

HEME SYNTHESIS

Structure of Heme

Heme is Iron porphyrin IX.
It has a tetrapyrrole ring structure with iron at the centre.

The 4 pyrrole rings are linked together by methenyl (=CH-) bridges. Certain groups like methyl vinyl and propionyl groups are attached to the pyrrole rings.

Heme Containing Protein

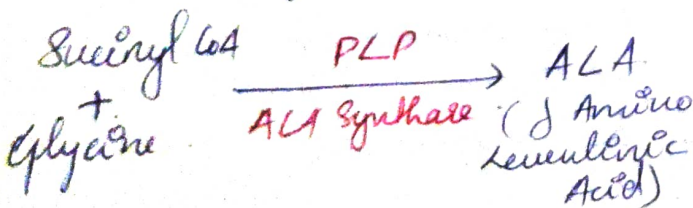
Hemoglobin
Myoglobin
Cytochromes etc

Synthesis of Heme

Site: Almost all tissues except mature RBC.
Organelle: mitochondria and cytoplasm.

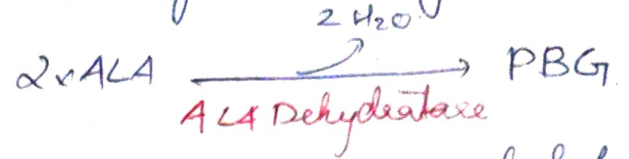
Steps

1. Succinyl CoA combines with glycine to form δ -aminolvalinic acid. This occurs in mitochondria and is catalyzed by ALA synthase.



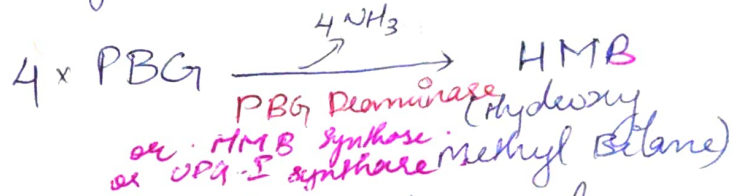
ALA synthase is the key enzyme.

(2) In cytoplasm, 2 ALA condense to form porphobilinogen (PBG) by ALA dehydratase.



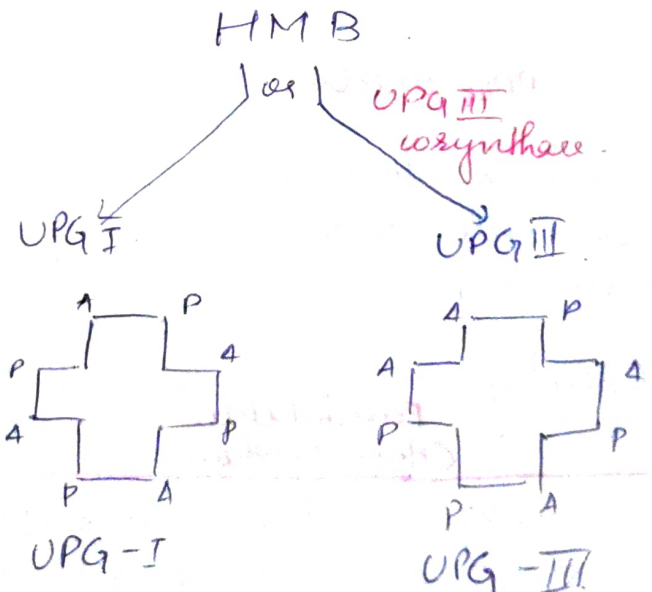
ALA dehydratase is inhibited by lead.

(3) 4 molecules of PBG combine to form a linear tetrapyrrole HMB (hydroxymethyl Bilane). Enzyme is PBG Deaminase.

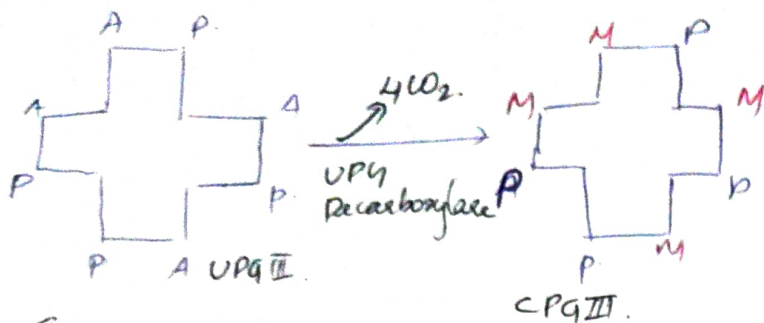


HMB can be spontaneously cyclized to form UFG I (uroporphyrinogen I).

