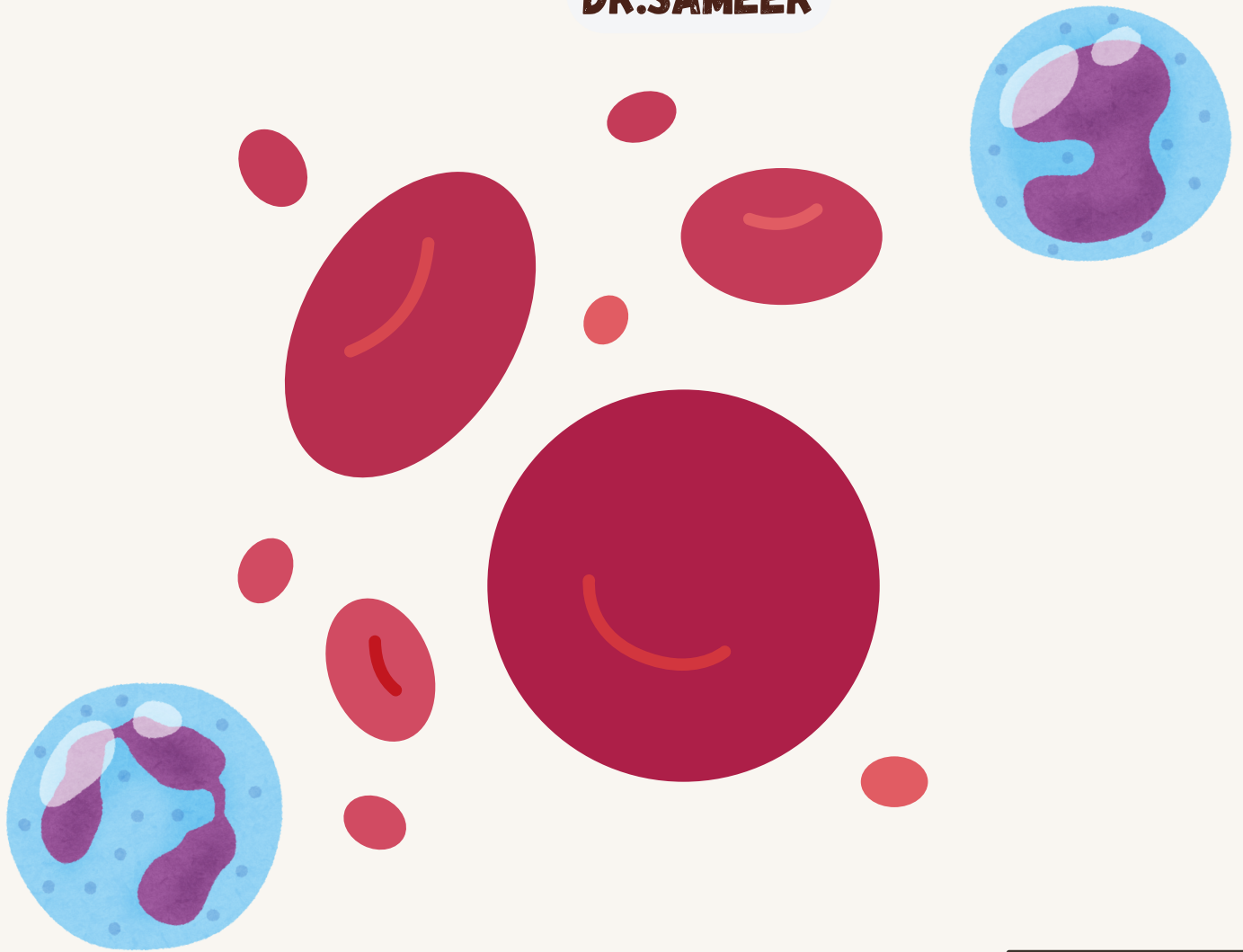


DOCTORS 2021 - رَوَح - MEDICINE- MU

(HLS)
BIOCHEM:

HEME DEGRADATION +
JAUNDICE

DR.SAMEER

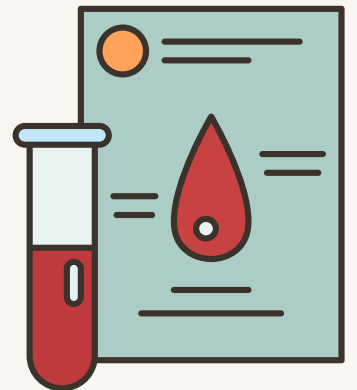


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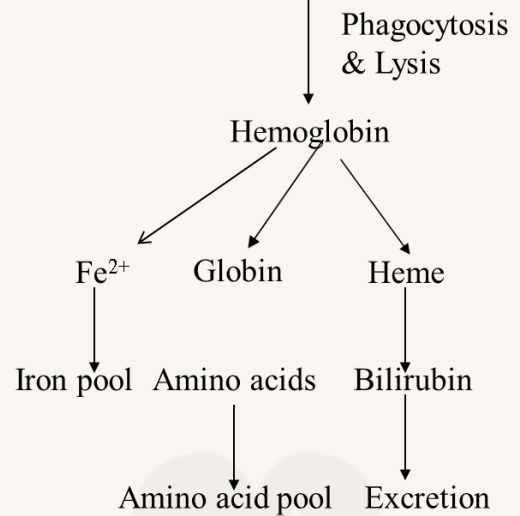
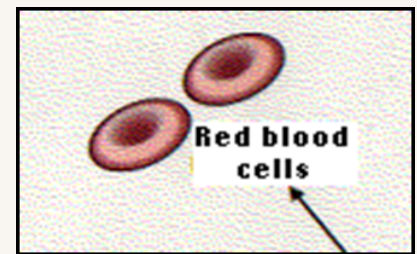


Heme degradation

Fate of RBCs

- Life span in blood stream is 90-120 days, RBCs are phagocytosed and/or lysed
- Normally, lysis occurs extravascularly in the ER of reticuloendothelial system (liver, spleen and bone marrow) subsequent to RBC phagocytosis
- Lysis can also occur intravascularly (in blood stream). (But to limited amounts)

- In the human body approx. 100 – 200 million RBCs are broken down every hour.
- Fe²⁺ → transported with transferrin and used in the next heme biosynthesis
- Not only Hb but other hemoproteins also contain heme groups which are degraded by the same pathway.



