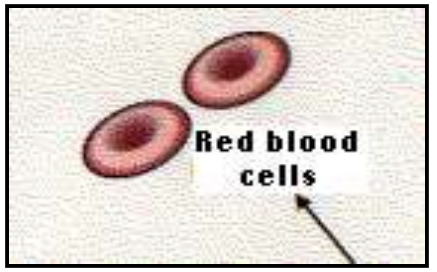
Heme degradation

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).
- In the human body approx. 100 – 200 million RBCs are broken down every hour.
- Fe^{2+} → 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.



Phagocytosis & Lysis

Hemoglobin

Fe^{2+} Globin Heme

Iron pool Amino acids Bilirubin

Amino acid pool Excretion

Handling of free (intravascular) hemoglobin

- Purposes: 1- Scavenge iron
  2- Prevent major iron losses
  3- Complex free heme (very toxic)

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.

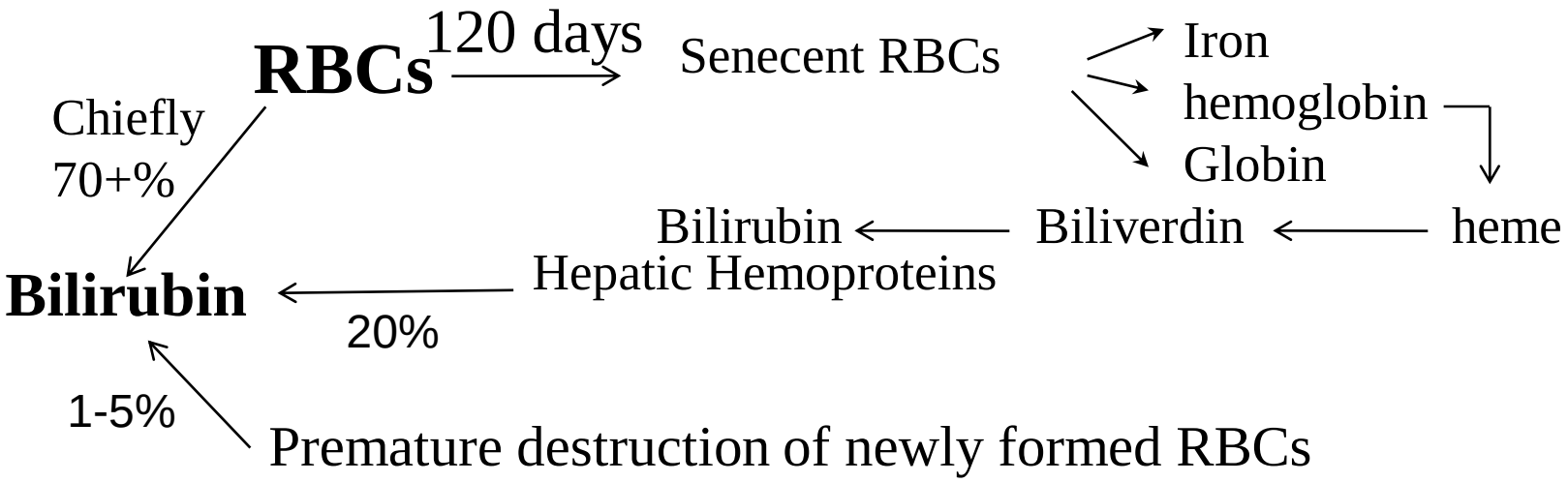
2- Hemopexin: binds free heme. The heme-hemopexin complex is taken up by the liver and the iron is stored bound to ferritin.

3- Methemalbumin: complex of oxidized heme and albumin.

Bilirubin metabolism

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

Bilirubin formation



Transport of bilirubin in plasma

Albumin + UB ⟶ UB ~ Albumin Complex

H affinity binding sites

Bilirubin ⟶ **Plasma protein Albumin**

2:1 Molar Ratio

L affinity binding sites

> 2:1 Bilirubin ⟶ **Plasma protein Albumin**

can be replaced by Other organic anions and low pH ⟶ ↑UCB

Hepatic Bilirubin Transport

1. Hepatic uptake of bilirubin

UCB ~ Albumin complex separated

(be) taken up

Bilirubin ⟶ Plasma membrane of the liver

2. Conjugation of bilirubin

bound to Z protein

UCB ⟶ carrier protein ⟶ ER

(Lipid soluble)

Conjugation (catalyzed by UDPGT)