Or, it can be converted to UFG II by UFG III cosynthase.

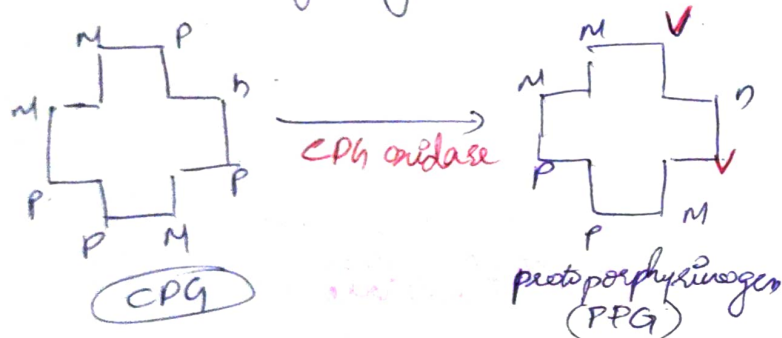


(4) UFG II is converted to CPG-III (coproporphyrinogen III) by UFG Decarboxylase.

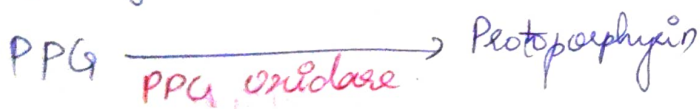


(Acetyl groups are decarboxylated to methyl groups)

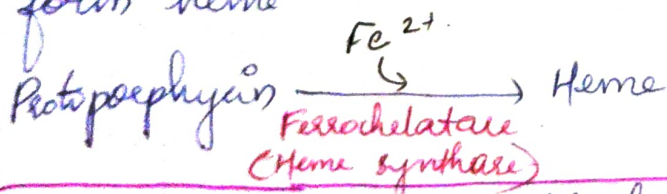
- ⑤ CPG III is taken to mitochondria and oxidized to PPG (protoporphyrinogen).



- ⑥ PPG is oxidized to protoporphyrin by PPG oxidase. Methylene ($-\text{CH}_2-$) bridges are oxidized to methenyl ($=\text{CH}-$) bridges.

