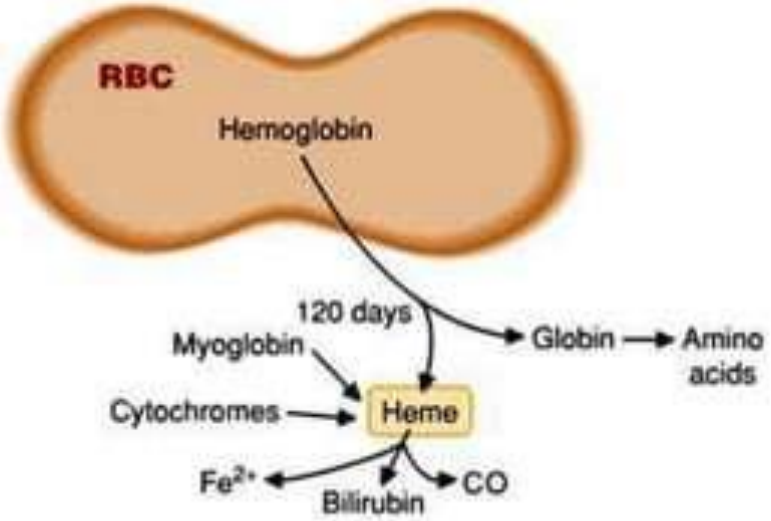
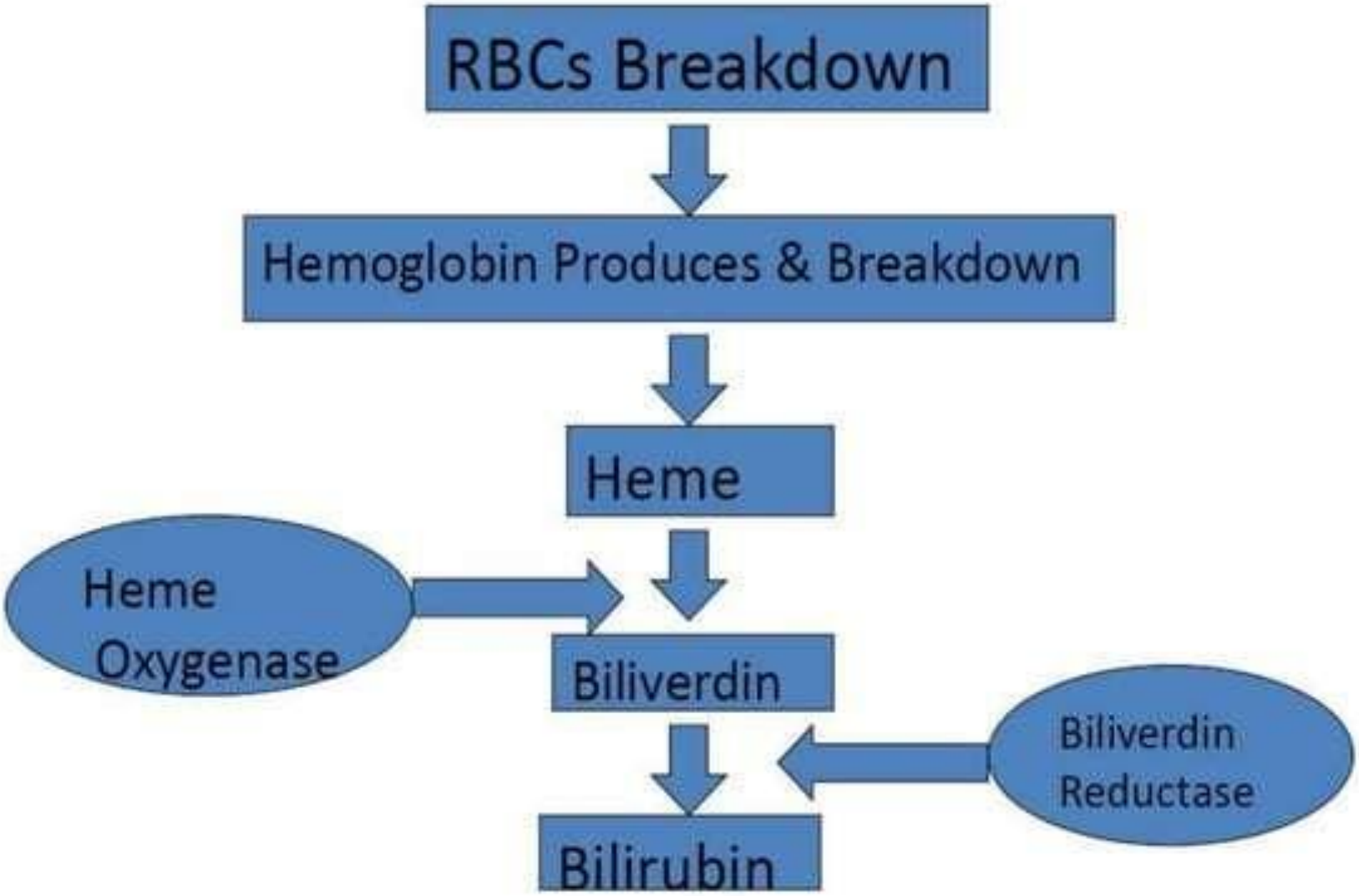
BILIRUBIN METABOLISM