(Water soluble) CB ⟵ CBGA

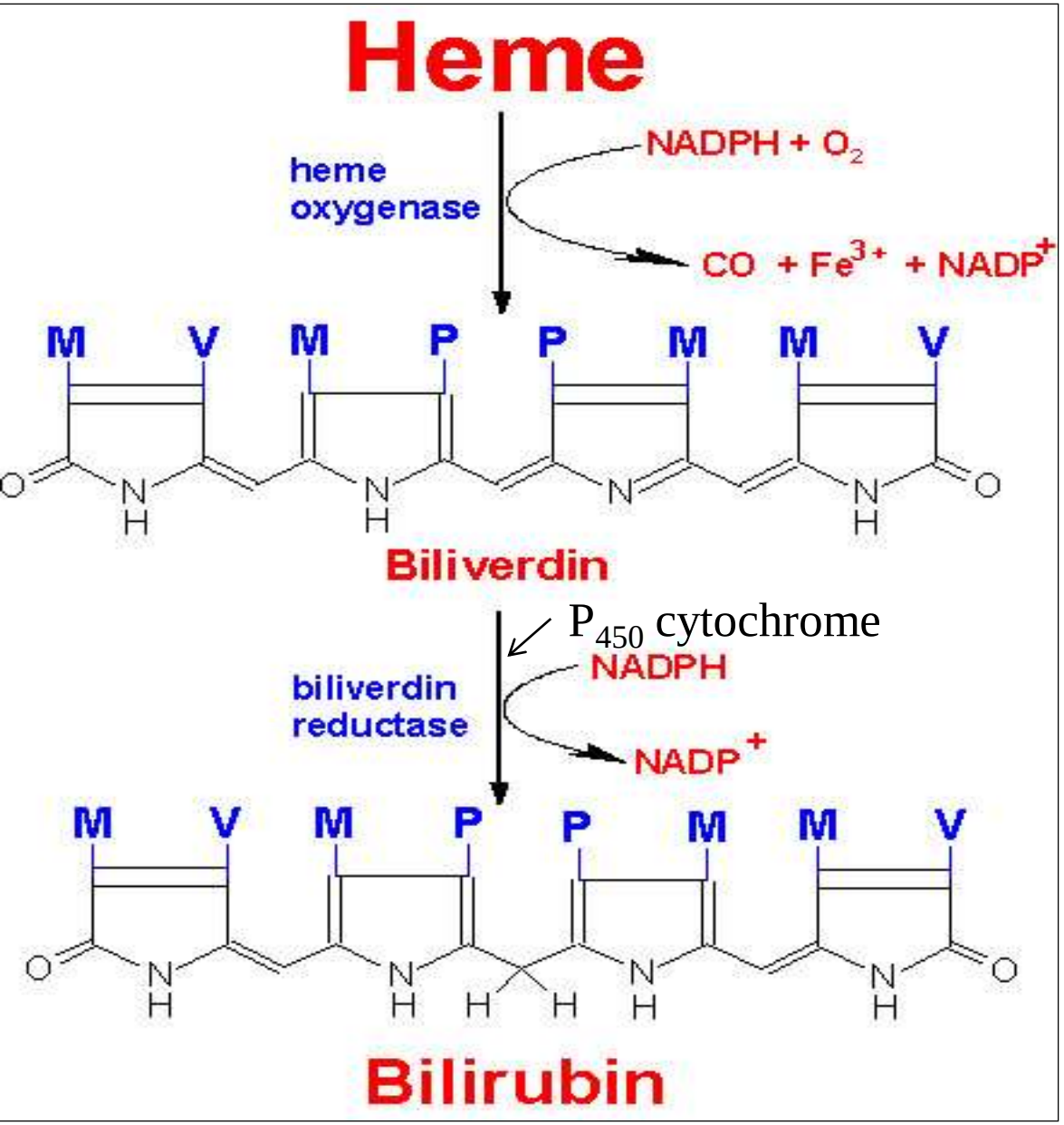
3. Biliary excretion of bilirubin

Transfer across

CB ⟶ Bile canaliculus

Microvillar membrane

Degradation of heme to bilirubin



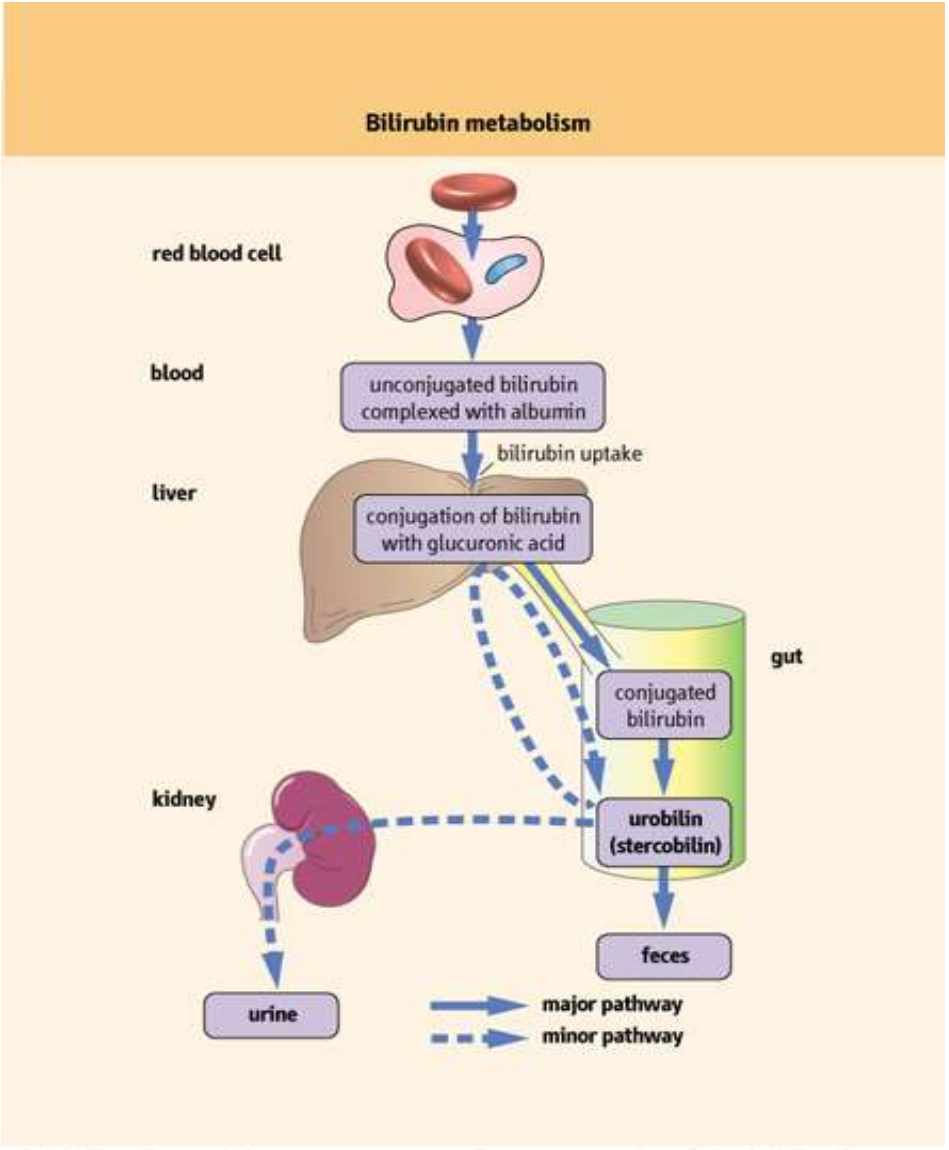
-75% is derived from RBCs

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

