The metabolic functions of the liver

Catabolism of haemoglobin, bilirubin
Metabolism of iron
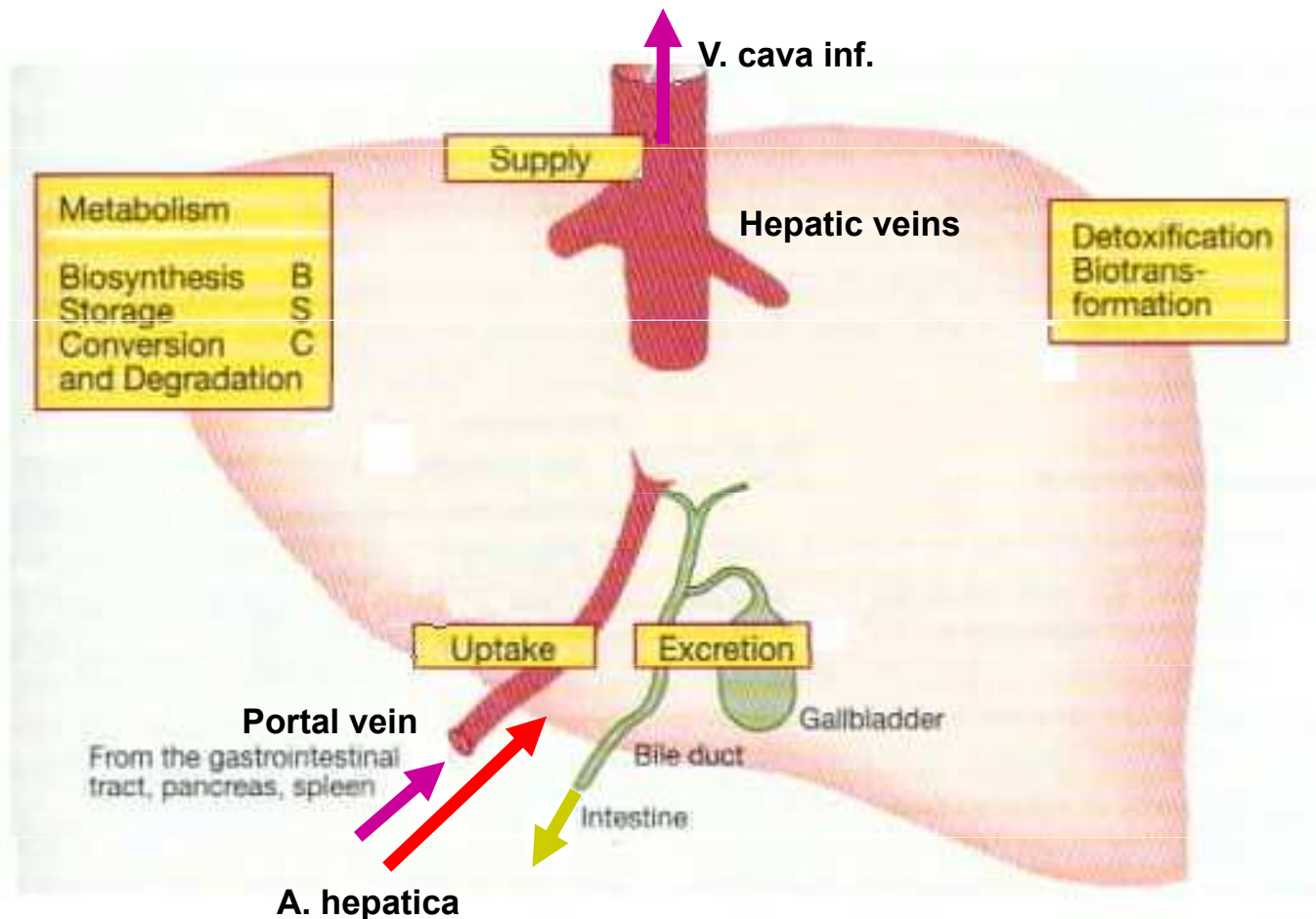
Biochemistry VFU

Lecture 15   2009 (J.S.)

The major metabolic functions of the liver:

- uptake of most nutrients from the gastrointestinal tract,
- intensive intermediary metabolism, conversion of nutrients,
- controlled supply of essential compounds (glucose, VLD lipoproteins, ketone bodies, plasma proteins, etc.),
- ureosynthesis, biotransformation of xenobiotics (detoxification),
- excretion (cholesterol, bilirubin, hydrophobic compounds, some metals).

V. cava inf.

Supply

Metabolism

Biosynthesis   B
Storage   S
Conversion   C
and Degradation

Hepatic veins

Detoxification
Biotrans-
formation

Uptake   Excretion

Portal vein

From the gastrointestinal
tract, pancreas, spleen

Gallbladder

Bile duct

Intestine

A. hepatica

The hepatocytes (the hepatic parenchymal cells) have an immensely broad range of synthetic and catabolic functions with a substantial reserve metabolic capacity.

Many of them are the **specialized metabolic functions of the liver**:

Metabolism of saccharides
- Primary regulation of the blood glucose concentration. E.g. in the postprandial state, there is an uptake of about 60 % of glucose supplied in portal blood and stored as glycogen, or in hypoglycaemia, glycogenolysis and gluconeogenesis is initiated.
- The liver cells meet their energy requirements preferentially from fatty acids, not from glycolysis. Glucose (also as glycogen store) is altruistically spared for extrahepatic tissues.

Metabolism of lipids
- Completion and secretion of VLDL and HDL.
- Ketogenesis produces ketone bodies, precious nutrients. They cannot be utilized in the liver, but they are supplied to other tissues.
- Secretion of cholesterol and bile acids into the bile represents the major way of cholesterol elimination from the body.
- Dehydrogenation of cholesterol to 7-dehydrocholesterol and 25-hydroxylation of calciols play an essential role in calcium homeostasis.

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Metabolism of nitrogenous compounds
- Deamination of amino acids that are in excess of requirements.
- Intensive proteosynthesis of major plasma proteins and blood-clotting factors.
- Uptake of ammonium, ureosynthesis.
- Bilirubin capturing, conjugation, and excretion.

Biotransformation of xenobiotics
- Detoxification of drugs, toxins, excretion of some metals.