HAEMOGLOBIN

- Haemoglobin is a conjugated protein made up of four subunits, each subunit contains a haem group and a polypeptide chain.
- The haem group is made up of porphyrin and a ferrous ion.
- Haemoglobin can reversibly combine with oxygen, and transports oxygen in the body.




RBCs Breakdown
Hemoglobin Produces & Breakdown
Heme
Heme Oxygenase
Biliverdin
Biliverdin Reductase
Bilirubin

CATABOLISM OF HAEMOGLOBIN

➢ **Generation of Bilirubin**

- When life-span of RBCs is over, they are broken down in reticulo-endothelial system.
- Haem and globin are separated.
- Globin is broken down into amino acids.
- The iron liberated from heme is reutilized.
- The porphyrin ring is broken down in reticulo-endothelial (RE) cells of liver, spleen and bone marrow to bile pigments, mainly bilirubin.
- About 6 g of Hb is broken down per day, from which about 250 mg of bilirubin is formed.

- From myoglobin and other heme containing proteins, another 50 mg of bilirubin is formed.
- Approximately 35 mg of bilirubin is formed from 1 g of Hb.
- A total of 300 mg of bilirubin is formed everyday; of which 80% is from destruction of old RBCs, 10% from ineffective erythropoiesis and the rest 10% from degradation of myoglobin and other heme containing proteins.
- Heme is degraded primarily by a microsomal enzyme system; heme oxygenase.
- It requires molecular oxygen and NADPH.
- The oxygenase enzyme specifically catalyzes the cleavage of the alpha methenyl bridge, which is linking the pyrrole rings I and II.

- The linear tetrapyrrole formed is biliverdin which is green in color.
- In mammals it is further reduced to bilirubin, a redyellow pigment, by an NADPH dependent biliverdin.
- In the first reaction, a bridging methylene group is cleaved by heme oxygenase to form Linear Biliverdin from Cyclic Heme molecule.
- Fe^{2+} is released from the ring in this process.

➢ **Transport to liver**

- The bilirubin formed in the reticuloendothelial cells isinsoluble in water.
- The lipophilic bilirubin is therefore transported in plasma bound to albumin.
- One molecule of albumin can bind 2 molecules of bilirubin.

- Albumin binds bilirubin in loose combination. So when present in excess, bilirubin can easily dissociate from albumin.
- The binding sites for bilirubin on albumin can be occupied by aspirin, penicillin, etc. Such drugs can, therefore, displace bilirubin from albumin. Hence, care should be taken while administering such drugs to newborn babies to avoid kernicterus.
- When the albumin–bilirubin complex reaches the sinusoidal surface of the liver, the bilirubin is taken up.
- Bilirubin is not very water-soluble, so most of it is carried to the liver bound to albumin.