- Normal plasma concentrations are less then 1 mg/dL

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

Normal bilirubin metabolism



- 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 glucuronyl transferase

-"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

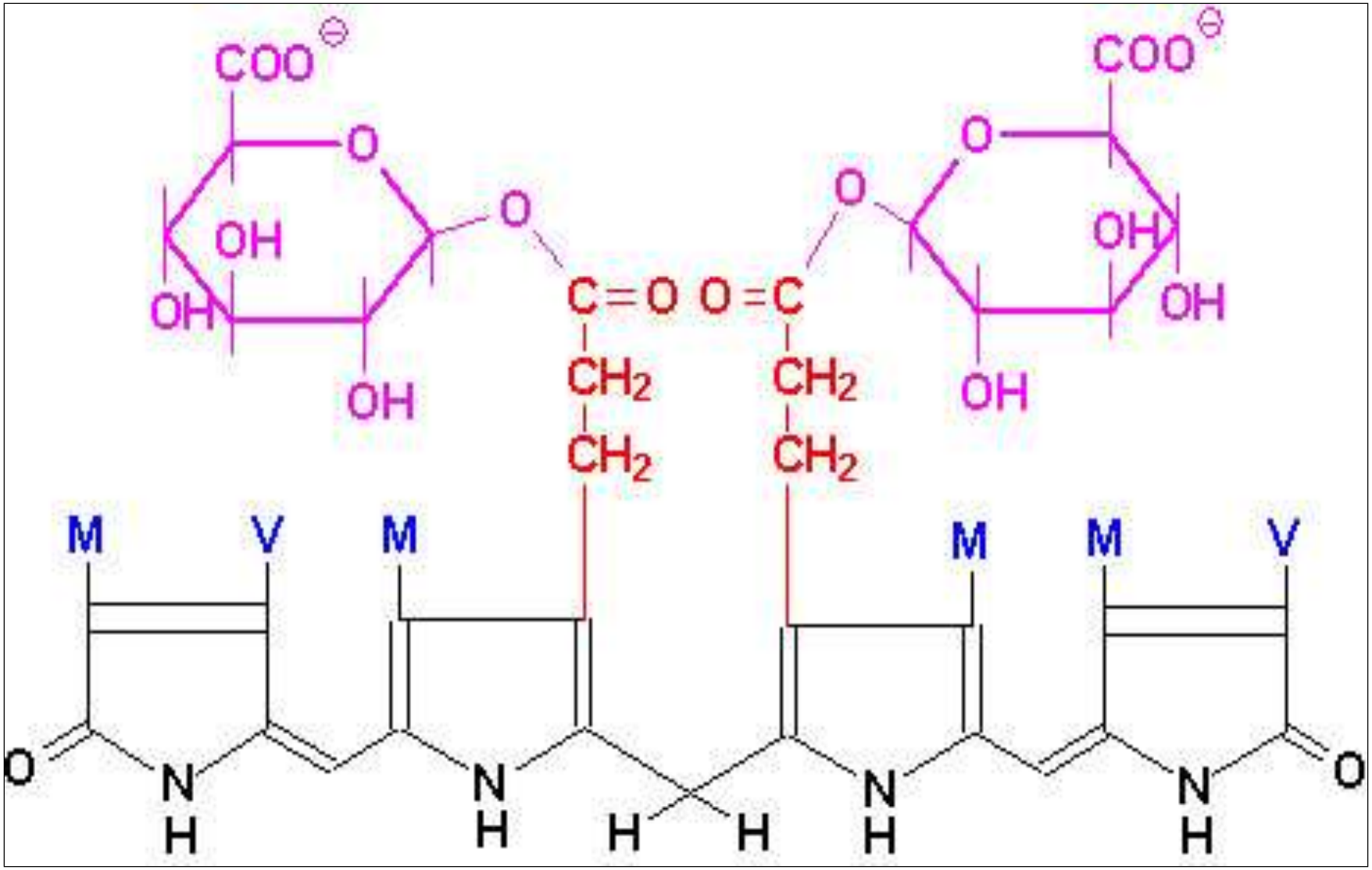
- Oxidized to stercobilin which is colored