Transformation of hormones
- Inactivation of steroid hormones – hydrogenation, conjugation.
- Inactivation of insulin, about 50 % insulin inactivated in its only passage through the liver (GSH:insulin transhydrogenase splits the disulfide bonds, then proteolysis of the two chains).
- Inactivation of catecholamines and iodothyronines, conjugation of the products.

Vitamins
- Hydroxylation of calciols to calcidiols, splitting of β-carotene to retinol.
- The liver represent a store of retinol esters and cobalamin (B_{12}).

Iron and copper metabolism
- Synthesis of transferrin, coeruloplasmin, ferritin stores, excretion of copper.

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The liver parenchymal cell (hepatocyte)

Columns (cords) of hepatocytes are surrounded by sinusoids lined by endothelial fenestrated layer (without a basement membrane) and Kupffer cells (mononuclear phagocytes).
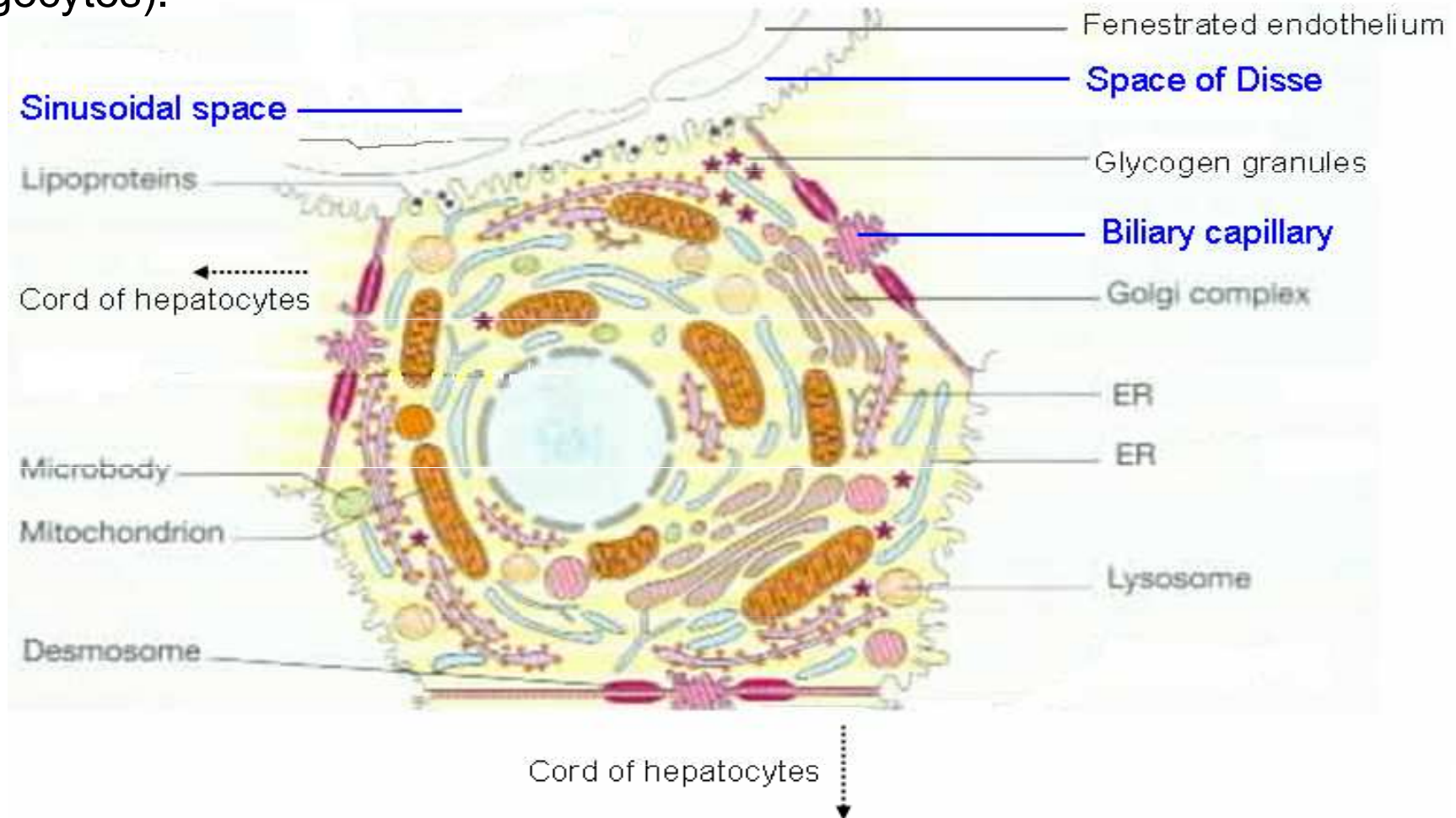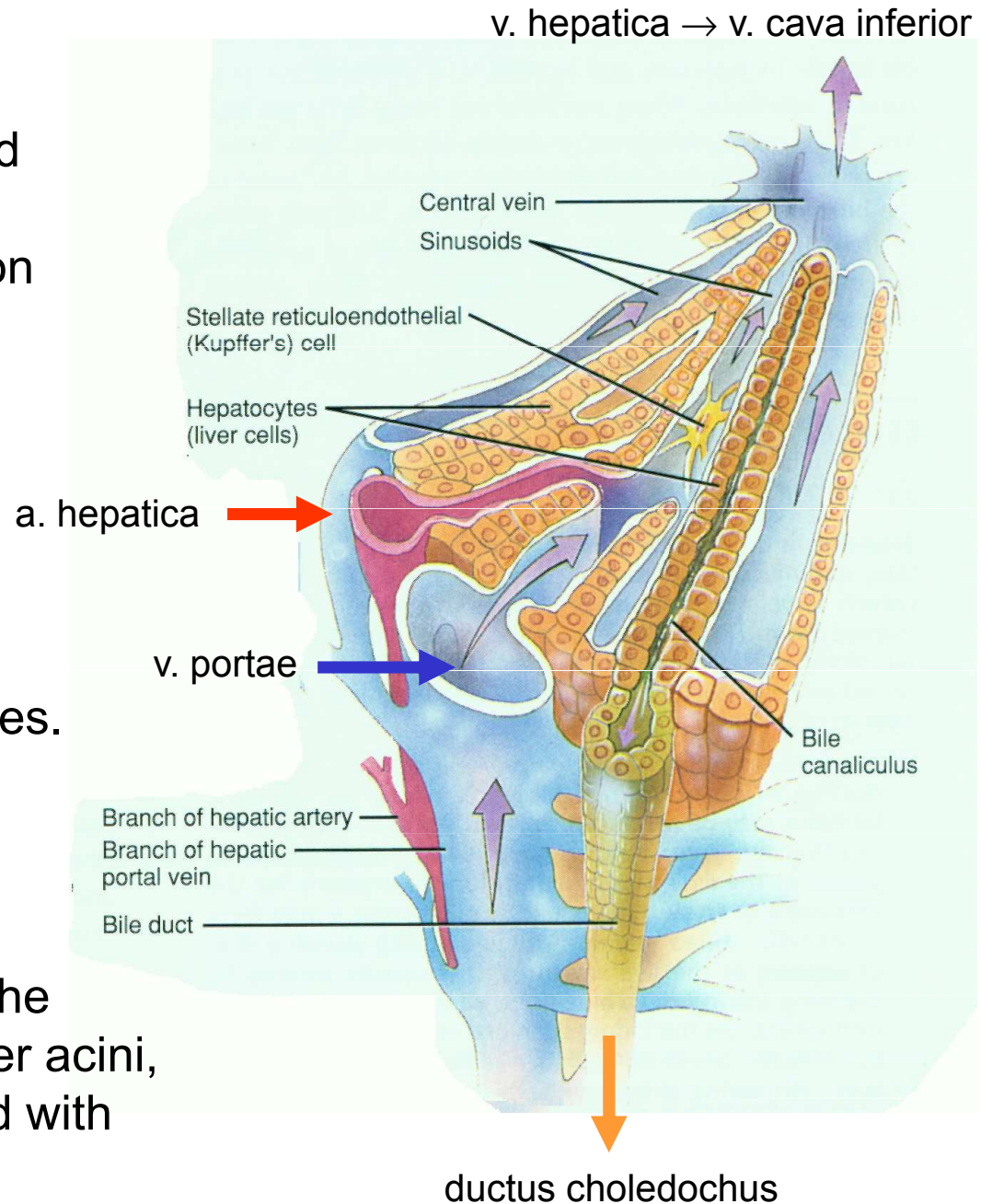