➢ **Hepatocellular bilirubin transport**

- Albumin-bound bilirubin in sinusoidal blood passes through endothelial cell fenestrae to reach the hepatocyte surface, entering the cell by both facilitated and simple diffusional processes. Within the cell, it is bound to glutathione-S-transferases and conjugated by bilirubin-UDP-glucuronosyl-transferase (UGT1A1) to mono- and diglucuronides, which are actively transported across the canalicular membrane into the bile. In addition to this direct excretion of bilirubin glucuronides, a portion are transported into the portal circulation by MRP3 and subjected to reuptake into the hepatocyte by OATP1B1 and OATP1B3.

➢ **Conjugation in liver**

- Inside the liver cell, the bilirubin is conjugated with glucuronic acid, to make it water soluble.

- The first carbon of glucuronic acid is combined with the carboxyl group of the propionic acid side chains of the bilirubin molecule.
- About 80% molecules are in the diglucuronide form, while 20% are monoglucuronides.
- Drugs like primaquine, novobiocin, chloramphenicol, androgens and pregnanediol may interfere in this conjugation process and may cause jaundice.
- Unconjugated bilirubin: Bilirubin that are not conjugated with glucuronic acid, also called hemobilirubin, indirect bilirubin.
- Conjugated bilirubin: Bilirubin that are conjugated with glucuronic acid, also called hepatic bilirubin, direct bilirubin.