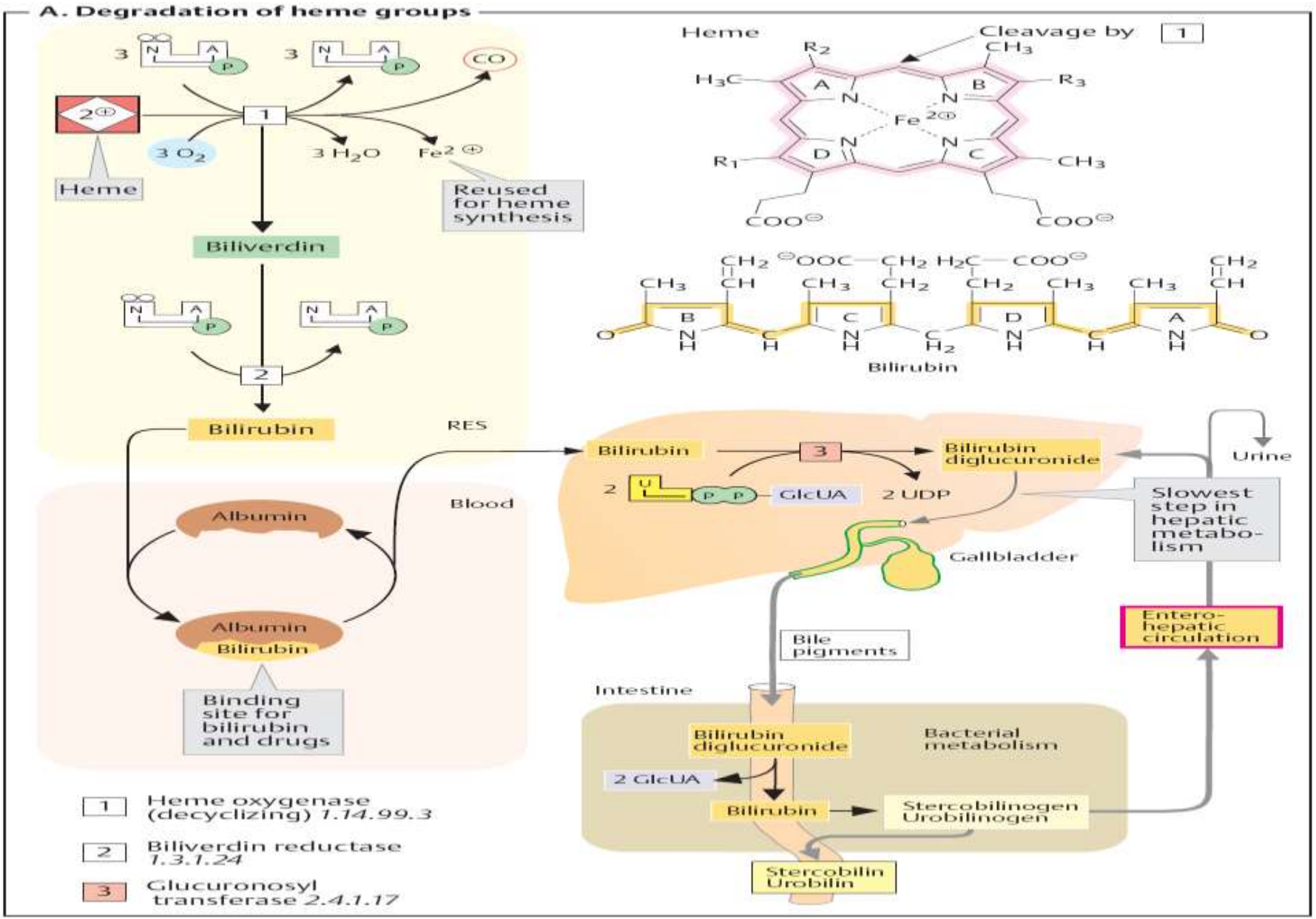
- Excreted in feces

- Some stercobilin may be re-adsorbed through enterohepatic circulation by the gut and re-excreted by either the liver or kidney

bilirubin-diglucuronide = conjugated bilirubin

is soluble in water → **„direct bilirubin“**





Bile pigments:

- Bilirubin **- urobilin** **- stercobilin**

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 µmol/L)

- 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 µmol/L)

-Total bilirubin measures both unconjugated and conjugated Bil (normal value up to 17 µmol/L).

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.