Plasmatic membrane directed to the space of Disse – the "blood" pole, the "bile" pole – lateral parts of the membrane with gap junctions and parts forming bile capillaries.
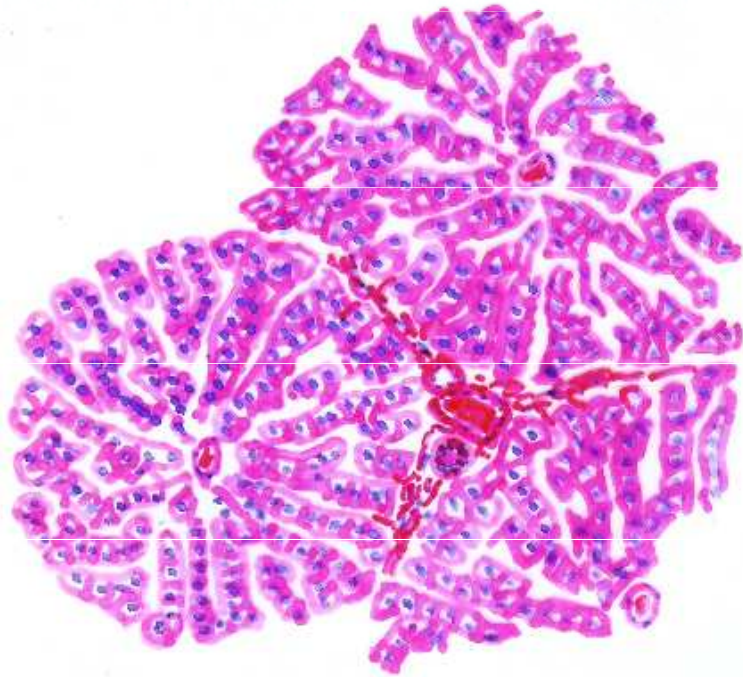
5

The liver receives venous blood from the intestine. Thus all the products of digestion, in addition to ingested drugs and other xenobiotics, perfuse the liver before entering the systemic circulation.

The mixed portal and arterial blood flows through sinusoids between columns of hepatocytes.

Hepatocytes are differentiated in their functions according to the decreasing pO_2. In a simple liver acini, there are <u>three zones</u> equipped with different enzymes.

v. hepatica $\rightarrow$ v. cava inferior

Central vein
Sinusoids
Stellate reticuloendothelial (Kupffer's) cell
Hepatocytes (liver cells)

a. hepatica

v. portae

Bile canaliculus

Branch of hepatic artery
Branch of hepatic portal vein
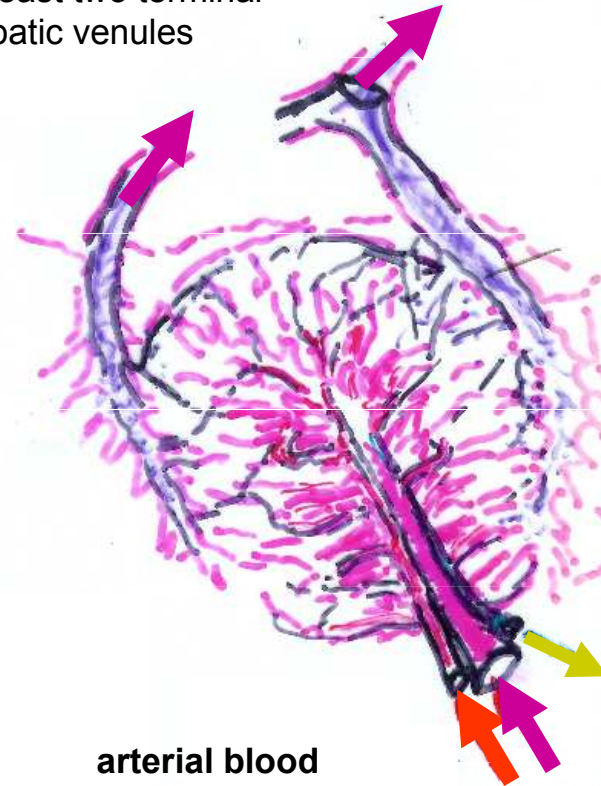Bile duct

ductus choledochus

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Hexagonal hepatic <u>lobules</u>
round terminal hepatic venules
<u>are not functional units</u>