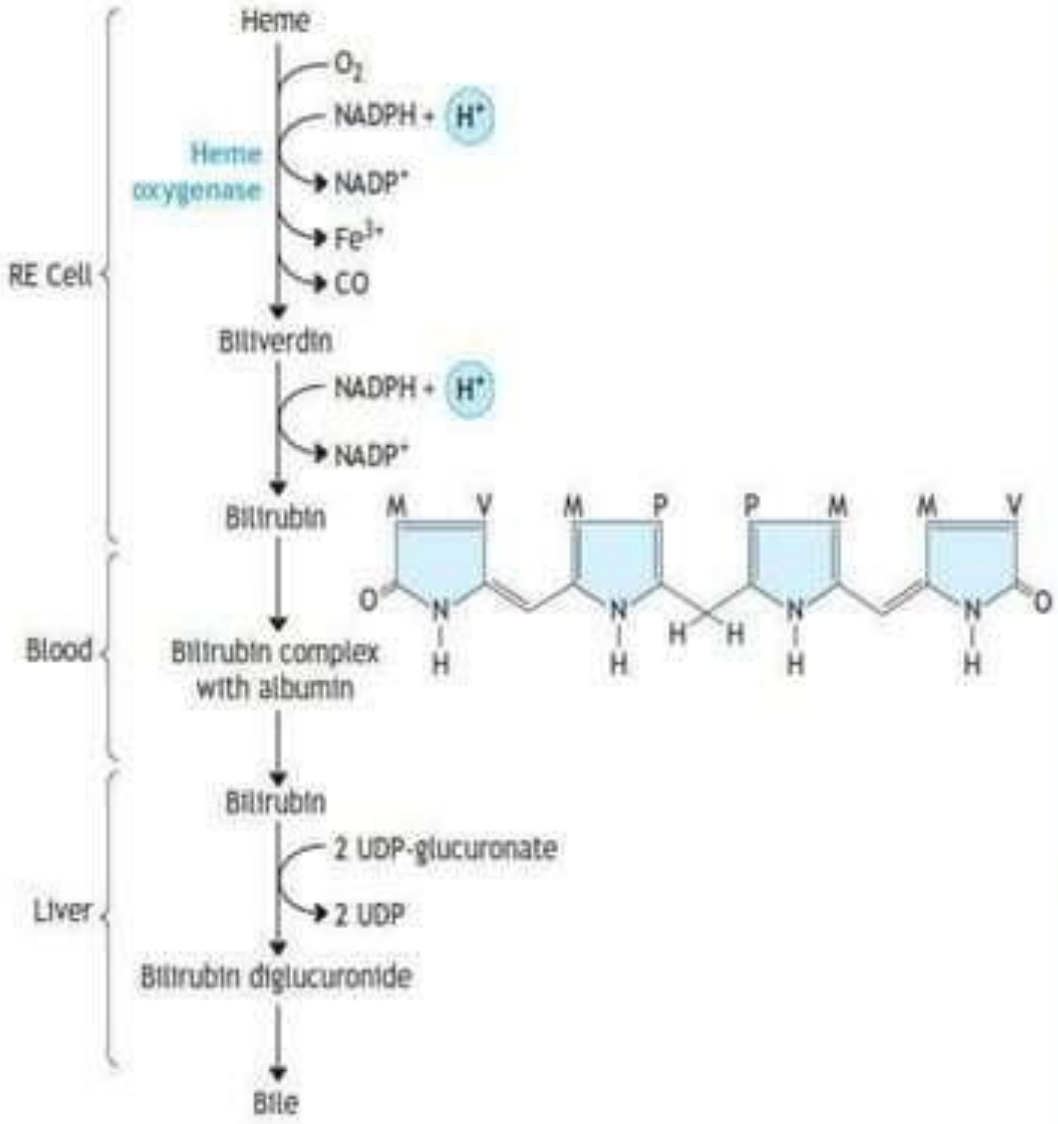
➢ **Uptake of Bilirubin by the liver**

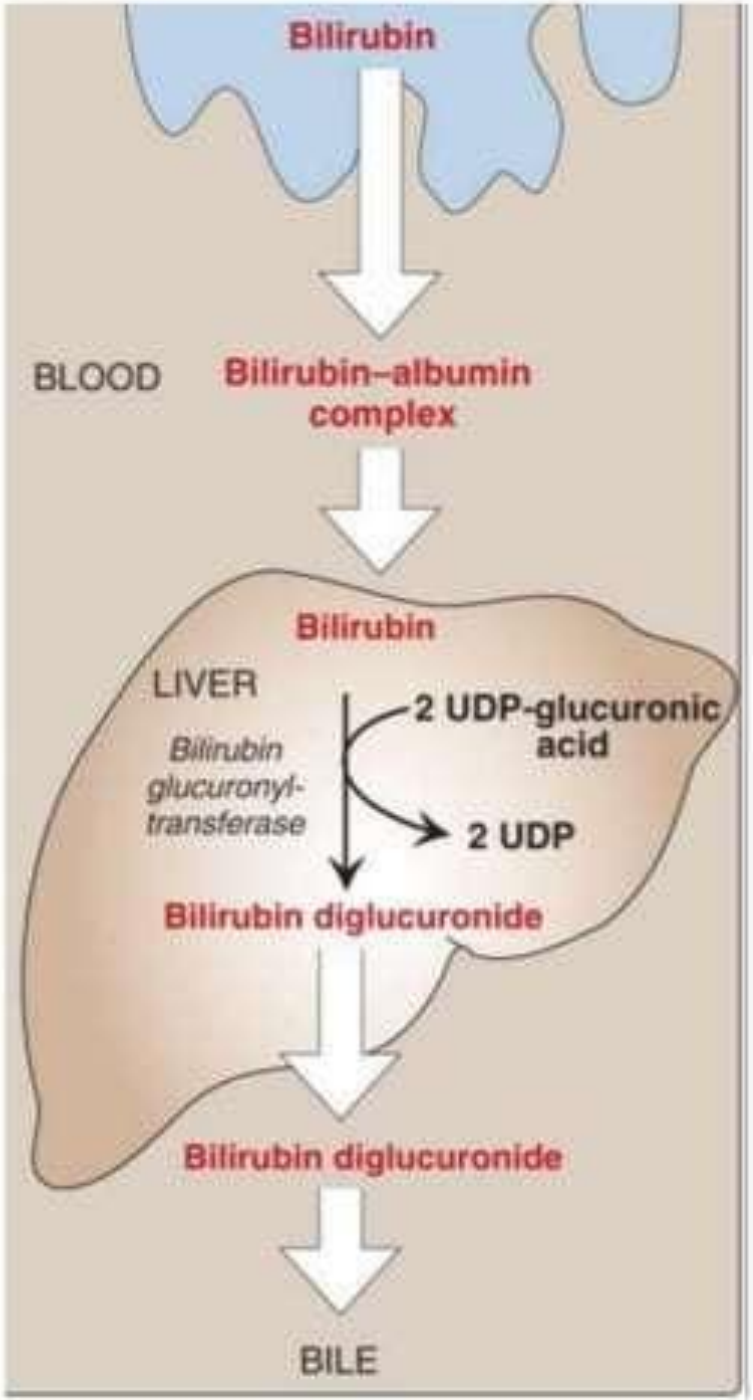
- Bilirubin is only slightly soluble in plasma thus transported to the liver by binding non-covalently to albumin.