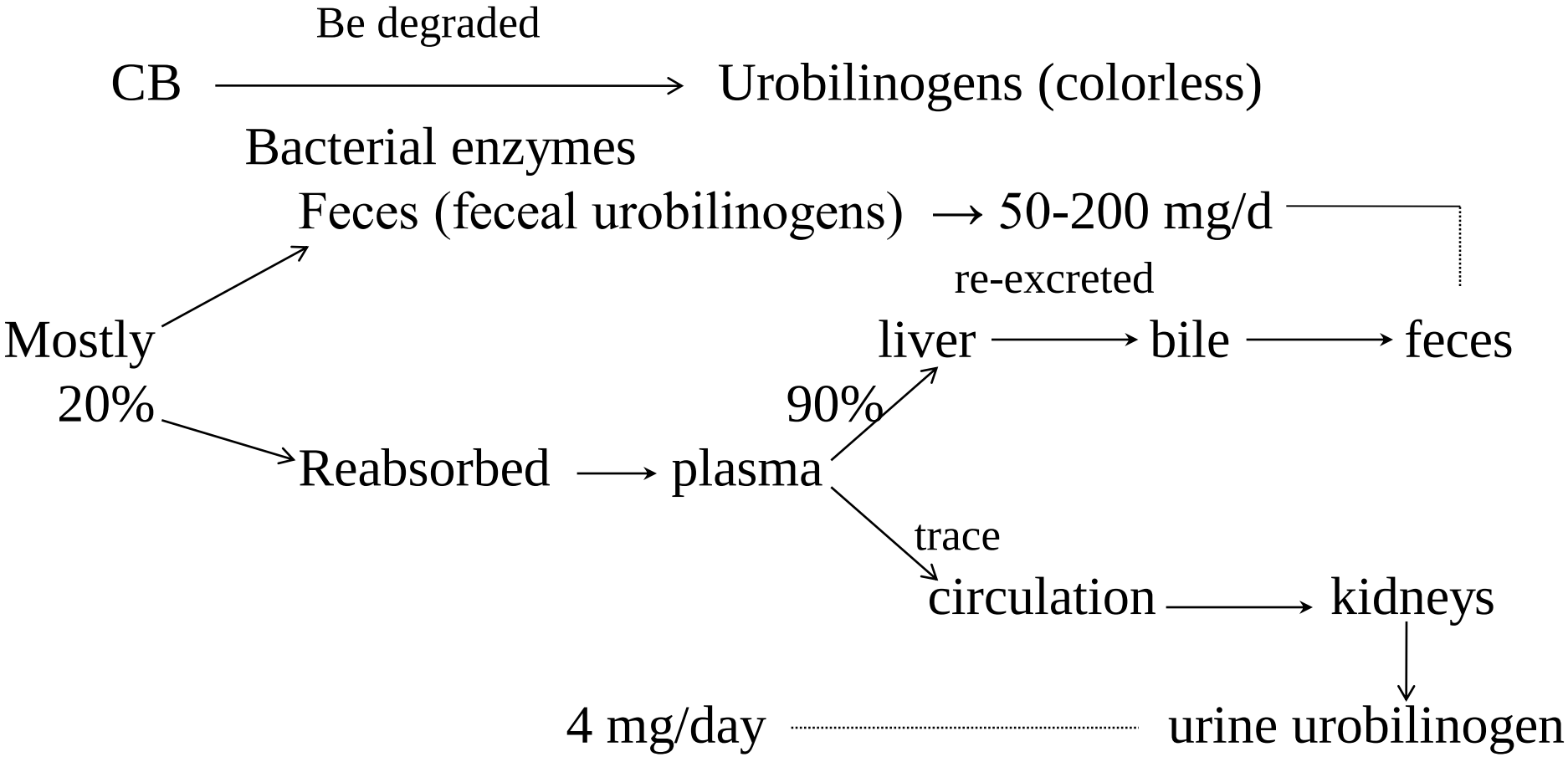
- Bilirubin conjugated in presence of UDPGT (uridine diphosphate glucuronyl transferase) to mono and diglucoronides, which are then excreted into bile canaliculi.

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.

Entero - hepatic circulation



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

Jaundice

Definition of Jaundice

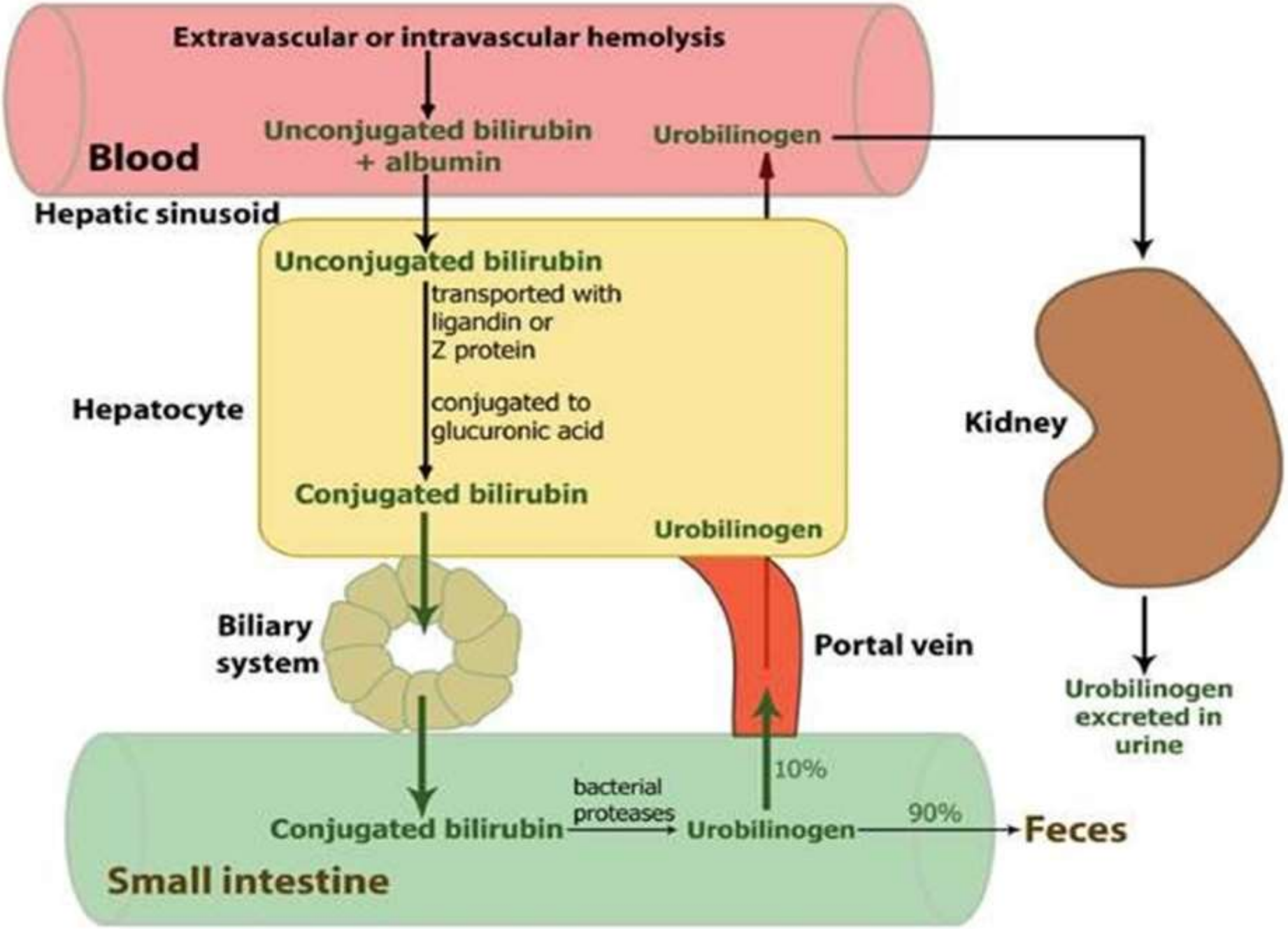
- Also called icterus
- A yellowish straining of the skin, conjunctiva, base of tongue palms and soles with bile pigments which are increased in plasma
- Can be seen on examination at serum bilirubin levels 27-35 μmol/l (1.5 – 2 mg/dl)