simple liver ACINUS – a functional unit

efferent vessels
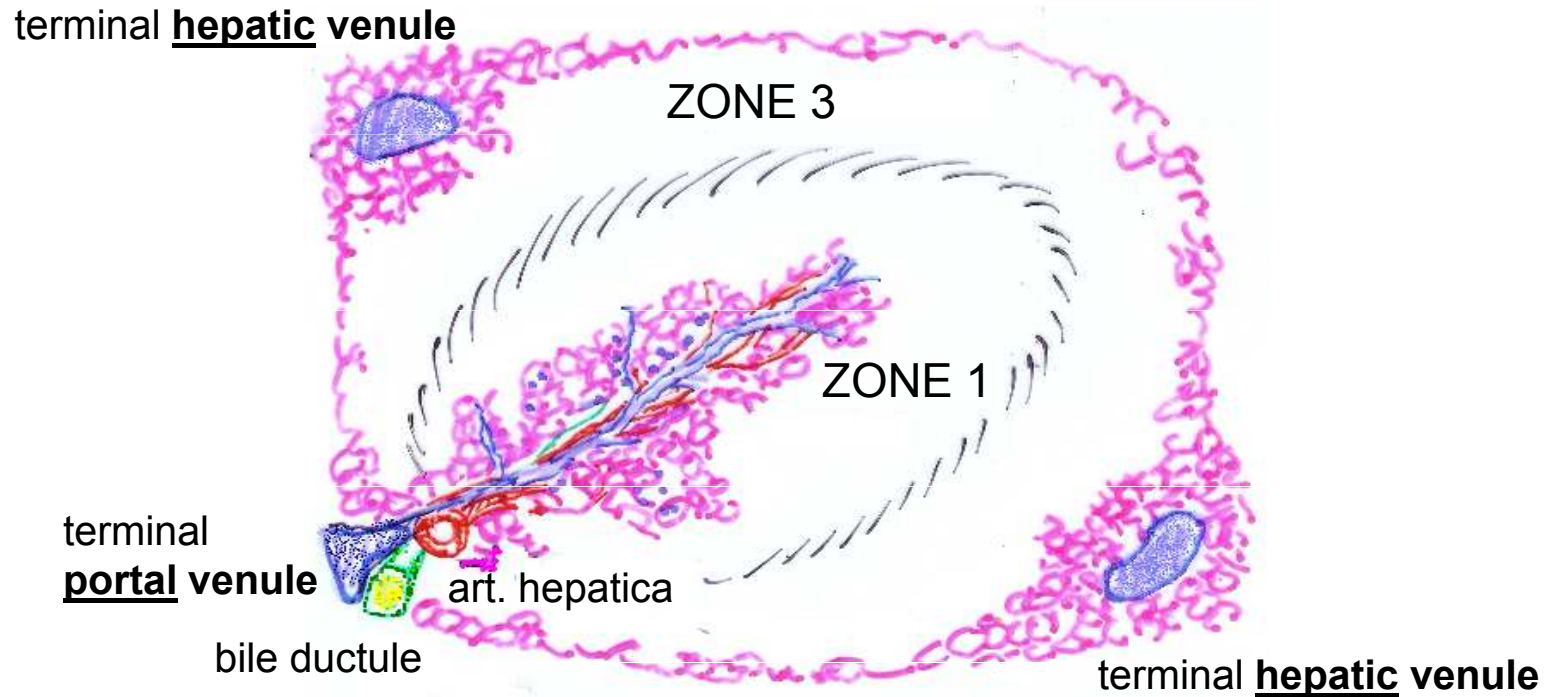at least two terminal
hepatic venules

bile ductules

arterial blood
terminal branches
aa. hepaticae

portal (venous) blood
from intestine, pancreas,
and spleen

portal field with afferent vessels

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Metabolic areas in the acini

terminal **hepatic venule**

ZONE 3

ZONE 1

terminal
portal venule

art. hepatica

bile ductule

terminal **hepatic venule**

Zone 1 – **periportal area**

high pO_2
cytogenesis, mitosis
numerous mitochondria
glycogenesis and glycogenolysis
proteosynthesis
ureosynthesis

Zone 3 – **microcirculatory periphery**

low pO_2
high activity of ER (cyt P450, detoxification)
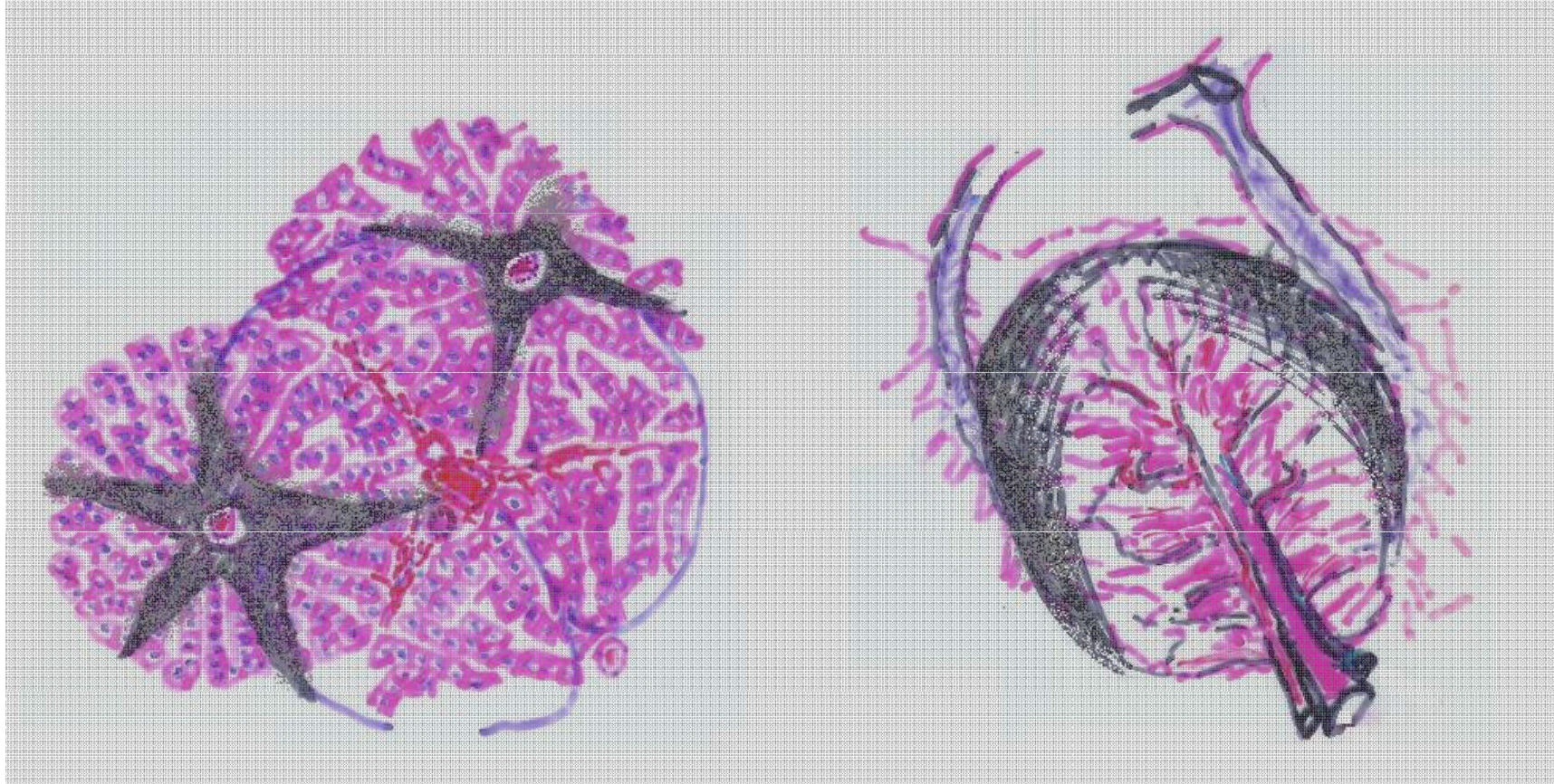pentose phosphate pathway
hydrolytic enzymes
glycogen stores, fat and pigment stores
glutamine synthesis