Handling of free (intravascular) hemoglobin

- Purposes: 1- Scavenge iron that results from the hemolysis of erythrocytes.
- 2- Prevent major iron losses
- 3- Complex free heme (very toxic) preventing the toxic effect of the heme.

1- **Haptoglobin**: hemoglobin-haptoglobin complex is readily metabolized in the liver and spleen forming an iron-globin complex and bilirubin. Prevents loss of iron in urine.

- **Hb still as it is (no degradation)**

2- **Hemopexin**: binds free heme. The heme-hemopexin complex is taken up by the liver and the iron is stored bound to ferritin.

- **If Hb dissociates into heme and globin, hemopexin will bind to heme.**

3- **Methemalbumin**: complex of oxidized heme and albumin.

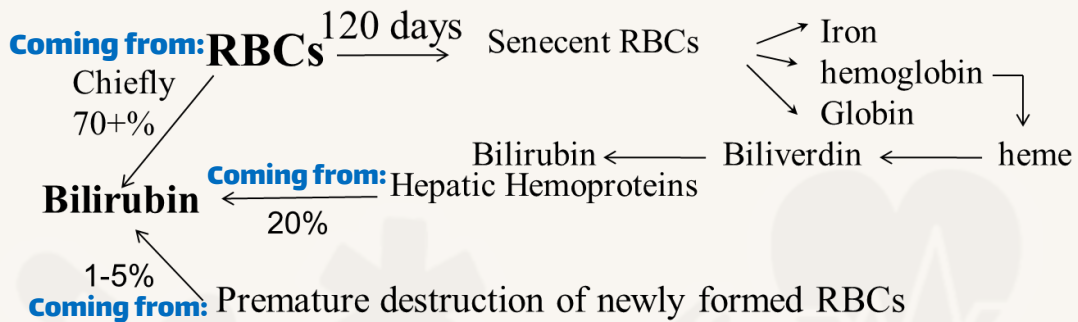
- **Haemoglobin**: has low molecular weight, if it release free It will pass through the glomerular filtration membrane in the kidney, and it will precipitate in the renal tubules, leading to renal failure.
- **Haptoglobin** is a glycoprotein produced by the liver. After the hemolysis of erythrocytes, it binds with hemoglobin to increase the molecular weight, preventing hemoglobin from passing through the glomerular filtration membrane. This prevents Hb deposition in the renal tubules.
- So among the investigations performed during hemolytic crises, not only in G6PD deficiency but also in different hemolytic diseases, we measure the concentration of haptoglobin. If there is hemolysis of the erythrocytes, there will be a decrease in the concentration of haptoglobin.

Bilirubin metabolism

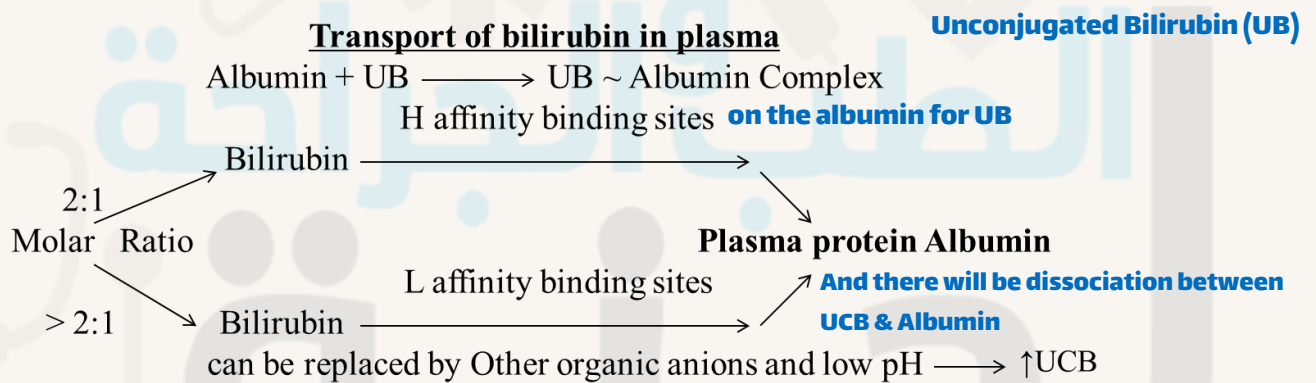
- Bilirubin formation
- Transport of bilirubin (**unconjugated**) in plasma
- Hepatic bilirubin transport
- A- Hepatic uptake B- Conjugation C- Biliary excretion
- Enterohepatic circulation

- **Unconjugated bilirubin, being hydrophobic, cannot freely pass through the bloodstream. Therefore, it binds to albumin,**

Bilirubin formation



Transport of bilirubin in plasma



- **The molar ratio refers to the ratio between unconjugated bilirubin and albumin.**
- **Albumin serves as a generalised carrier, including fatty acids, hormones, calcium, medication. This means there can be competition between bilirubin and other compounds for binding sites on albumin**
- **if the ratio was >2:1, here albumin is not a specific carrier, thus, it has different competitors for binding of UCB to Albumin such as Organic anions and Low pH and they will bind to Albumin instead, so rejecting some UB from binding sites of albumin.**
- **In uncontrolled diabetic patients who have yellow eye/skin discoloration → Ketone bodies is formed by Acetoacetic acid & Beta Hydroxybutyric acid and they are found in blood in ionized form, so H⁺ will be at higher level in circulation then pH will decrease and UCB will be dissociated from binding sites on albumin and patient will show characteristic of Jaundice**