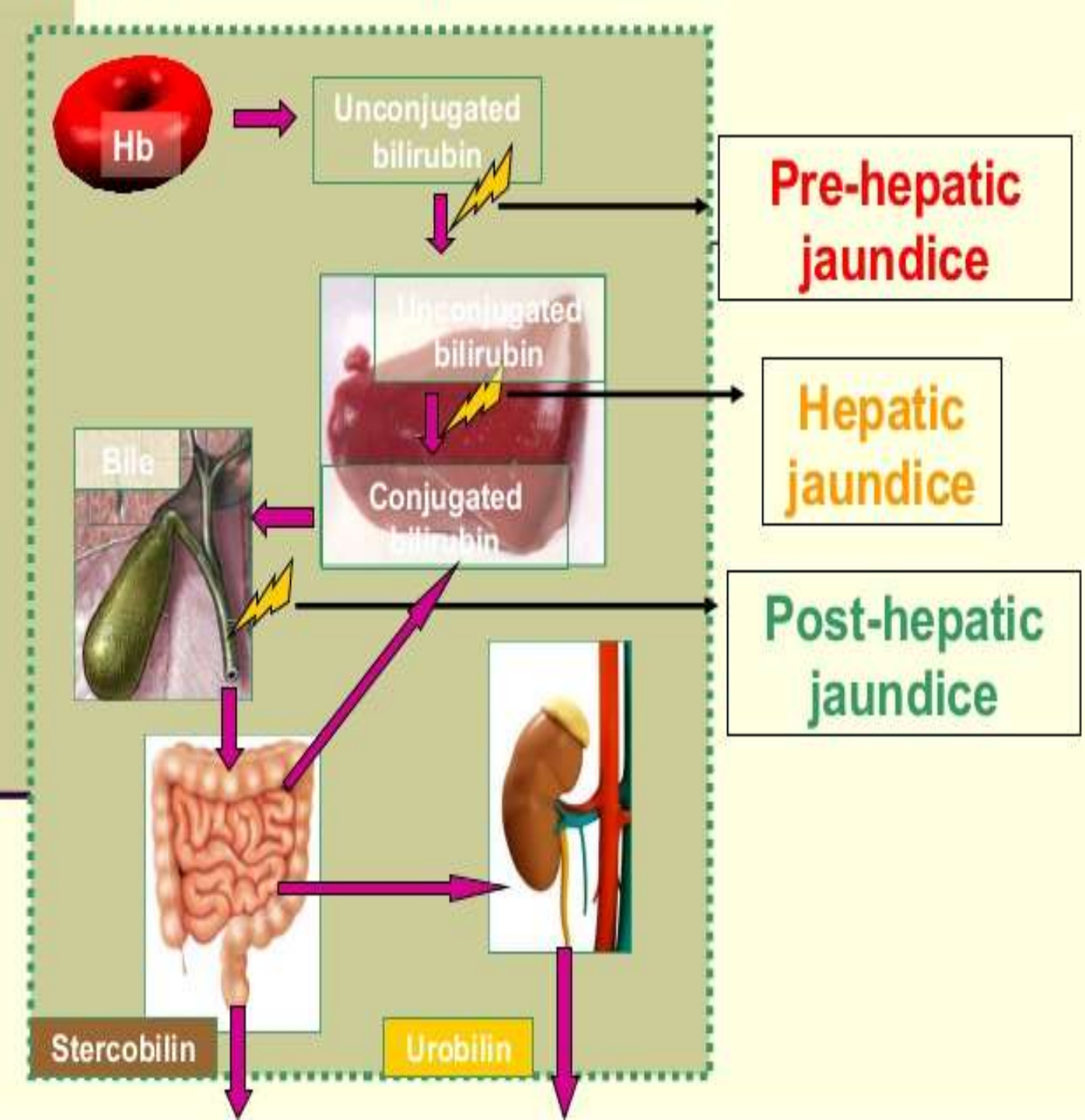
Pathophysiologic classification of Jaundice

- 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%


Extravascular or intravascular hemolysis
Blood
Unconjugated bilirubin
+ albumin
Urobilinogen
Hepatic sinusoid
Unconjugated bilirubin
transported with
ligandin or
Z protein
conjugated to
glucuronic acid
Hepatocyte
Conjugated bilirubin
Urobilinogen
Kidney
Biliary
system
Portal vein
Urobilinogen
excreted in
urine
10%
bacterial
proteases
Conjugated bilirubin
Urobilinogen
90%
Feces
Small intestine