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Liver of a patient who died in hepatic coma:.

Seastar-shaped necrotic lesion around the terminal hepatic venule. This shape is produced by necrosis creeping along zones 3 of the simple acini, intercalating between them and reaching portal spaces.

Liver – production of bile

Composition of bile	Mass concentration / g/l	
	Hepatic bile	Gall-bladder bile
Inorganic salts	8.4	6.5
Bile acids	**7 – 14**	**32 – 115**
Cholesterol	**0.8 – 2.1**	**3.1 – 16.2**
Bilirubin glucosiduronates	**0.3 – 0.6**	**1.4**
Phospholipids	2.6 – 9.2	5.9
Proteins	1.4 – 2.7	4.5
pH	7.1 – 7.3	6.9 – 7.7

Functions
The **bile acids** emulsify lipids and fat-soluble vitamins in the intestine. High concentrations of bile acids **and phospholipids** stabilize micellar dispersion of cholesterol in the bile (crystallization of cholesterol → cholesterol gall-stones).
Excretion of cholesterol and bile acids is the major way of removing cholesterol from the body. Bile also removes hydrophobic metabolites, drugs, toxins and metals (e.g. copper, zinc, mercury).
Neutralization of the acid chyme in conjunction with HCO_3^- from pancreatic secretion.

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Degradation of haemoglobin to bilirubin – bile pigments

Erythrocytes are taken up by the **reticuloendothelial cells** of the spleen, bone marrow, and Kupffer cells in the liver by phagocytosis.

Haemoglobin

$3 O_2$

CO

Haem oxygenase
(*NADPH:cyt* P450 *oxidoreductase*)