- Bilirubin dissociates from the carrier albumin molecule and enters a hepatocyte and binds to intracellular proteins; ligandin and Z protein.
- Note: drugs, such as salicylates and sulfonamides can displace bilirubin from albumin, permitting bilirubin to enter the central nervous system.
- This causes the potential for neural damage in infants.

Unconjugated Bilirubin VS Conjugated Bilirubin

Normal serum level	More	Less (less than 0.2mg/dl)
Water solubility	Absent	Present
Affinity to lipids (alcohol solubilty)	Present	Absent
Serum albumin binding	High	Low
Van den Bergh rxn	Indirect(Total minus direct)	Direct
Renal excretion	Absent	Present
Affinity to brain tissue	Present(kernicterus)	Absent


Heme
O_2
NADPH + H^+
Heme oxygenase
$NADP^+$
Fe^{3+}
CO
RE Cell
Biliverdin
NADPH + H^+
$NADP^+$
Bilirubin
Blood
Bilirubin complex with albumin
Bilirubin
2 UDP-glucuronate
2 UDP
Liver
Bilirubin diglucuronide
Bile


Bilirubin
BLOOD
Bilirubin–albumin complex
Bilirubin
LIVER
2 UDP-glucuronic acid
Bilirubin glucuronyl-transferase
2 UDP
Bilirubin diglucuronide
Bilirubin diglucuronide
BILE

➢ Excretion of Bilirubin to Bile

- The water soluble conjugated bilirubin is excreted into the bile by an active process and this occurs against a concentration gradient.
- This is the rate limiting step in the catabolism of heme. It is induced by phenobarbitone.
- Excretion of conjugated bilirubin into bile is mediated by an ATP binding cassette protein which is called Multispecific organic anion transporter (MOAT), located in the plasma membrane of the biliary canaliculi.

➢ Fate of Conjugated Bilirubin in Intestine

- The conjugated bilirubin reaches the intestine through the bile.

- In the small intestine, conjugated bilirubin is poorly reabsorbed and partly hydrolyzed back to unconjugated bilirubin by catalytic action of bacterial ß-glucuronidases.
- In the distal ileum and colon, anaerobic flora mediate further catabolism of bile pigments.

a. hydrolysis of conjugated bilirubin to unconjugated bilirubin by bacterial β-glucuronidases;

b. multistep hydrogenation (reduction) of unconjugated bilirubin to form colorless urobilinogens (44 Hydrogen)

c. Further reduction of UBG leads to formation of stercobilinogen (SBG,48 hydrogen)