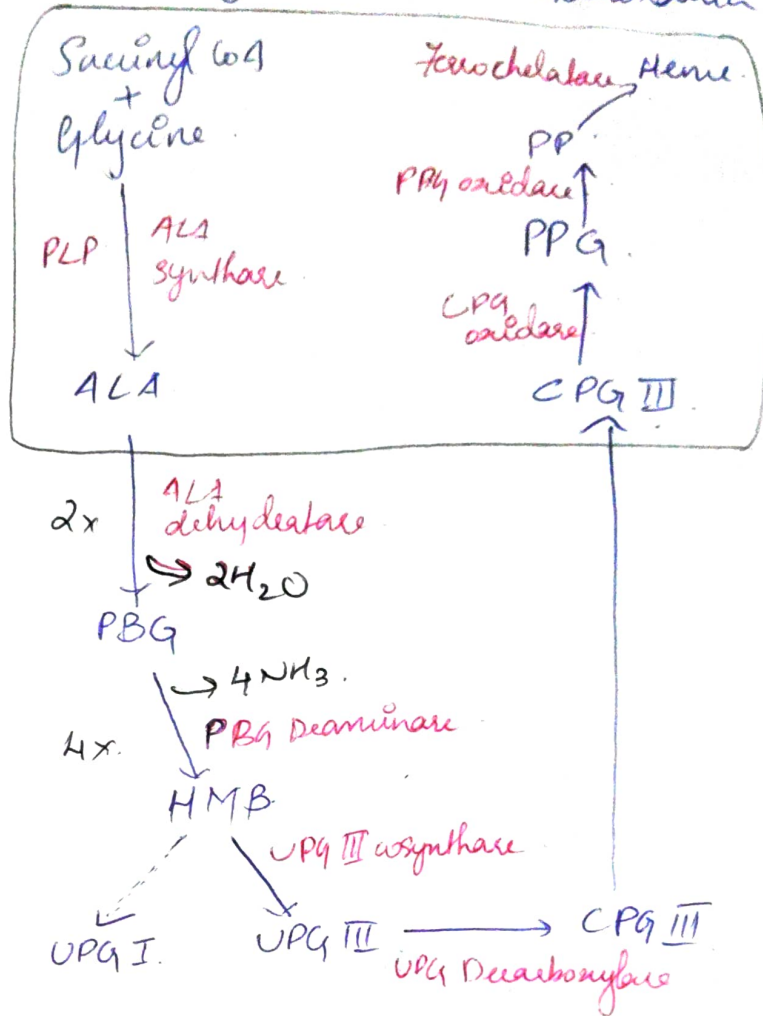


- ⑦ Ferrochelatase incorporates iron into protoporphyrin to form heme.

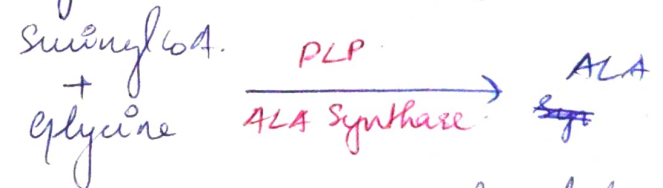


Ferrochelatase and ALA dehydratase are inhibited by lead (Pb).

Summary



Regulation of Heme Synthesis



- ALA synthase is induced by
- ① Oestrogens, progesterone.
 - ② Starvation, low calorie diet.
 - ③ Barbiturates, alcohol and drugs metabolized by cytochrome P450.
 - ④ Iron supplements.

→ ALA synthase is repressed by

- ① High intracellular glucose.
- ② Heme (Heme act as a co-repressor).

ALA synthase is allosterically inhibited by Heme.

DISORDERS OF HEME SYNTHESIS

Enzyme defect of heme synthesis

Enzyme	Disease
ALA synthase	X-linked sideroblastic anemia
ALA dehydratase	Doss porphyria
PBG deaminase	Acute intermittent porphyria
UGT III cosynthase	congenital erythropoietic porphyria
UGT decarboxylase	Porphyria cutanea tarda
CPG oxidase	Hereditary coproporphyria
PPG oxidase	Porphyria variegata
Ferrochelatase	Hereditary erythropoietic porphyria

If PBG or porphyrinogens are excreted through urine, colour of urine darkens on exposure to sunlight. This is due to photo oxidation.

Elevation of ALA may lead to neurological manifestations. Porphyrins have AD inheritance except Doss porphyria and CEP which are AR.

Acute Intermittent Porphyria
Due to deficiency of PBG deaminase

Succinyl CoA + Glycine

↓
ALA

↓

PBG

↓

HMB

PBG deaminase

Porphyrias are also classified as

(A) Erythropoietic

CEP
Hereditary Erythropoietic protoporphyria

(B) Hepatic

Doss (rarest)

AIP

Coproporphyria

Porphyria variegata

(C) Porphyrias with both features
porphyria cutanea tarda

General features of Porphyria

If porphyrinogens are elevated in blood, there will be photosensitivity

PBG and ALA increase in blood and are excreted through urine.

Clinical Features

AD inheritance

Acute and intermittent attacks of abdominal pain and neuropsychiatric manifestation.

Urine turns dark on exposure to sunlight because PBG is oxidized to porphobilin.

No photosensitivity

Precipitating factors

Anything which induces ALA synthase will aggravate the symptoms.

- ① Alcohol, Barbiturates, drugs metabolized by CYP450
- ② Starvation, low calorie diet
- ③ Iron supplements
- ④ Oestrogens, progesterone

Relieving factors

Anything which suppresses ΔA synthase will decrease the symptoms

- ① Heme.
- ② Glucose.

Diagnosis

Watson - Schwartz test

to detect PBG in urine.

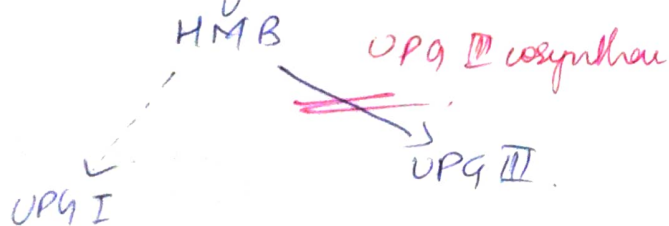
Treatment

- ① Avoid all precipitating factors
- ② Oral or intravenous glucose.