Hepatic Bilirubin Transport

1. Hepatic uptake of bilirubin

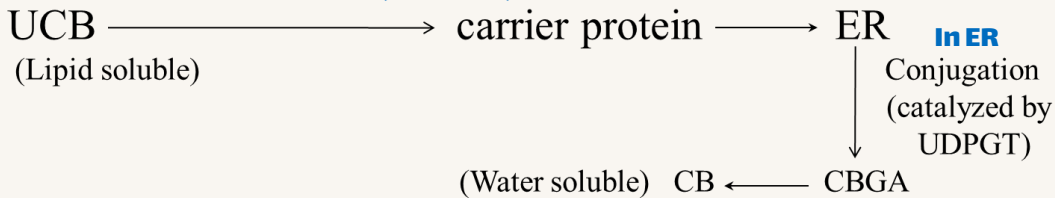
UCB ~ Albumin complex separated
(be) taken up

Bilirubin \longrightarrow Plasma membrane of the liver

- Bilirubin uptake is reduced: in neonates, cirrhosis, some drugs effects

2. Conjugation of bilirubin Inside liver cells

bound to Z protein (Ligandin)



- Conjugation of UCB with two molecules of glucuronic acid.

3. Biliary excretion of bilirubin

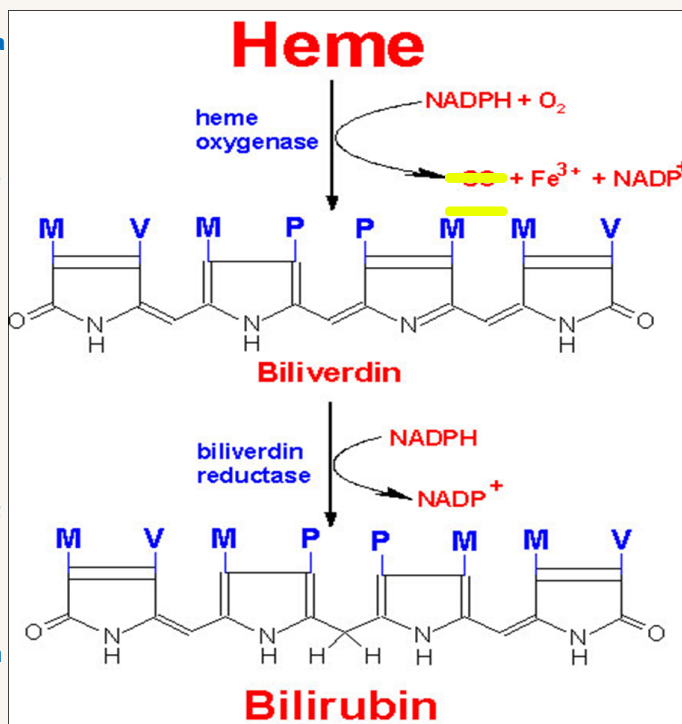
Transfer across

CB \longrightarrow Bile canaliculus

Microvillar membrane

Degradation of heme to bilirubin

- When someone get a bruise (contusion), initially, its color appears red due to the extravasation of erythrocytes from injured capillaries. Then, after a few days, it may appear bluish, which is actually a deep green color which is colour of the biliverdin. Finally, it will be change into a yellowish-brownish coloration, which is colo of UCB bilirubin



-75% is derived from
RBCs

- In normal adults this
results in a daily load of
250-300 mg of bilirubin

Liver can covert UCB to CB : 250-300 mg daily

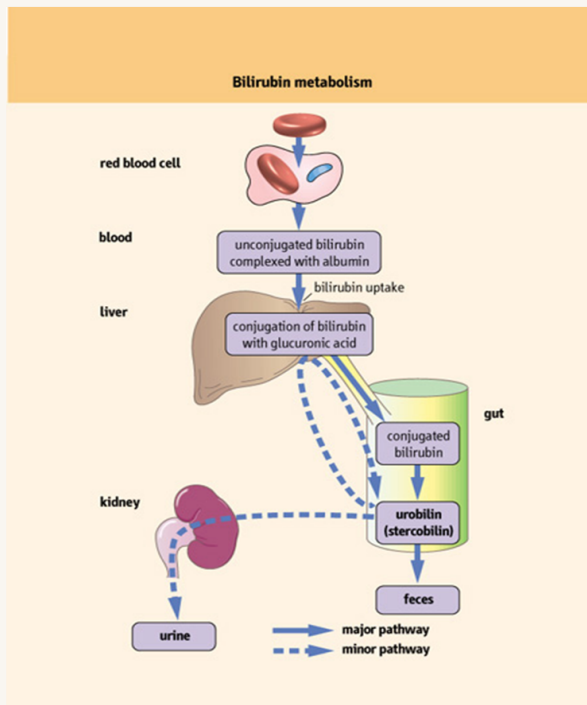
- Normal plasma
concentrations are less
then 1 mg/dL.

- Hydrophobic -
transported by albumin to
the liver for further
metabolism prior to its
excretion

- The only reaction in our cells normally producing carbon monoxide (CO) is the first reaction in heme degradation catalyzed by heme oxygenase. Carbon monoxide is more dangerous than carbon dioxide (CO2) because its affinity for binding to hemoglobin is 210 times higher than the affinity for binding oxygen.