Verdoglobin IX-α

Fe^{3+} globin

NADPH

Biliverdin reductase

Biliverdin IX-α
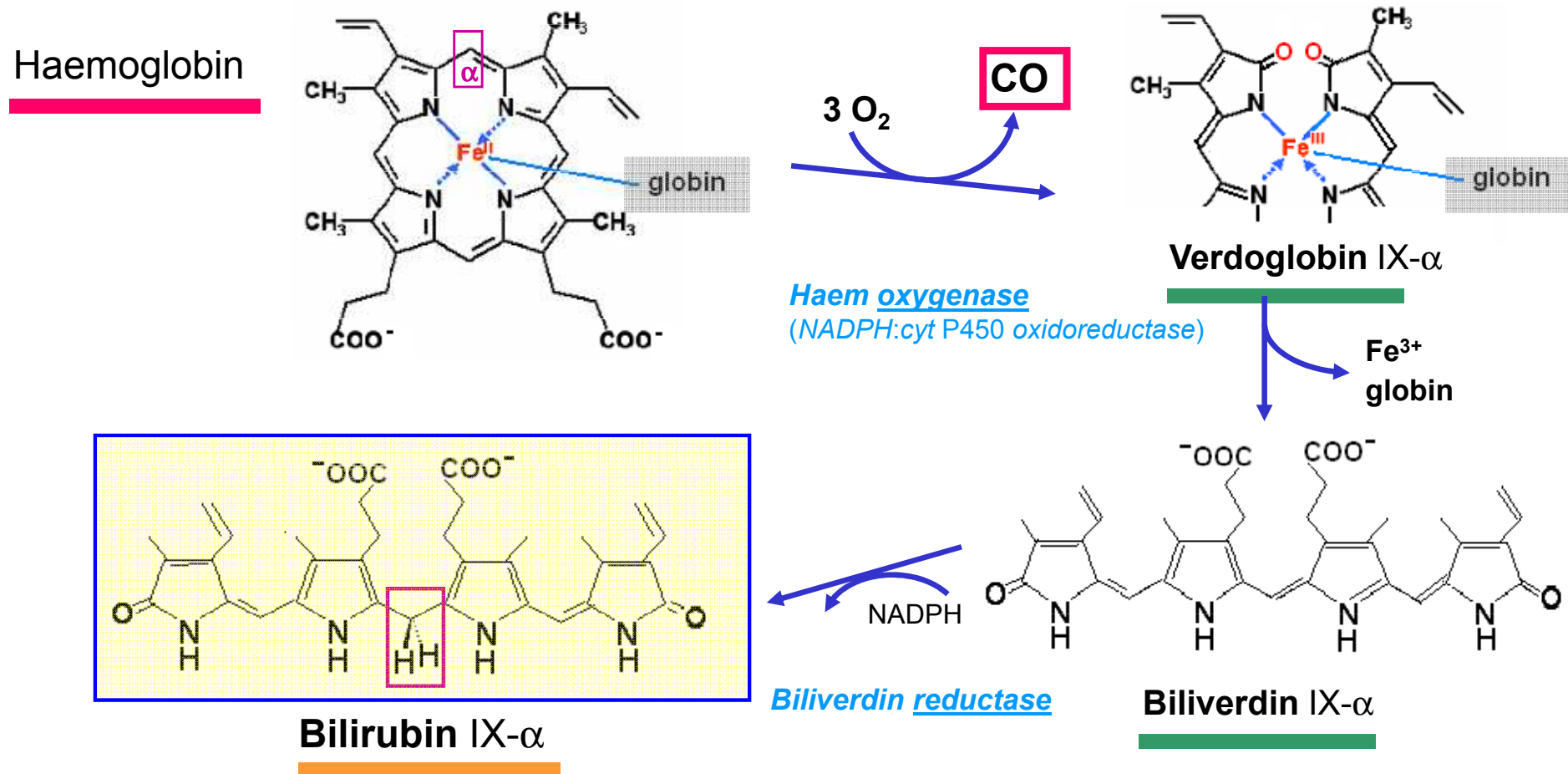
Bilirubin IX-α

In blood plasma, these **hydrophobic bilirubin molecules** (called **unconjugated bilirubin**) are transported in the form of **complexes bilirubin-albumin**.

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Bilirubin IXα

(configuration 4Z,15Z)
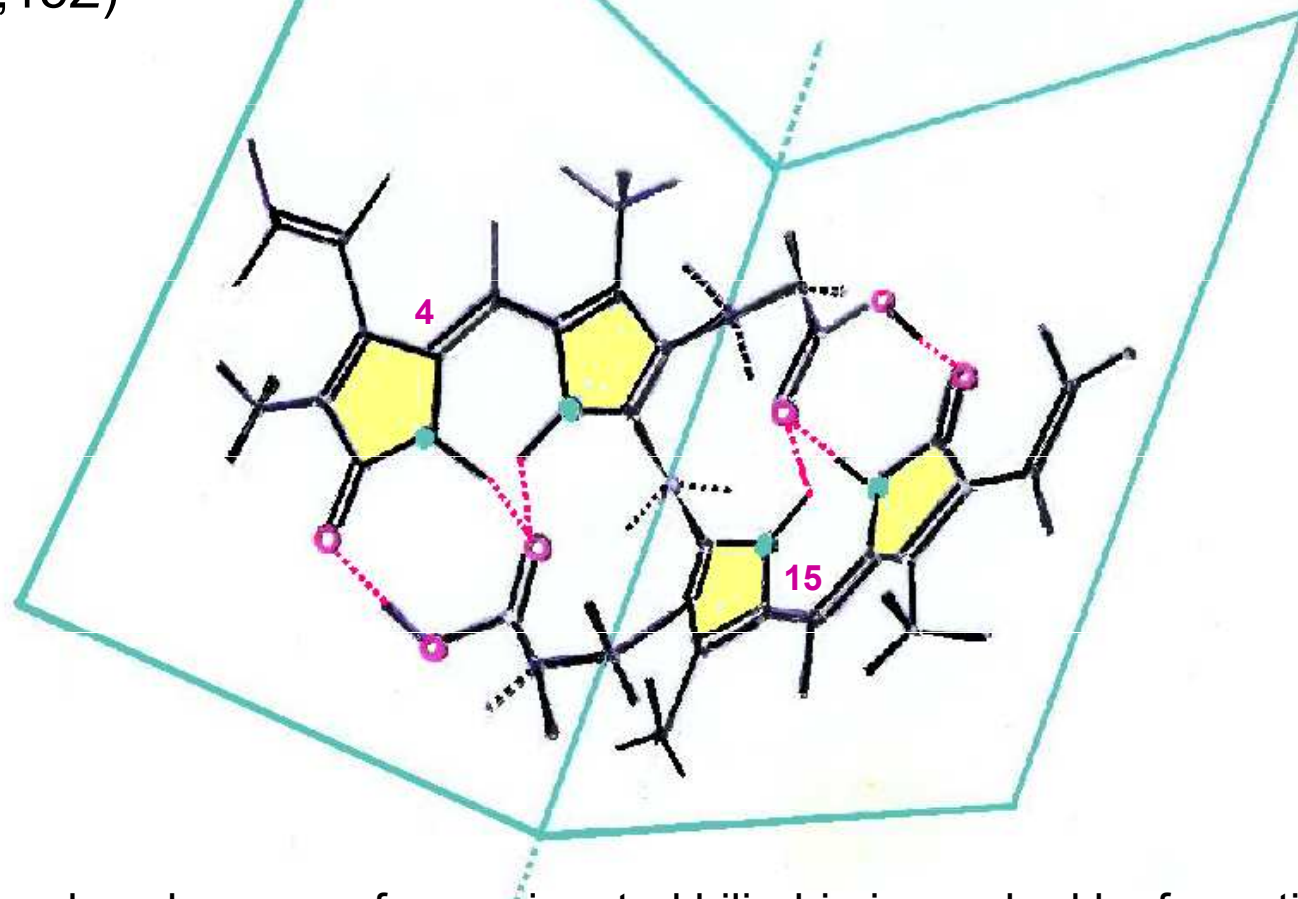
Polarity of the two carboxyl groups of unconjugated bilirubin is masked by formation of <u>hydrogen bonds</u> between the carboxyl groups and the electronegative atoms within the opposite halves of bilirubin molecules.
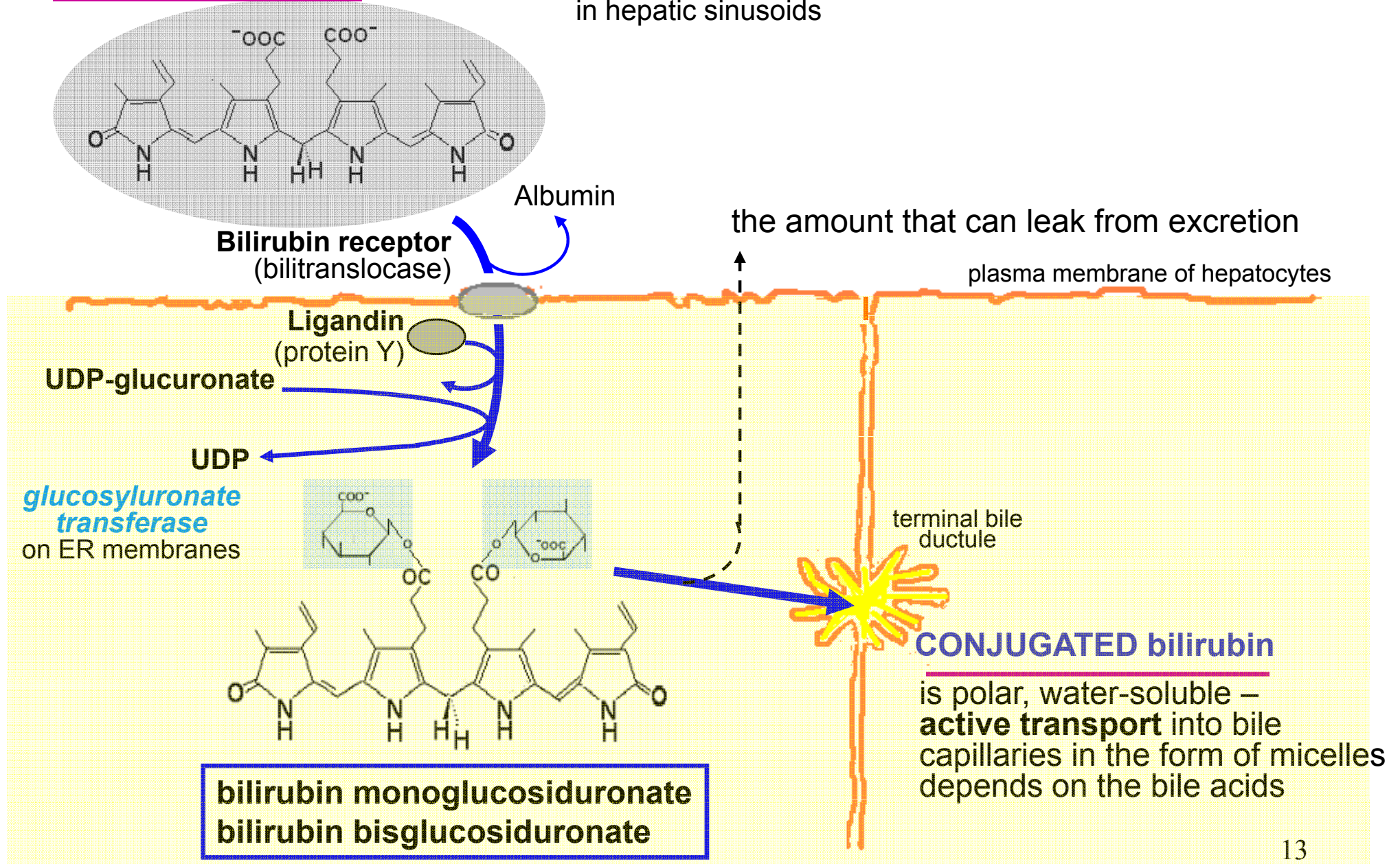
The molecules of unconjugated bilirubin are hydrophobic – insoluble in water; they dissolve only in the presence of proteins forming complexes, e.g., bilirubin-albumin.