Hemolytic jaundice

Intrahepatic jaundice

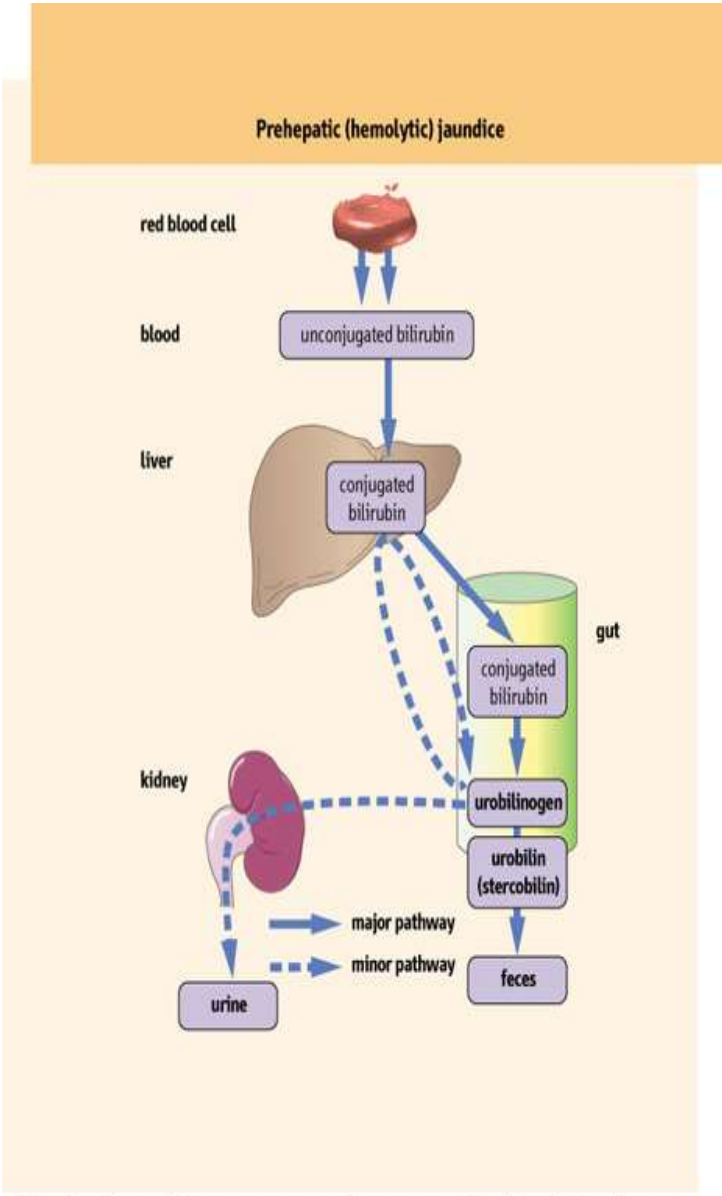
Obstructive/ Surgical jaundice

Prehepatic (hemolytic, unconjugated) jaundice

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

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
- Ineffective erythropoiesis



- Impaired bilirubin conjugation
  Gilbert's and Crigler-Najarr syndromes
  Hyperthyroidism
  Liver diseases as in chronic hepatitis, cirrhosis, Wilson's disease

Laboratory findings

- UB↑ without bilirubinuria (50-150 μmole/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