Therefore, individuals exposed to CO poisoning are treated with 95% oxygen (O2) instead of 100% to avoid respiratory center failure

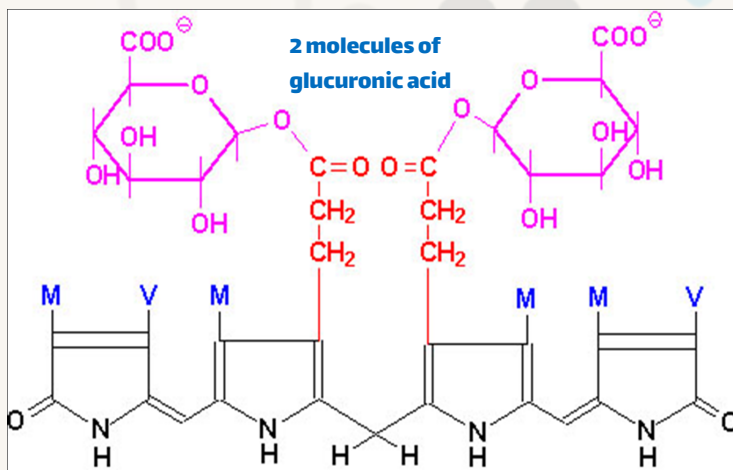
Normal bilirubin metabolism



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- **Mutation of UDPGT leads to:**
- Gilbert syndrome
- Crigler-najjar syndrome type 1&2&3

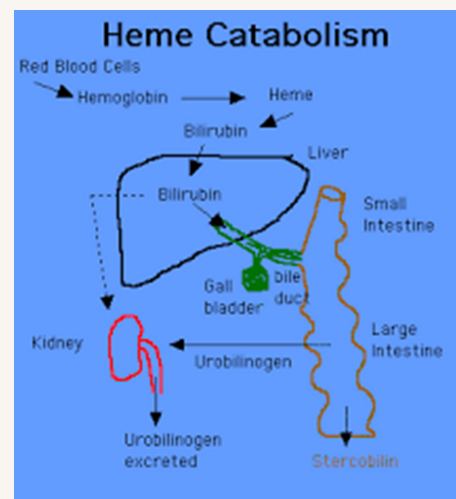
bilirubin-diglucuronide = conjugated bilirubin
is soluble in water → „direct bilirubin“



- **Physiological jaundice in newborns occurs after birth. This happens because either the baby isn't receiving a sufficient amount of glucose for the production of glucuronic acid, which is necessary for bilirubin conjugation, or the baby's liver is still immature and not able to efficiently convert a sufficient amount of glucose into glucuronic acid.**

- Uptake of bilirubin by the liver is mediated by a carrier protein (receptor)
- Uptake may be competitively inhibited by other organic anions
- On the smooth ER, bilirubin is conjugated with glucuronic acid, xylose, or ribose
- Glucuronic acid is the major conjugate – catalyzed by UDP glucuronyltransferase
- “Conjugated” bilirubin is water soluble and is secreted by the hepatocytes into the biliary canaliculi
- Converted to stercobilinogen (urobilinogen) (colorless) by bacteria in the gut
- Oxidized to stercobilin which is colored (brown) then excreted in feces
- Some stercobilin may be re-adsorbed through enterohepatic circulation by the gut and re-excreted by either the liver or kidney (yellow colored)

- **Glucuronic acid is derived from the oxidation of glucose at carbon 6.**
- **For example, if an individual does not receive a sufficient amount of glucose, He may develop jaundice. This is because he doesn't has sufficient amount of glucose to be oxidised by normal reaction in our cells to be converted to glucuronic acid, Consequently, he has a high level of unconjugated bilirubin due to the absence of sufficient amount glucuronic acid which is necessary for bilirubin conjugation**



Clinical correlations

Determination of bilirubin (Bil) in serum

Blood tests

-Bil reacts directly when reagents are added to the blood sample → **conjugated bilirubin = direct Bil (up to 3.4 $\mu\text{mol/L}$)** Give deep purple colouration

-free Bil does not react to the reagents until alcohol (methanol) or caffeine is added to the solution. Therefore, the measurement of this type of bilirubin is indirect → **unconjugated bilirubin = indirect Bil (up to 13.6 $\mu\text{mol/L}$)**

- So they use solvent: Methanol or caffeine.
- Then the deep purple colouration appear (indirect bil)

-**Total bilirubin** measures both unconjugated and conjugated Bil (**normal value up to 17 $\mu\text{mol/L}$**).

	Results of Vanden Bergh	Type of Hyperbilirubinemia/Jaundice
1	Direct Vanden Bergh's Reaction Positive	Conjugated Hyperbilirubinemia Obstructive Jaundice
2	Indirect Vanden Bergh's Reaction Positive	Unconjugated Hyperbilirubinemia. Hemolytic Jaundice
3	Both Direct and Indirect Vanden Bergh's Reaction positive	Biphasic Hyperbilirubinemia means Both conjugated and Unconjugated Bilirubin increased. Hepatic Jaundice.

Bilirubin physiology

-Ligandins responsible for transport from plasma membrane to endoplasmic reticulum. They are necessary for intracellular transport of bilirubin, are also low at birth and reach adult levels by 3-5 days.

- Bilirubin conjugated in presence of UDPGT (uridine diphosphate glucuronyltransferase) to mono and diglucuronides, which are then excreted into bile canaliculi.