Congenital Erythropoietic Porphyrria

Quantitative disease

Enzyme deficient $\rightarrow \text{UFG III}$ ΔA synthase



Since HMB cannot be converted to UFG III, formation of UFG I increases.

UFG I accumulates in blood and is excreted through urine.

Inheritance AR.

Clinical features.

Starts early
Severe photosensitivity.

This leads to

Itching $\rightarrow$ scarring $\rightarrow$ Monkey faces

Red fluorescence of teeth

$\rightarrow$ Crythrodontia

Portwine urine

Acquired porphyrias.

Lead poisoning can inhibit ΔA dehydratase and ferro-chelatase and can minimize the features of acute intermittent porphyria.

Catabolism of Heme

Average life span of RBCs - 120 days

When RBCs lyse, Hb is liberated.

Hb $\rightarrow$ Globin $\rightarrow$ 4 α -pol
 $\rightarrow$ Heme $\rightarrow$ Fe^{2+} is released
 porphyrin ring is catabolized to bilirubin

Daily production of Bb

1g Hb $\rightarrow$ 35 mg Bb.

6g Hb/day

$\downarrow$
260 mg Bb/day

other heme containing proteins

$\downarrow$
50 mg Bb/day

① Formation of Bilirubin

Broken down to bilirubin in the R.E cells of liver, spleen and bone marrow.

This is by Mg dependent Heme oxygenase system (MHOs) This

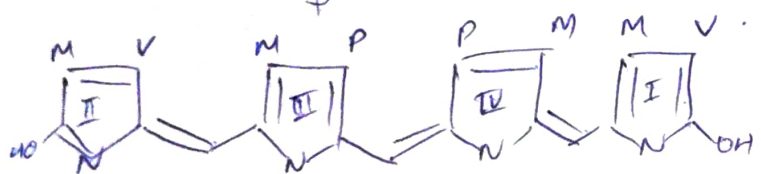
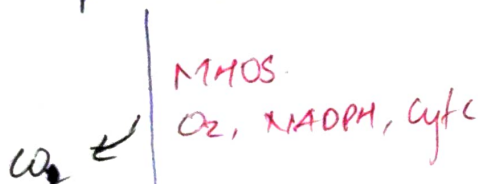
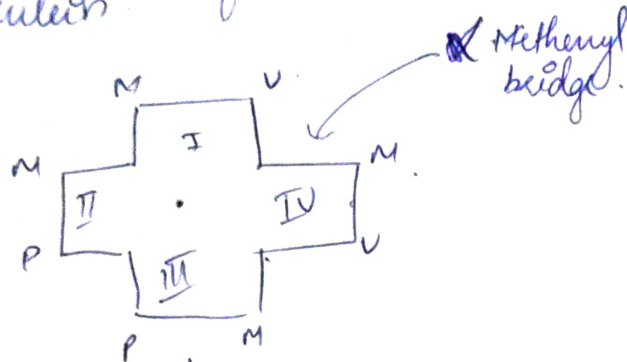
(4) requires Oxygen, NADPH & cytochrome C .

(2)

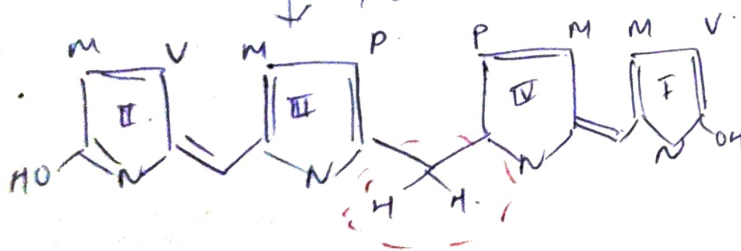
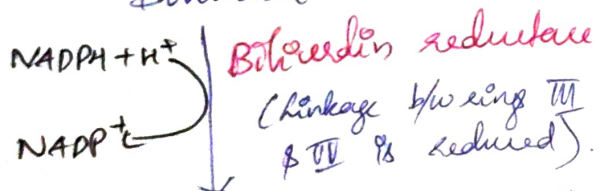
2 Methylene bridge (between rings I and II) is removed as carbon monoxide.