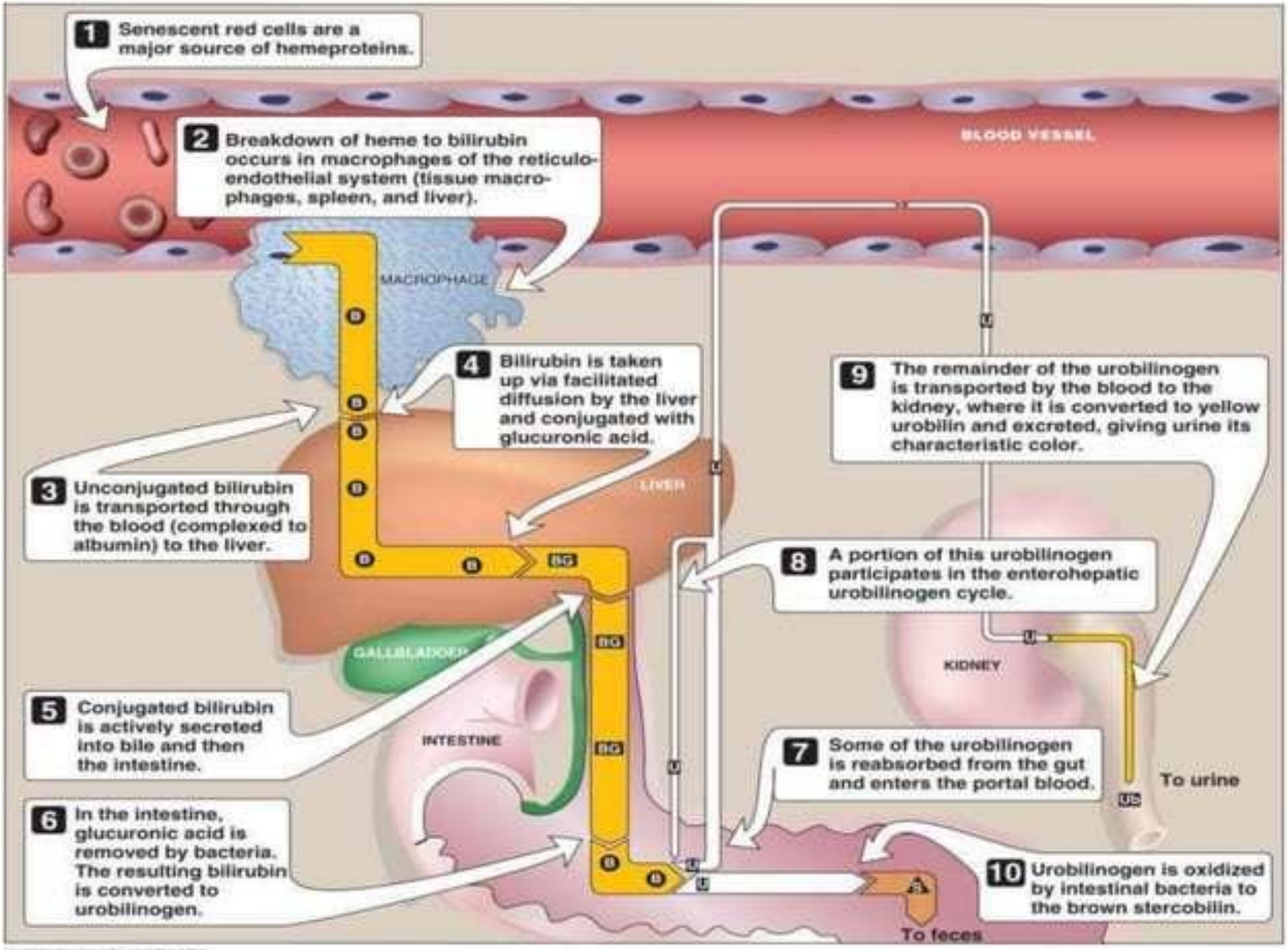
- The SBG is mostly excreted through feces (250-300 mg/day).
- Upto 20 % of urobilinogen produced daily is reabsorbed from the intestine & enters the entero-hepatic circulation.

➢ Final Excretion

- UBG and SBG are both colorless compounds but are oxidized to colored products, urobilin (42 hydrogen) or stercobilin (46 hydrogen) respectively by atmospheric oxidation.
- Black color is seen in constipation. If intestinal flora is decreased by prolonged administration of antibiotics, bilirubin is not reduced to bilinogens, and in the large gut, it is re-oxidized by O2 to form biliverdin. Then green tinged feces is seen, especially in children.

SUMMARY OF BILIRUBIN METABOLISM

- Old red blood cells are major source of haem proteins.
- Breakdown of haem to bilirubin occur in macrophage of reticulo-endithelial system (tissue macrophages, spleen and liver).
- Unconjugated bilirubin is transported through blood (complex to albumin) to liver.
- Bilirubin is taken into liver and conjugated with glucuronic acid.
- Bile is secreted into intestine where glucuronic acid is removed and the resulting bilirubin is converted to urobilinogen.
- A portion of urobilinogen is reabsorbed into blood, where it is converted to the yellow urobilin and excreted by kidneys.
- Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.