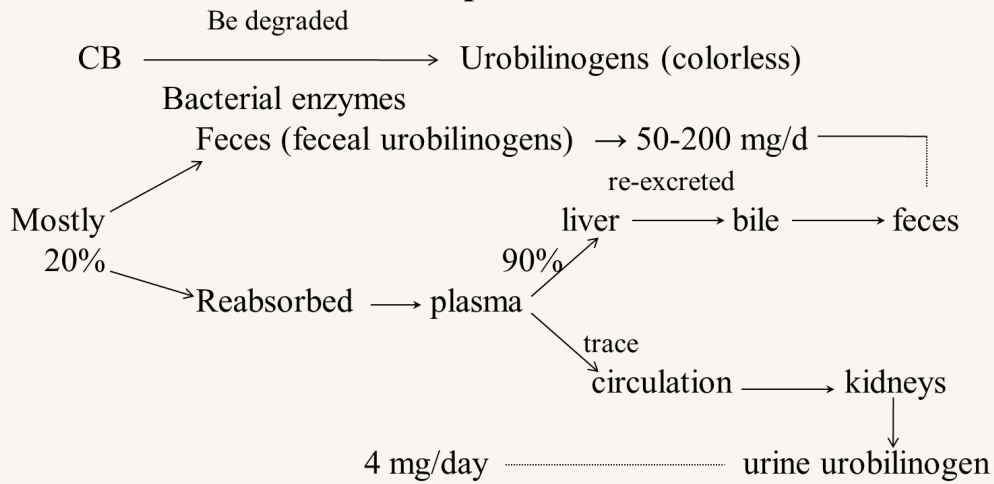
Enterohepatic Circulation

- Conjugated bilirubin is unstable and easily hydrolyzed to unconjugated bilirubin.

-This process occurs nonenzymatically in the duodenum and jejunum and also occurs in the presence of β glucuronidase, an enteric mucosal enzyme, which is found in high concentration in newborn infants and in human milk.

- After (CB) descending from the biliary tract it go to the large intestine, the bacterial flora secretes β -glucuronidase, which dissociates the two molecules of glucuronic acid from CB. Thus get (UCB). Subsequently, UCB then it will be oxidised by enzymes secreted by bacteria flora to be Urobilinogen

Entero - hepatic circulation



- The serum of normal adults contains ≤ 1 mg of bilirubin per 100 ml.
- In healthy adults \rightarrow The direct fraction is usually < 0.2 mg/100 ml
- \rightarrow The indirect fraction is usually < 0.8 mg/100 ml

Definition of Jaundice

Also called icterus

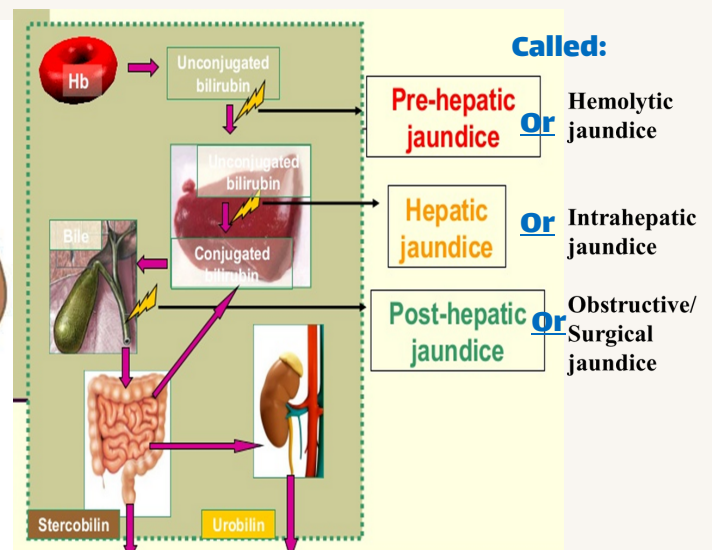
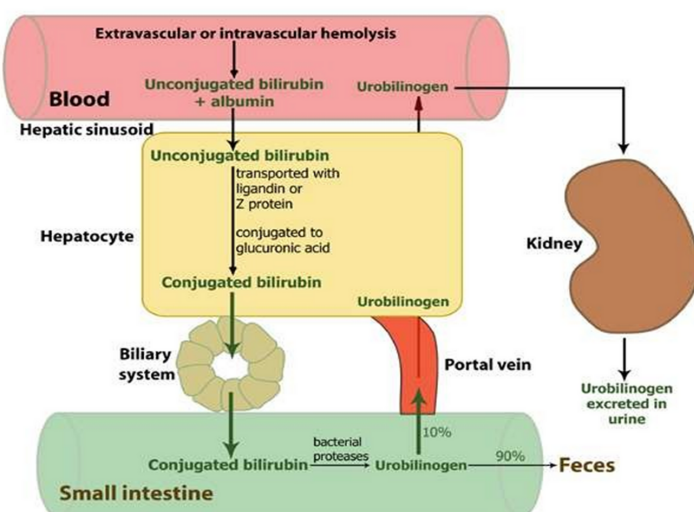
- **A yellowish straining of the skin, conjunctiva, base of tongue palms and soles with bile pigments which are increased in plasma**
- **Can be seen on examination at serum bilirubin levels 27-35 $\mu\text{mol/l}$ (1.5 – 2 mg/dl)**

Pathophysiologic classification of Jaundice

- **Hemolytic Jaundice**
- **Hepatic Jaundice**
- **Obstructive Jaundice (cholestasis)**
- **Genetic based jaundice**

Jaundice classification (according to type of bilirubin)

- **Unconjugated hyperbilirubinemia: when direct bilirubin level is less than 15% of total serum bilirubin.**
- **Conjugated hyperbilirubinemia: when direct bilirubin level is greater than 15%**



Prehepatic (hemolytic, unconjugated) jaundice

- Results from excess production of bilirubin (UCB) (beyond the ability of liver to conjugate) following hemolysis

- The capacity of liver to conjugate is 250-300 mg daily

Causes

- Increased production of bilirubin due to extravascular hemolysis, extravasation of blood into tissues, intravascular hemolysis and errors in production of red blood cells

- Pyruvate kinase and glucose 6-phosphate dehydrogenase deficiency
- Impaired hepatic bilirubin uptake as in CHF Congestive Heart Failure

- Ineffective erythropoiesis
- Impaired bilirubin conjugation
Gilbert's and Crigler-Najarr syndromes
Hyperthyroidism

Liver diseases as in chronic hepatitis, cirrhosis,

Wilson's disease

- Related to Cu metabolism
- Accumulation of Cu in liver, brain, cardiac muscle.