This forms a linear tetrapyrrole - Biliverdin

Biliverdin is further reduced to Bilirubin



Biliverdin



Bilirubin

→ Mammals secrete bilirubin.
Birds & amphibians secrete biliverdin

(2) Transport of Bb to liver
→ Bb is excreted through bile
→ So, Bb found in RE cells of spleen, bone marrow etc have to reach liver.
→ But Bb is soluble in plasma. So, it is transported bound to albumin.

→ 1 Albumin can bind 2 Bb molecules (Maximum 25 mg/dL)

→ Bb-Alb Binding is loose. Bilirubin will dissociate from albumin and cause kernicterus under the following conditions:

① when Bb level is very high.

② Use of drugs [eg: Aspirin, penicillins etc] which bind to albumin and so displace Bb from Alb.

→ When Bb-Alb complex reaches liver, Bb is taken up by a carrier mediated uptake process.

(3) Conjugation in Liver

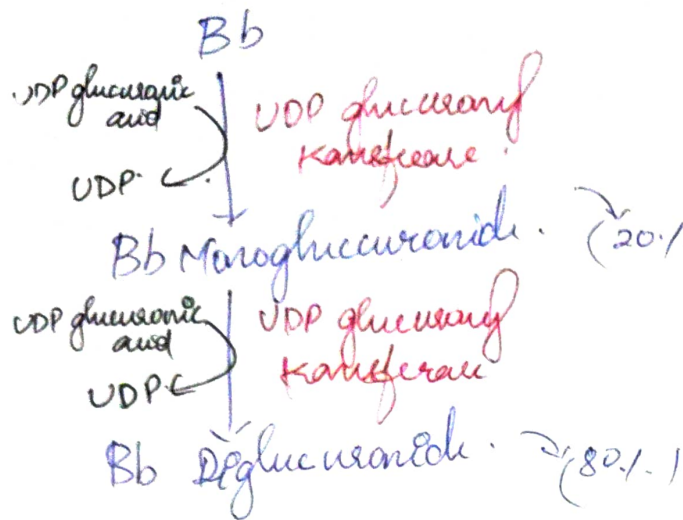
Conjugation is done to make Bb water soluble.

Bb is conjugated with glucuronic acid. Glucuronic acid combines with pyronic acid side chains of Ring III & IV.

→ conjugation can be induced by barbiturates

(5)

(7)



Some drugs may interfere with conjugation.

eg. Pregnenolone
Chloramphenicol
Primidone
Novobiocin
Androgens.

These drugs can cause increase in the level of unconjugated Bb in blood.

(A) Secretion of Bb into bile.

- Rate limiting step of biliary excretion.
- Done by a Multispecific organic anion transporter (MOAT) located in the plasma membrane of biliary canaliculi.
- Induced by barbiturates.

(B) Fate of Bb in the Intestine

- Deconjugated by intestinal bacteria.
- Bb is reduced to urobilinogen (UBG) - A colourless tetrapyrrole.
- 20% of UBG is reabsorbed

and undergo enterohepatic circulation and is re-excreted. A small fraction is excreted through urine.