The hepatic uptake, conjugation, and excretion of bilirubin

UNCONJUGATED bilirubin (bilirubin-albumin complexes)
in hepatic sinusoids

Albumin

Bilirubin receptor
(bilitranslocase)

the amount that can leak from excretion

plasma membrane of hepatocytes

Ligandin
(protein Y)

UDP-glucuronate

UDP

*glucosyluronate
transferase*
on ER membranes

terminal bile
ductule

CONJUGATED bilirubin

is polar, water-soluble –
active transport into bile
capillaries in the form of micelles
depends on the bile acids

bilirubin monoglucosiduronate
bilirubin bisglucosiduronate

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The formation of urobilinoids by the intestinal microflora
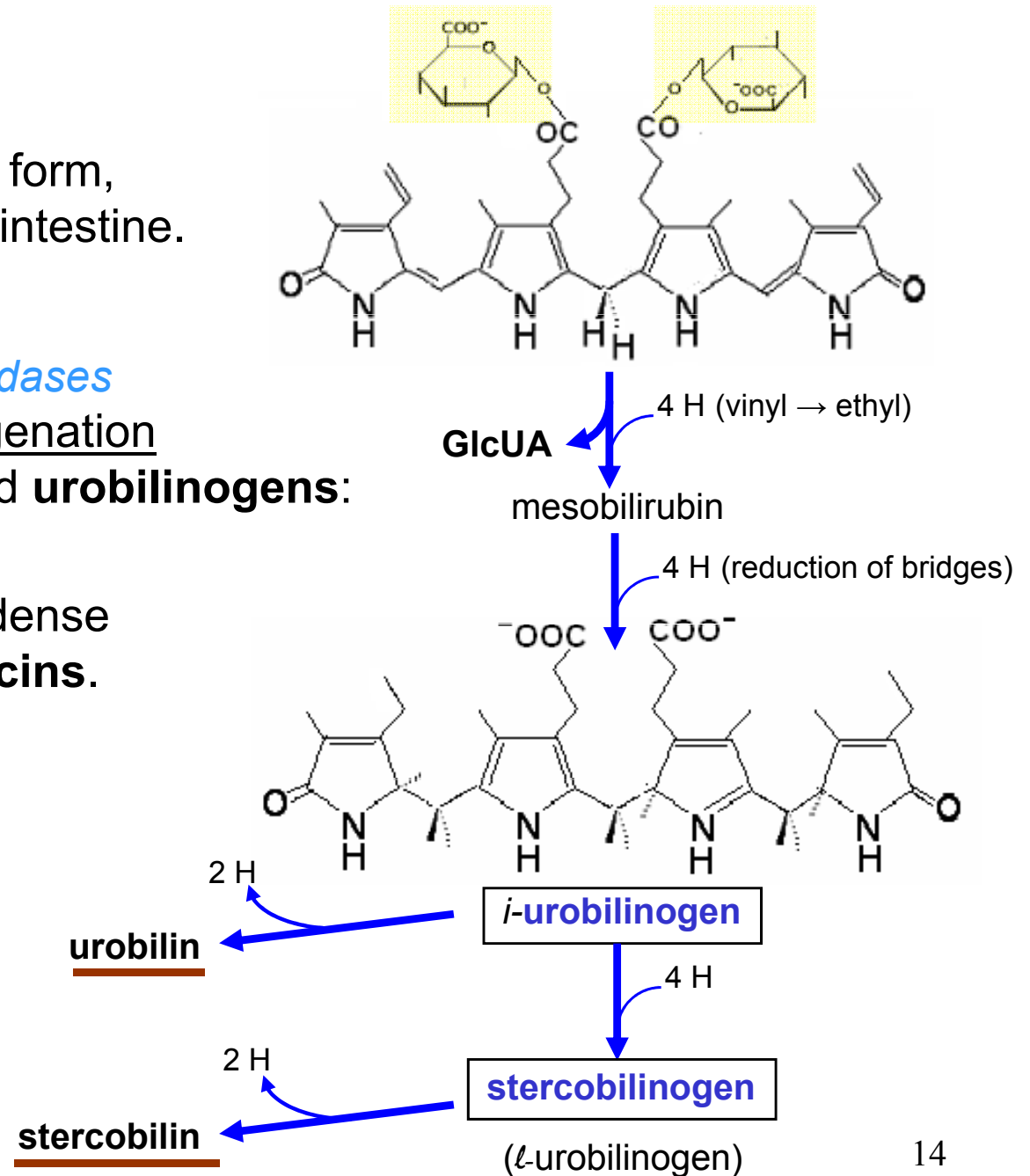
Conjugated bilirubin
is secreted into the bile. As far as
bilirubin remains in the conjugated form,
it cannot be absorbed in the small intestine.