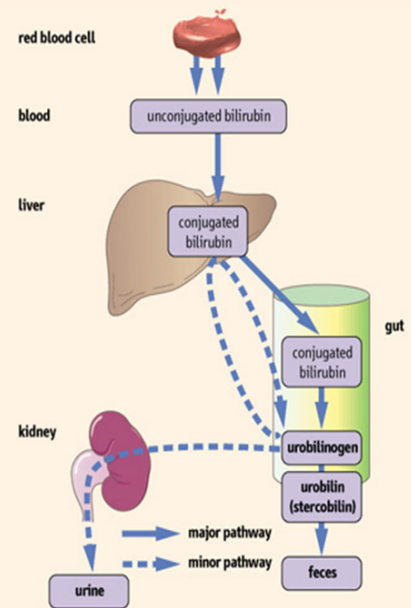
Laboratory findings

- (No excretion of conjugated bilirubin in extra amount in urine)

- **UB** ↑ **without bilirubinuria** (50-150 $\mu\text{mole/l}$)
- Hemolytic anemia
- Hemoglobinuria (in acute intravascular hemolysis)
- Reticulocyte counts ↑ (10-30 %; normal range <1 %)
- Urinary changes:
- Bilirubin: absent
- Urobilinogen: increased or normal
- Faecal changes: stercobilinogen: normal

- Changing in coloration isn't used as an indicator of disease, other investigations should be undertaken, but it can serve as supportive for early diagnosis of the disease

Prehepatic (hemolytic) jaundice



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- Despite the increase in unconjugated bilirubin levels, **bilirubinuria is not observed in hemolytic jaundice** due to the liver's capacity to uptake up to 250-300 mg of unconjugated bilirubin and conjugate them. And slightly increase its capacity if there is an extra production of unconjugated bilirubin.
- In hemolytic jaundice: The UCB increase (**early**) due to the excessive production of UCB & coupled with the liver's inability to uptake and conjugate all different amounts of UCB, but later on (in **advance stage**) we will find **both types UCB & CB level increase, why?**
- because as the liver attempts to uptake more and more UCB, it eventually becomes exhausted, leading to impaired liver function in uptaking normal amount of UCB (which is already high), & impairment in the conjugating, and excreting. Consequently, both UCB and CB will increase. So Early stage is totally different from the advanced stage of jaundice

Intrahepatic (conjugated) jaundice

-Due to a disease affecting hepatic tissues either congenital or acquired diffuse hepatocellular injury

- Impaired uptake, conjugation, or secretion of bilirubin

- Reflects a generalized liver (hepatocyte) dysfunction

- In this case, hyperbilirubinemia is usually accompanied by other abnormalities in biochemical markers of liver function

Causes

- Impaired or absent hepatic conjugation of bilirubin

- Gilbert's and Grigler–Najjar

- Acquired disorders

- Hepatocellular necrosis

- Hepatitis, Cirrhosis, Drug-related

- Sepsis

- Infiltrative: TB, amyloid, lymphoma

- Toxins

- Hepatic crisis in sickle cell disease

Laboratory findings

- liver function tests are abnormal

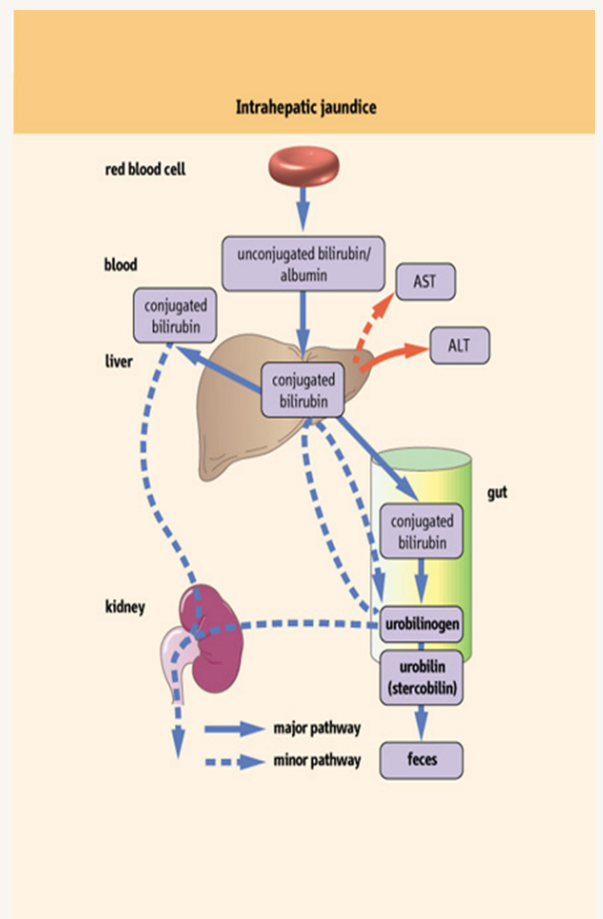
- Both CB and UCB ↑

- Bilirubinuria (↑750-250 $\mu\text{mole/l}$)

- Urobilinogen: normal or reduced

- Stercobilinogen: normal or reduced

• the liver is unable to excrete a normal amount of conjugated bilirubin, so will be a decrease in the production of urobilinogen and stercobilinogen.



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• In intrahepatic jaundice, both UCB and CB levels will be increase ,due to the impairment in the liver's uptake of UB,UB will elevate .Even after conjugation, the liver will be unable to excrete properly, leading to diffusion of CB into the circulation.