1 Senescent red cells are a major source of hemeproteins.
2 Breakdown of heme to bilirubin occurs in macrophages of the reticulo-endothelial system (tissue macrophages, spleen, and liver).
BLOOD VESSEL
MACROPHAGE
4 Bilirubin is taken up via facilitated diffusion by the liver and conjugated with glucuronic acid.
3 Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.
LIVER
9 The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.
8 A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.
KIDNEY
GALLBLADDER
5 Conjugated bilirubin is actively secreted into bile and then the intestine.
INTESTINE
7 Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.
To urine
6 In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.
10 Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.
To feces

JAUNDICE

- Jaundice is a yellow discoloration of the skin, mucous membranes, and sclera caused by increased amounts of bilirubin in the blood.
- Jaundice is a sign of an underlying disease process.
- Jaundice results from the accumulation of bilirubin.
- Hyperbilirubinemia may be due to abnormalities in the formation, transport, metabolism, and excretion of bilirubin.
- Total serum bilirubin is normally 0.2-1.2 mg/dL and jaundice may not be recognizable until levels are about 3 mg/dL.
- In the normal adult the rate of systemic bilirubin production is equal to the rates of hepatic uptake, conjugation, and biliary excretion.

- Jaundice occurs (bilirubin levels may reach 30-40 mg/dL in severe disease) when the equilibrium between bilirubin production and clearance is disturbed by one or more of the following mechanisms.

a. Excessive production of bilirubin,
b. Reduced hepatic uptake,
c. Impaired conjugation,
d. Decreased hepatocellular excretion,
e. Impaired bile flow (both intra hepatic and extra hepatic).

- The first three mechanisms produce unconjugated hyperbilirubinemia, and the latter two produce predominantly conjugated hyperbilirubinemia.
- More than one mechanism may operate to produce jaundice, especially in hepatitis, which may produce Unconjugated and conjugated hyperbilirubinemia.

CLASSIFICATION OF JAUNDICE

- **Pre-hepatic (before bile is made in the liver)**

- Jaundice in these cases is caused by rapid increase in the breakdown and destruction of the red blood cells (hemolysis), overwhelming the liver's ability to adequately remove the increased levels of bilirubin from the blood.
- Causes:

a. Malaria,
b. Sickle cell crisis,
c. Spherocytosis,
d. Thalassemia,
e. Glucose-6-phosphate dehydrogenase deficiency,

f. Drugs or other toxins,

g. Autoimmune disorders.

➢ **Hepatic (the problem arises within the liver)**

- Jaundice in these cases is caused by the liver's inability to properly metabolize and excrete bilirubin.
- Causes:

a. Viral hepatitis (A, B, or C)

b. Infections (yellow fever, bacterial sepsis, tuberculosis)

c. Alcohol,

d. Drugs e.g. estrogens, contraceptive pills

e. Pregnancy

f. Carcinoma (metastases, lymphoma, adenocarcinoma of kidney)

- **Post-hepatic (after bile has been made in the liver)**

- Jaundice in these cases, also termed obstructive jaundice, is caused by conditions which interrupt the normal drainage of conjugated bilirubin in the form of bile from the liver into the intestines.
- The obstruction may be intrahepatic or extra hepatic.
- Causes:

1. Intra hepatic Obstruction;

a. Biliary atresia,

b. Primary Biliary Cirrhosis,

c. Malignant infiltration of ducts.

2. Extra hepatic obstruction;

a. Gallstones in the bile ducts,

b. Cancer (pancreatic and gallbladder/bile duct carcinoma),

c. Strictures of the bile ducts,
d. Pressure on the common bile duct from enlarged lymph nodes,
e. Cholangitis,
f. Congenital malformations,
g. Pancreatitis.

PHYSIOLOGICAL NEONATAL JAUNDICE

- Most infants (around 60%) develop visible jaundice due to elevation of unconjugated bilirubin concentration during their first week. This common condition is called Physiological Neonatal Jaundice.
- Bilirubin produced by the fetus is cleared by the placenta and eliminated by the maternal liver.
- Immediately after birth, the neonatal liver must assume responsibility for bilirubin clearance and excretion.
- However, many hepatic physiologic processes are incompletely developed at birth.

