hypoglycemia) exacerbates acute porphyria attack due to **activation** of the transcription factor, **PGC-1α**, in the **liver** which **induces** synthesis of gluconeogenic genes and the **ALAS1** gene resulting in accumulation of heme intermediates. Therefore, in **porphyrias**, giving glucose would **reduce** the synthesis of **the ALAS1** gene in the liver.

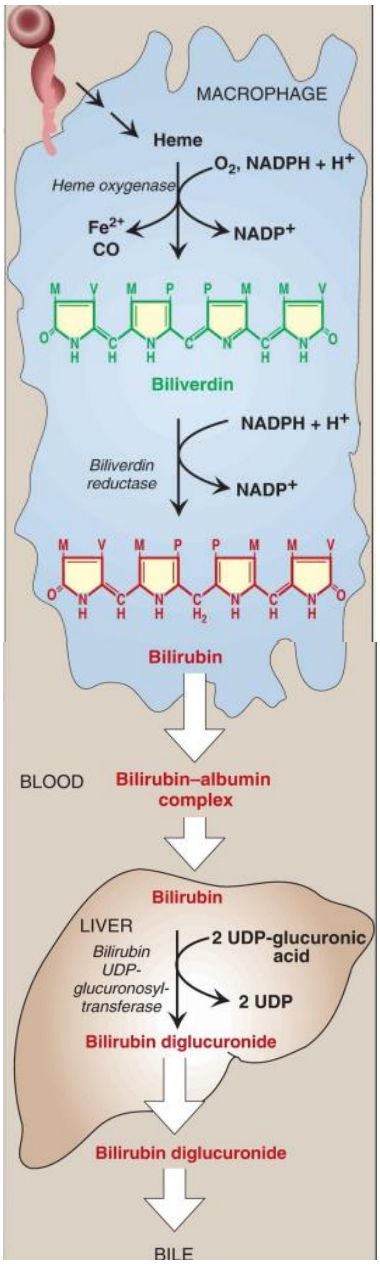
Heme Degradation (Catabolism)

Challenges

- RBCs are the largest storage place of heme.
- Erythrocytes are mainly destroyed by macrophages in the **spleen** and **bone marrow**, releasing **hemoglobin**, which is degraded to **heme.** The **protein** is metabolized into **amino acids**.
- **6 g/day** of hemoglobin are turned over, but:
  1- The porphyrin ring is **hydrophobic**; it cannot be excreted alone.
  2- Iron must be **conserved**.

Heme degradation

- Heme needs to be **hydrophilic** to be excreted.
- **Heme oxygenase** catalyzes 3 reactions catalyzed by **NADPH** resulting in breaking the heme into a **linear** molecule.
- This is the only process in the body that forms **CO**.
- It goes through many colors:
  1- **Red**: from the released Hb.
  2- **Blue**: as Hb loses oxygen, the iron becomes in the ferric state.
  3- **Green**: from the formation of biliverdin.
  4- **Yellow**: from the formation of bilirubin.