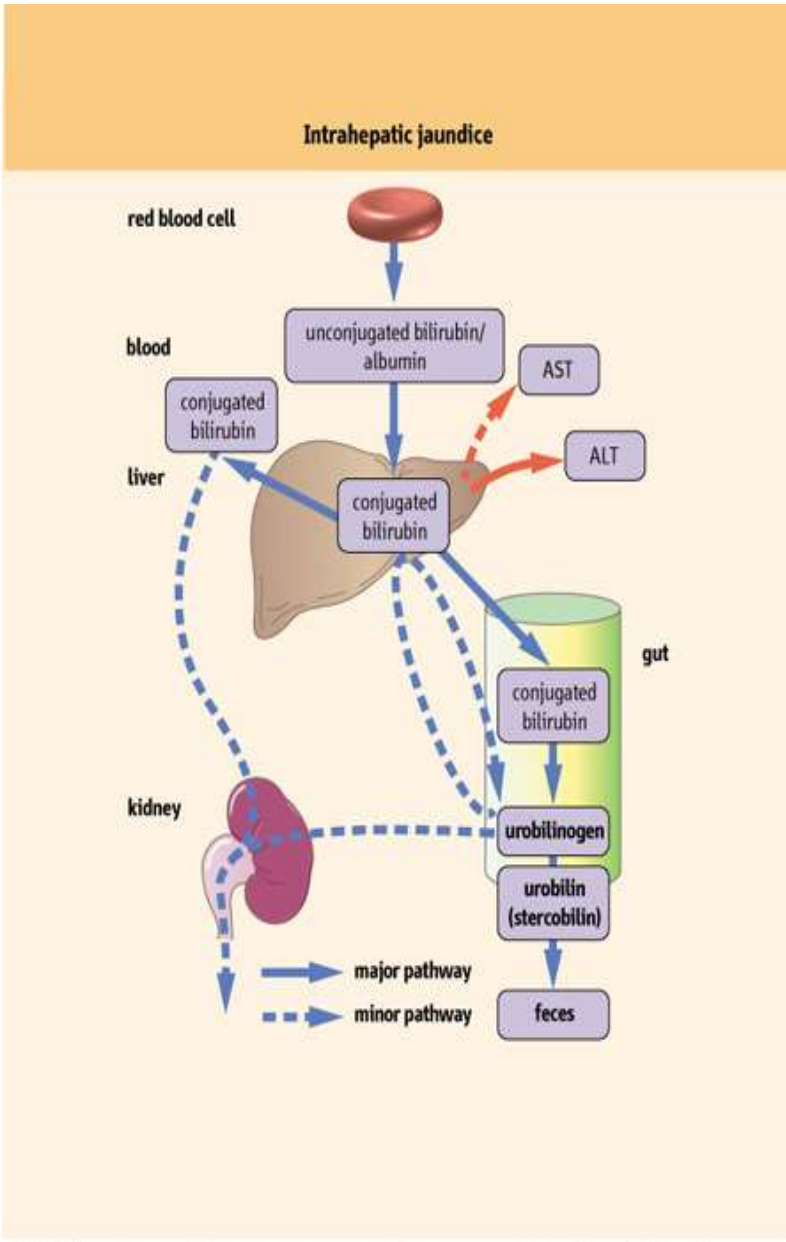
Intrahepatic (conjugated) jaundice

- 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 **(**↑ 50-250 μmole/l)
- Urobilinogen: normal or reduced
- Stercobilinogen: normal or reduced

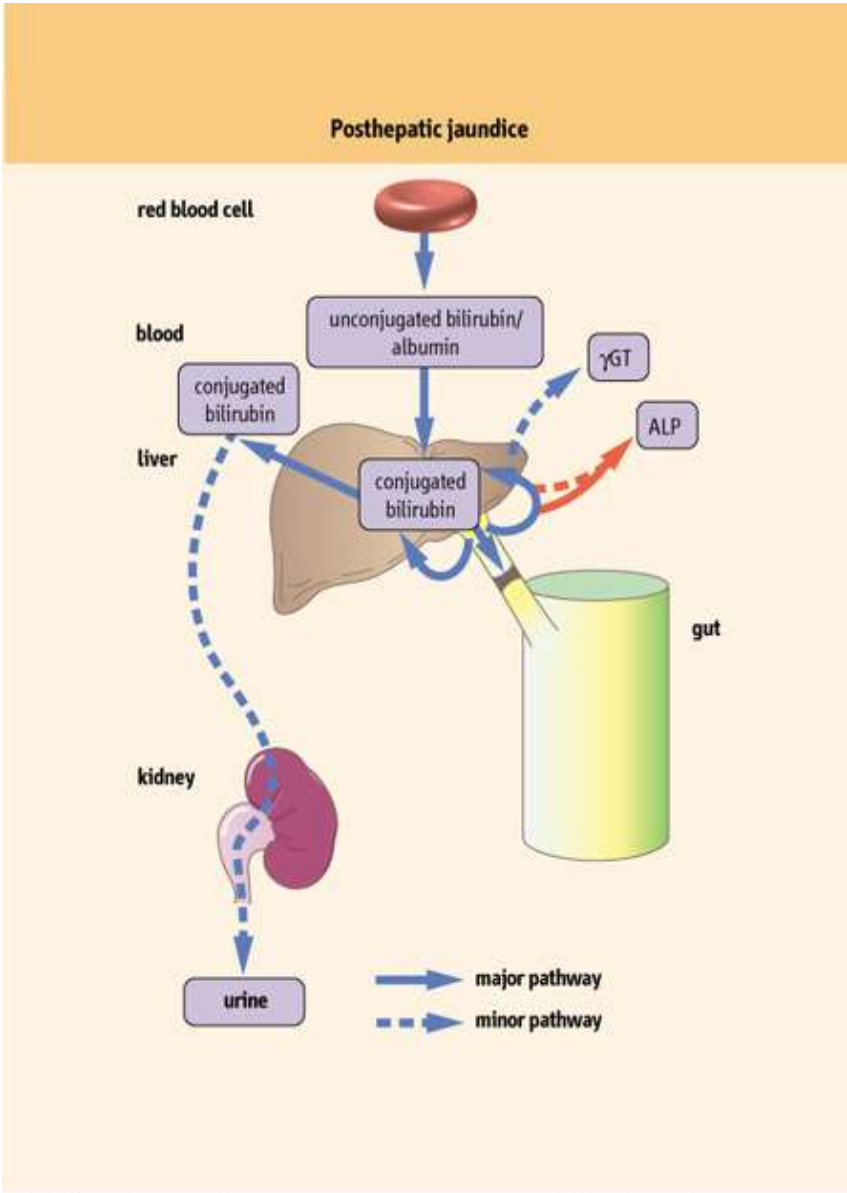
Posthepatic (Obstructive) jaundice

- 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↑ (100-500 μmole/l)
- Fecal urobilinogen ↓ (incomplete obstruction) or absent in (complete obstruction)
- Urobilinogenuria is absent in complete obstructive jaundice
- Bilirubinuria ↑
- Cholesterol ↑
- Urinary changes:

1- Bilirubin: increased

2- Urobilinogen: reduced or absent

- Faecal changes: stercobilinogen: reduced or absent

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 bilary cirrhosis cholangitis	common uncommon common
	extrahepatic bile ducts	gall stones pancreatic tumor cholangiocarcinoma	very common uncommon rare

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

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 then 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 glucuronyl transferase, 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.

- ½ 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 glucuronyl transferase activity

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 µmol/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,
- 12 hours/day phototherapy
- heme oxygenase 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.

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Crigler-Najjar syndrome, type II

Differs from type I in several aspects:

1- bilirubin levels are generally below 345 µmol/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.

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