- **Mechanism of neonatal jaundice**

1. More bilirubin produced

a. Destruction of fetal haemoglobin (Hbf),

b. Shorter life span of fetal red blood cells.

2. The low capability of albumin on unconjugated bilirubin transportation.

3. The low capability of hepatocytes for conjugation of bilirubin.

4. High workload of the hepato-enteric circulation

a. Less bacterial flora (low conversion of bilirubin to urobilinogen by the intestinal flora),

b. Low enzymatic activity in intestine.

CONGENITAL CONDITIONS FOR JAUNDICE

1. **Crigler-Najjar syndrome:**

- An inherited condition that may lead to severe Unconjugated hyperbilirubinemia (high bilirubin concentrations); a gene mutation leads to a deficiency in an enzyme (UDP-glucuronosyl-transferase) production necessary for bilirubin conjugation.
- In Crigler-Najjar patients the enzyme is either inactive (type I) or severely reduced (type II).
- Therefore bilirubin cannot be excreted into the bile and remains in the blood.
- The high plasma level of Unconjugated bilirubin leads to jaundice and may lead to kernicterus (bilirubin encephalopathy).

2. Dubin-Johnson syndrome:

- An inherited disorder that causes the retention of conjugated bilirubin (and other compounds that turn the liver black) in liver cells; patients may have intermittent jaundice.
- This is an autosomal recessive disorder in which patients have an increase in conjugated bilirubin in the blood.
- This is caused by a defect in secretion of bilirubin glucuronides (already conjugated) across the canalicular membrane (patients are missing a canalicular protein that transports bilirubin glucuronides into bile).
- The protein was originally termed the canalicular multiple organic anion transporter (cMOAT) but is also known as multidrug resistance protein 2 (MRP2).

3. Rotor's syndrome:

- Is another autosomal recessive disorder in which patients have an increase in conjugated bilirubin in the blood.
- The exact molecular defect is unknown, but it seems these patients have multiple defects in hepatocyte uptake and excretion of bilirubin pigments.
- The liver looks normal, and similar to Dubin-Johnson syndrome, most patients are asymptomatic.

4. Gilbert syndrome:

- This syndrome is common which is estimated around 5-10% of the population has it.
- In this disorder, patients have a decreased activity of UGT1A1.

- It sounds just like type II CNS. However, Gilbert syndrome (which is an autosomal recessive syndrome) has a UGT1A1 activity level of about 30% of normal, which is quite a bit higher than the amount of activity we see in CNS.
- Patients usually have only mild Unconjugated hyperbilirubinemia.

Jaundice

Increased Bilirubin Production

1. Hemolytic anaemia
   - ABO incomp.
   - Rhesus incomp.
   - Spherocytosis
   - Elliptocytosis
   - G6PD def
   - Pyruvate kinase def
   - AIHA
   - Mechanical trauma: prosthetic valves, hypersplenism, DIC
2. Dyserythropoiesis
   - Megaloblastic anaemias
   - Sideroblastic anaemias
3. Breakdown of large hematomas
4. Sepsis
5. Drugs

Unconjugated ↑bilirubin
Conjugated ↑bilirubin
UC and C ↑bilirubin

Impaired Bilirubin Excretion

Congenital Non-hemolytic ↑bilirubin

- Gilbert's syndrome
- Crigler-Najjar Types I & II
- Rotor syndrome
- Dubin-Johnson syndrome

Cholestatic

Intrahepatic

1. Primary biliary cirrhosis
2. Primary Sclerosing cholangitis
3. Viral hepatitis
4. Alcohol
5. Drugs
6. Autoimmune hepatitis
7. Wilson's dz
8. α1-antitrypsin deficiency
9. Severe bacterial infection
10. Pregnancy
11. Idiopathic recurrent cholestasis

Extrahepatic

1. Choledocholithiasis
2. CA
   - Ampulla
   - Pancreas
   - Bile duct (cholangioCA)
   - Secondary
3. Trauma to biliary structures
4. Fibrosis (eg postop)
5. Cystic fibrosis
6. Parasitic infection

**may result in ascding cholangitis*