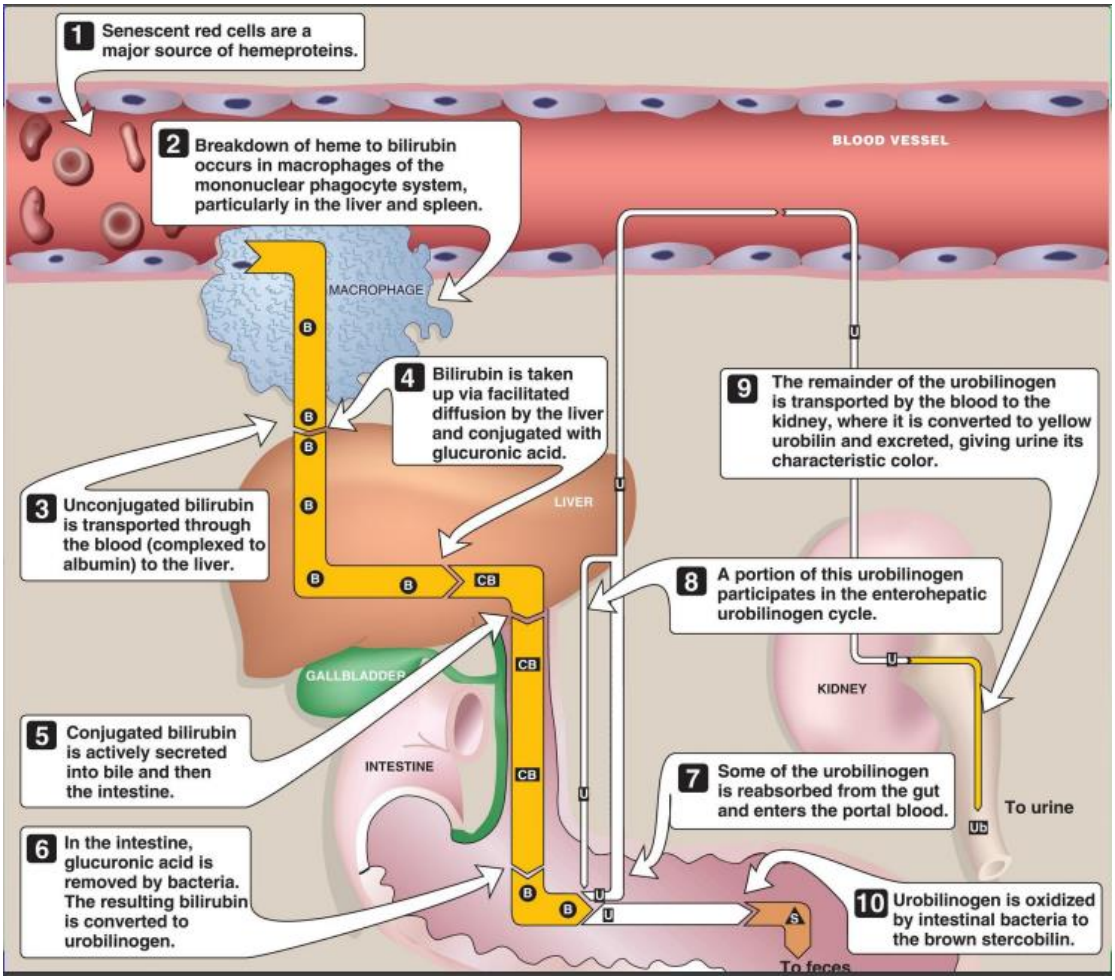
Transport of Bilirubin

- Bilirubin is released into the **blood** complexed with **albumin** to the liver.
  ⇨ **Salicylates** and **sulfonamides** can displace **bilirubin** from albumin permitting bilirubin to enter the central nervous system (**CNS**). This causes the potential for **neural damage** in infants.

- In the **liver**, bilirubin gets conjugated with 2 **glucuronide** molecules forming **Bilirubin Diglucuronide**, where it then passes with **bile** into the **intestine**.
  - ⇨ **Crigler-Najjar** (I, II) and **Gilbert syndrome** are disorders of bilirubin conjugation.
  - ⇨ Defection in bilirubin transport into bile causes **Dubin-Johnson syndrome**. It is manifested by chronic conjugated hyperbilirubinemia.

<u>The following picture shows all the steps in Heme catabolism:</u>

Any defect in these steps would cause different types of jaundice.



Measurement of bilirubin

- It is done via a reaction known as **Van den Bergh** reaction. It takes place in 2 different solvents:

  1- **In water:** Direct measurement of conjugated bilirubin (direct bilirubin).
  2- **In Ethanol or methanol**: Total measurement of bilirubin.

  ⇨ Indirectly, unconjugated bilirubin = total bilirubin – direct bilirubin.

			Unconjugated hyperbillirubinemia		Conjugated hyperbillirubinemia
Sample	Indices	Normal	Hemolytic Jaundice	Hepatic Jaundice	Obstructive Jaundice
Serum	Total Bil	< 1mg/dl	> 1mg/dl	> 1mg/dl	> 1mg/dl
	Direct Bil	0 ~ 0.8mg/dl		↑	↑↑
	Indirect Bil	< 1mg/dl	↑↑		
Urine	Color	normal	deeper	deep	deep
	Bilirubin	—	—	+ +	+ +
	Urobilinogen	A little	↑	uncertain	↓
	Urobilin	A little	↑	uncertain	↓ Clayish color
Stool	Color	normal	deeper	lighter or normal	Argilous (complete obstruction)

➔ In Hemolytic Jaundice:

High levels of **unconjugated** bilirubin are present, leading to **dark stool** and **urine**.