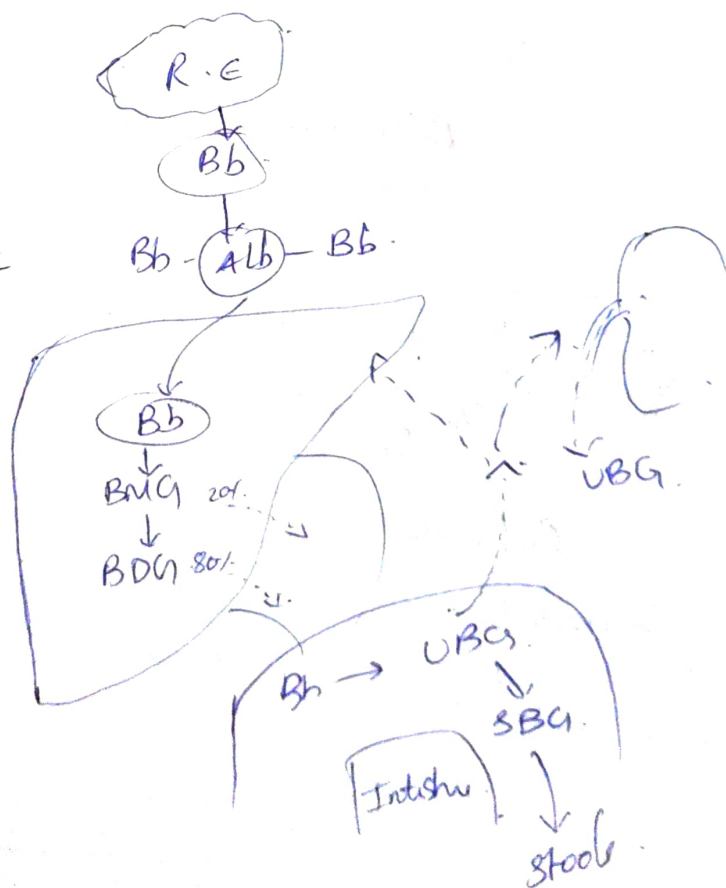
Remaining UBG is further reduced

UBG
↓
Mesobilinogen

Stercobilinogen → stool

→ Colour of feces is due to urobilin & stercobilin. Urine turns dark in constipation.

→ on prolonged antibiotic therapy, green coloured stools are seen - because Bb is not reduced due to absence of intestinal bacteria - Bb is oxidised to Bv.



JAUNDICE

Normal plasma bilirubin

0.2 - 0.8 mg/dL

conjugated Bb 0 - 0.2 mg/dL

Unconjugated Bb 0.2 - 0.6 mg/dL

Total Bb > 1 mg/dL - Hyperbilirubinemia

→ Jaundice - yellowish discoloration of skin and mucous membranes due to hyperbilirubinemia.

→ Jaundice usually manifests when total plasma Bb > 2 mg/dL (1-2 mg/dL = latent jaundice)

→ Jaundice is clearly visible on sclera, because Bb has high affinity to elastin

Tests in Jaundice

- ① van den Bergh reaction - to detect plasma Bb
- ② Fouchet's test - to detect bile pigments in urine
- ③ Hay's test - to detect bile salts in urine
- ④ Ehrlich's test - to detect urobilinogen in urine
- ⑤ Liver enzymes in blood - ALT, AST and ALP are usually measured.

van den Bergh reaction

To detect Bb in plasma.

Reagent : Diazotized sulphamic acid

Bb reacts with the reagent

to give a purple colour at pH 5

Conjugated Bb gives colour immediately (as it is water soluble)

- this is called direct reaction.

→ Unconjugated Bb gives colour immediately only if alcohol is added. (Indirect reaction)
If both direct & indirect reactions - Biphasic

Fouchet's test

Test to detect bile pigments in urine (bilirubin in urine)

Fouchet's reagent is Ferric chloride in trichloroacetic acid.

First, Bb is adsorbed to Barium SO₄. Reagent then oxidises Bb to biliverdin and biligaymen - this gives a green colour.

Hay's test

To detect bile salts. Bile salts are present in urine. When there is an obstruction to the flow of bile, sprinkle sulphur powder to a sample of urine.

Normally, S powder floats. If bile salts are present, S powder will sink to the bottom because bile salts reduce the surface tension of urine.

Ehrlich's test

Test to detect urobilinogen (UBG)

SBG also gives a positive test.

Paradimethyl amino benzaldehyde reacts with UBG and SBG to form a red colour.

UBG is increased in urine in hemolytic jaundice and is absent or decreased when there is an obstruction to the flow of bile.

[Another test for UBG - Schlesinger's test].

Enzymes:

Serum ALT } Elevated in
AST } hepatocellular damage.

Serum ALP - Elevated in obstructive jaundice.

Types of jaundice:

→ Congenital.

→ Acquired.

Congenital hyperbilirubinemia

• Gilbert's disease

Due to defective uptake of Bb by the liver.

Most common cause of congenital unconjugated hyperbilirubinemia.
Bb level usually $< 6 \text{ mg\%}$.

• Crigler - Najjar Syndrome

Defect in conjugation.
Deficiency of glucuronyl transferase.

unconjugated hyperbilirubinemia.
There are 2 types:

Type 1 - is severe due to severe deficiency of glucuronyl transferase.
Kernicterus can occur.