• The enzymes that be measured to investigate liver function include:

1. ALT (Alanine Aminotransferase)

2. AST (Aspartate Aminotransferase)

3. ALP (Alkaline Phosphatase)

4. γ GT (Gamma-Glutamyl Transferase)

5. 5' Nucleotidase (expensive Test)

• No increase in reticulocyte count occurs because the cause is not excessive hemolysis of erythrocytes.

Posthepatic (Obstructive) jaundice

- Caused by intra- and extra hepatic obstruction of bile ducts
- Plasma bilirubin is conjugated, and other biliary metabolites, such as bile acids accumulate in the plasma
- Characterized by pale colored stools (absence of fecal bilirubin or urobilin), and dark urine (increased conjugated bilirubin)
- In a complete obstruction, urobilin is absent from the urine

Causes

Intrahepatic

- Blockage of Bile Canaliculi
- Dubin-Johnson syndrome
- Hepatitis-viral, chemical
- Infiltrative tumors

Extrahepatic

- Obstructive of bile ducts by tumors, CBD or CHD stone and Stenosis
- Acute and chronic pancreatitis
- Parasitic infections as *Ascaris lumbricoides* and liver flukes

Laboratory Findings

- Serum Bilirubin \uparrow (100-500 $\mu\text{mole/l}$)
- Fecal urobilinogen. \downarrow (incomplete obstruction) or absent in (complete obstruction)
- Urobilinogenuria is absent in complete obstructive jaundice

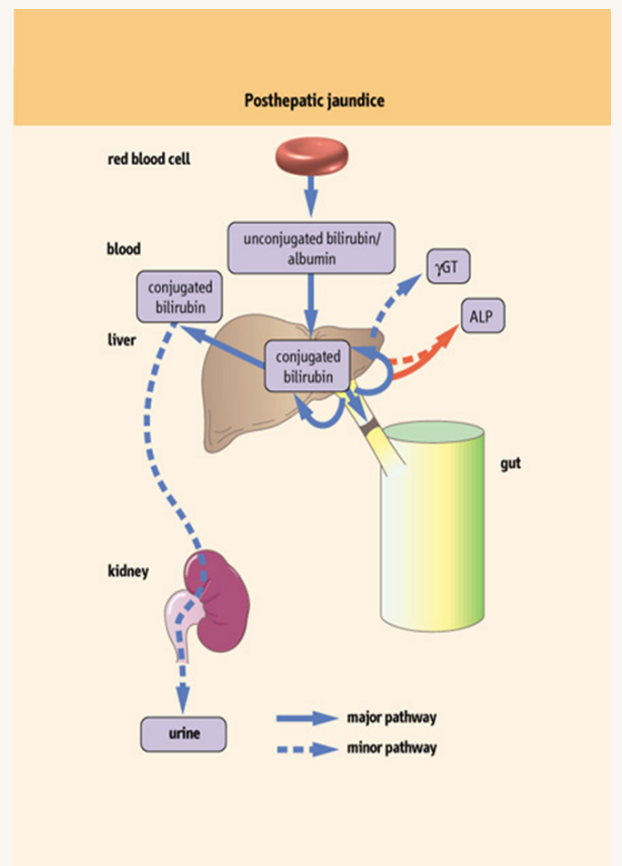
- Bilirubinuria \uparrow - Cholesterol \uparrow

Urinary changes:

1- Bilirubin: increased. 2- Urobilinogen: reduced or absent

- Faecal changes: stercobilinogen: reduced or absent

- The distinctive clinical sign for individuals with obstructive jaundice is: severe itching, which results from the diffusion of bile salts that did not descend with the bile to the large intestine. Some of them will exist subcutaneously, causing itching for the individual.



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- In Post hepatic jaundice: Liver is normal, its up taking normal amount of UCB, & its conjugating the normal amount of the UCB.

- Here (CB): Which increase

- The urine is very dark, but the Darkness of the urine is not caused by an increase in the production or excretion of urobilinogen but rather due to increase production & excretion of (CB).

The causes of jaundice

Type	Cause	Clinical example	Frequency
Prehepatic	hemolysis	autoimmune abnormal hemoglobin	uncommon depends on region
intrahepatic	infection	hepatitis A, B, C	common/very common
	chemical/drug	acetaminophen alcohol	common common
	genetic errors: bilirubin metabolism	Gilbert's syndrome Crigler–Najjar syndrome Dubin–Johnson syndrome Rotor's syndrome	1 in 20 very rare very rare very rare
	genetic errors: specific proteins	Wilson's disease α_1 antitrypsin	1 in 200 000 1 in 1000 with genotype
	autoimmune	chronic active hepatitis	uncommon/ rare
	neonatal	physiologic	very common
Posthepatic	intrahepatic bile ducts	drugs primary biliary cirrhosis cholangitis	common uncommon common
	extrahepatic bile ducts	gall stones pancreatic tumor cholangiocarcinoma	very common uncommon rare

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	Pre-hepatic	Hepatic	Post-hepatic
Urine	No Bilirubin Urobilinogen \uparrow	There is bilirubin Normal urobilinogen	There is bilirubin Urobilinogen is absent
Faeces	Dark	Pale	Pale
Blood	\uparrow Reticulocyte count \uparrow Unconjugated bilirubin (up to $100\mu\text{mol/L}$) Normal ALP and γ GT Normal AST and ALT PT Normal	Normal reticulocyte count \uparrow Bilirubin – mixed conjugated & unconjugated \uparrow ALP and γ GT \uparrow AST and ALT \uparrow PT – not correctable with Vit K	Normal reticulocyte count \uparrow Bilirubin (up to $1000\mu\text{mol/L}$) – conjugated \uparrow ALP and γ GT Normal AST and ALT \uparrow PT – correctable with Vit K

- **Intra hepatic jaundice**

There are damage of liver cells from the early beginning of the disease & this damage will not allow to storage of vit. k .