➔ In Hepatic Jaundice:

Liver damage **decreases** the **conjugation** efficiency, thus there is a defect in the secretion of conjugated bilirubin into bile causing stool to be **lighter** in color. However, lab results **vary** a lot depending on the **cause** of liver damage.

➔ In Obstructive Jaundice:

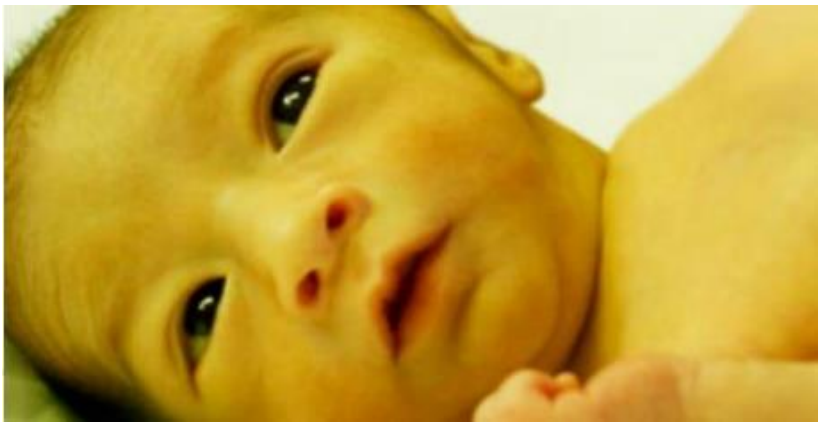
There is a **high** buildup of **conjugated** bilirubin that will not be delivered into the intestine. Therefore, it leaves into the **blood** causing **conjugated hyperbilirubinemia**.

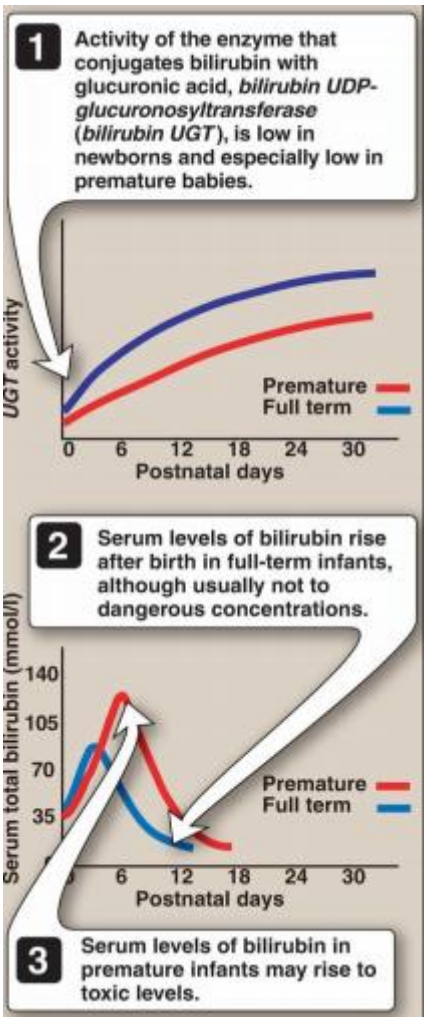
The conjugated bilirubin is excreted through **urine** in **high levels** causing **dark** urine.

Unconjugated bilirubin amounts are **low** since they aren't being transported into the intestine. Stool is thus **argilous** (clayish) in color in the case of complete obstruction.

Jaundice in Newborns

- Newborn jaundice is very common and can occur when babies have a **high** level of **bilirubin**, the main cause is that the **conjugating enzyme** is not efficient yet.
- Rarely, an **unusually high** blood level of bilirubin can place a newborn at **risk** of **brain damage** where bilirubin may cross the BBB into the CNS, particularly in the presence of certain risk factors.





Good Luck