Type 2 - is partial deficiency of glucuronyl transferase and is less severe.

• Dubin - Johnson syndrome
Due to defect in transporter that secretes Bb into bile.
conjugated hyperbilirubinemia.

• Rotor Syndrome.

due to defective secretion of Bb into bile. Mechanism is unknown.
Leads to conjugated hyperbilirubinemia.

• Acquired hyperbilirubinemia

classified into → pre hepatic jaundice
→ hepatic jaundice
→ post hepatic jaundice

Prehepatic jaundice

This is usually due to red paction of unconjugated Bb (eg: Hemolysis).

Physiological jaundice

(Neonatal jaundice)

• Unconjugated hyperbilirubinemia after 2nd day of life.

• Due to ↑ destruction of RBCs.

• Also due to immaturity of conjugating enzyme.

• Bb level will be approximately 8-12 mg/dL.

• If there is a tendency to ↑ Bb to higher levels, it is treated.

by phototherapy.

- 400 - 500 nm light (blue) converts bilirubin to a soluble form (photobilirubins) which can be excreted through urine.
- Exposure to morning sunlight is also useful.

Hemolytic disease of the Newborn can occur due to incompatibility of blood groups between father and mother.

Commonly it is due to Rh incompatibility between mother and fetus. Rh⁺ve child born to Rh⁻ve mother is at risk. The first child usually does not develop the problem. But in the next pregnancy onwards, antibodies in maternal blood pass to the fetus and cause hemolysis.

- Severe hemolysis can cause (erythroblastosis fetalis).
- ABO incompatibility may also cause hemolysis in newborn.
- If Bb level increases above 20 mg/dL there is chance of kernicterus.

Treatment

- Phototherapy with blue light to make Bb soluble so that it may be excreted.
- Barbiturates to induce conjugating enzyme system.
- Exchange transfusion.

Hemolytic disease in Adult

causes

- ① G-6-PD deficiency.
- ② Congenital spherocytosis.
- ③ Auto immune hemolytic anemia.
- ④ Carbon tetrachloride poisoning.

Leads to unconjugated hyperbilirubinemia.

Hepatic Jaundice.

(Hepatocellular jaundice).

causes

- ① Viral hepatitis (A, B, C, D or E).
- ② Drug / toxin induced liver damage.

During early phase of hepatitis there is defect in conjugation. This causes unconjugated hyperbilirubinemia.

In late phase, inflammatory edema causes obstruction to the intrahepatic biliary canaliculi. So, conjugated Bb also ↑ in blood. [Biphasic van den Bergh reaction].

Post hepatic Jaundice.

(Obstructive jaundice).

cholestatic jaundice.

→ Defective excretion of conjugated Bb to the intestines. Leads to conjugated hyperbilirubinemia. The obstruction can be

- Intra hepatic.
- Extra hepatic.

Intrahepatic

- Biliary cirrhosis
- Chronic active hepatitis
- Hepatoma
- Late phase of hepatitis

Extrahepatic

- Gall stones / stones in bile duct.
- Ca head of pancreas.

Blood and urine findings in jaundice

Finding	Pre Hepatic	Hepatic	Post Hepatic
Blood Bb	↑	↑ in early phase	Normal
Indirect	↑	↑ in early phase	Normal
Direct	Normal	↑ in late phase	↑
Urine Bile pigment	—	+ in late phase	+
Bile salt	—	+ in late phase	+
UBG	↑	↓ or (—)	—
Enzymes (Blood) ↑	LDH	ALT, AST	ALP

Stools are dark coloured in hemolytic jaundice because of increase in UBG and SBG, and clay coloured in obstructive jaundice due to their decrease.

Why is Bb toxic?

- ① Bb is an uncoupler. So, it inhibits oxidative phosphorylation.
- ② Inhibits various enzymes and

synthesis of DNA and RNA

Shunt Bilirubin

This is Bb produced from heme that is not incorporated into Hb. When radiolabelled glycine is injected, it will be incorporated into heme. After 100-120 days radioactive Bb is elevated.

But about 15% of radioactive Bb is excreted within 10 days.

This is called shunt Bb. Its level increases in ineffective erythropoiesis and in erythropoietic porphyrias.