(Normally fat soluble vitamins (ADEK) can be stored in liver),

-The \uparrow PT so there are increase in the bleeding tendency in the patient which cannot be corrected with vit.K

- However, in obstructive jaundice, there is no early damage to the liver cells. Vitamin K can be store and the condition can be corrected with vit k..

- In intraheptic jaundice due to damaging liver cells All enzymes elevated (there is impairment of liver function in the uptaking , conjugation and excretion)

يعبروا عن
Damaging level of liver cells

- In obstructive jaundice , enzymes which related to obbstctive jaundice (colestaiss)..will increase only :
 \uparrow ALP and γ GT.

- In obstructive jaundice All enzyme in liver will increase in the advance state

In early there isn't damaging in liver cells ,But later on due to regurgitation of bile (cant pass through billiry tract to large intestines) this will damage liver cells ,So later on all enzymes will increase

\uparrow ALP and γ GT
 \uparrow AST and ALT (also will be increase)

Neonatal Jaundice

- Common, particularly in premature infants
- Transient (resolves in the first 10 days), due to immaturity of the enzymes involved in bilirubin conjugation

- High levels of unconjugated bilirubin are toxic to the newborn – due to its hydrophobicity it can cross the blood-brain barrier and cause a type of mental retardation known as kernicterus

- If bilirubin levels are judged to be too high, then phototherapy with UV light is used to convert it to a water soluble, non-toxic form

- If necessary, exchange blood transfusion is used to remove excess bilirubin

- Phenobarbital is oftentimes administered to Mom prior to an induced labor of a premature infant – crosses the placenta and induces the synthesis of UDP glucuronyl transferase

- Jaundice within the first 24 hrs of life or which takes longer than 10 days to resolve is usually pathological and needs to be further investigated

Gilbert's syndrome

- **Benign** liver disorder considered the most common hereditary cause of increased bilirubin.

- A major characteristic is jaundice, caused by elevated levels of unconjugated bilirubin in the bloodstream.

- The cause of this hyperbilirubinemia is the reduced activity of the glucuronyltransferase, which conjugates bilirubin and some other lipophilic molecules.

- It is caused by a **70%-80% reduction** in the glucuronidation activity of the enzyme UDP-glucuronosyltransferase 1A1.

- 1/2 of the affected individuals inherited it
- Males more frequently affected than females
- Onset of symptoms in teens, early 20's or 30's
- Can be treated with small doses of phenobarbital to stimulate UDP glucuronyltransferase activity

• **Phototherapy with UV light :**
Do isomerization to convert UCB (hydrophobic) to CB (hydrophilic) water soluble

• If phototherapy is not effective and (UCB) levels continue to increase, we will do exchange blood transfusion.

• Means replacing a volume of blood (which containing a high concentration of UCB) with an equivalent volume of blood (which contains a normal level of) UCB. وهكذا . .

• This approach to avoid increasing in blood volume which could lead to heart failure.

• Performing a blood transfusion for the baby is not appropriate, as it will cause overloading on cardiac muscle which will result in heart failure

• Its (**benign**): **20%-30%** of Activity present just and its still can do conjugation to the normal level of UCB.

• So It's benign, (the amount of unconjugated bilirubin (UCB) does not rise to high levels that could lead to kernicterus)

• **Gilber's syndrome (benign) case of jaundice, means: there is normal level of UCB.**

• If the Gilbert's syndrome patient state is complicated means : has problem in gene encoding for **UDP-glucuronosyltransferase 1A1. & with any other cause of: Excessive hemolysis of erythrocytes , in this state the case will not be benign but complicated.**

• If case be (Complicated) with another thing causing hemolysis of erythrocytes .there will be excessive production of UCB and the activity of the enzyme which is (20%-30% of normal activity of the enzyme) will not be able to conjugate the excessive amount of UCB.

Crigler - Najjar syndrome, type I

- A very rare disease (estimated at 0.6 - 1.0 per million live births), and consanguinity increases its risk.
- Inheritance is autosomal recessive.
- Type 1 is characterized by a serum bilirubin usually above 345 $\mu\text{mol/L}$ (310 - 755)
- No UGT1A1 (UDP glucuronosyltransferase 1 family, polypeptide A1) expression can be detected in the hepatic tissue.
- These children died of kernicterus (=bilirubin encephalopathy), or survived until early adulthood with clear neurological impairment.

Today, therapy includes:

- exchange transfusions in the immediate neonatal period, **To decrease level of UCB**
- 12 hours/day phototherapy
- hemeoxygenase inhibitors to reduce effect of hyperbilirubinemia
- oral calcium phosphate and -carbonate to form complexes with bilirubin in the gut,
- liver transplantation prior to the onset of brain damage.

Form Complex with UCB in the large intestine to avoid re absorption ..thus decrease amount of UCB

Crigler-Najjar syndrome, type II

Differs from type I in several aspects:

- 1- bilirubin levels are generally below 345 $\mu\text{mol/L}$.
 - 2- Some cases are only detected later in life because of lower serum bilirubin, kernicterus is rare in type II.
 - 3- bile is pigmented, instead of pale in type I or dark as normal. (**<10% of normal activity of the enzyme**)
 - 4- UGT1A1 is present at reduced but detectable levels (**typically <10% of normal**), because of single base pair mutations
 - 5- therefore, treatment with phenobarbital is effective, generally with a decrease of at least 25% in serum bilirubin.
- The inheritance pattern of Crigler – Najjar syndrome type II has been difficult to determine, but is generally considered to be autosomal recessive.
 -

Dubin-Johnson and Rotor's syndromes

- Characterized by impaired biliary secretion of conjugated bilirubin
- Present with a conjugated hyperbilirubinemia that is usually mild