<u>In the large intestine</u>,
bacterial *reductases and β-glucuronidases*
catalyze <u>deconjugation and hydrogenation</u>
of free bilirubin to mesobilirubin and **urobilinogens**:

A part of urobilinogens is split to
dipyrromethenes, which can condense
to give intensively coloured **bilifuscins**.

Urobilinogens are partly
– absorbed (mostly removed by
   the liver), a small part appears
   in the urine,
– partly excreted in the feces;
   on the air, they are oxidized
   to dark brown faecal **urobilins**.

GlcUA

4 H (vinyl → ethyl)

mesobilirubin

4 H (reduction of bridges)

2 H

urobilin

i-**urobilinogen**

4 H

2 H

stercobilinogen

stercobilin

(*l*-urobilinogen)

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Healthy individuals:

Plasma: <u>unconj.</u> **bilirubin-albumin complex**
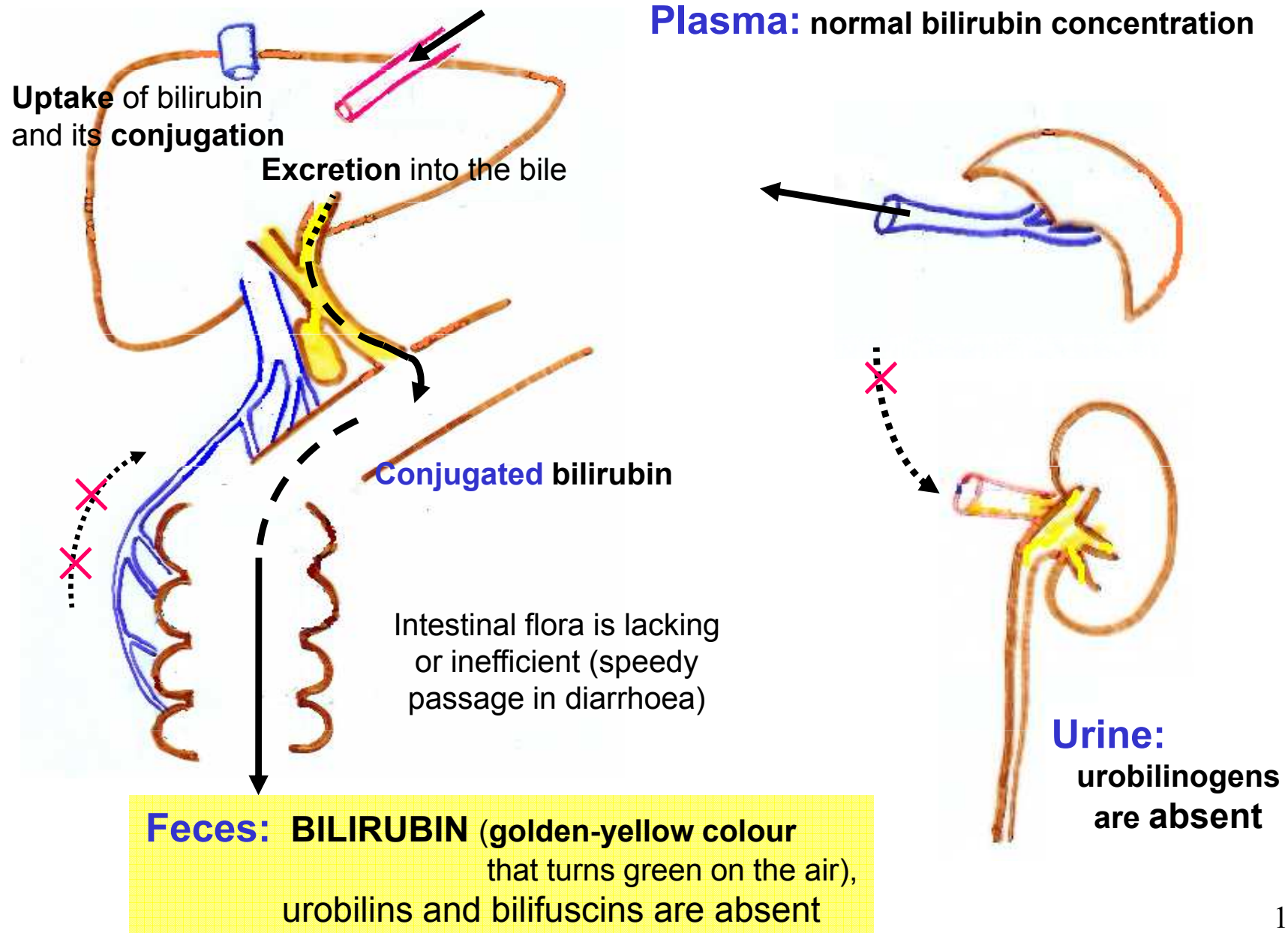**< 20 μmol / l**

bilirubin ⟵ haemoglobin

Uptake of **bilirubin**,
its conjugation and excretion

V. lienalis
(the blood from the spleen
flows into the portal vein)

Most of **urobilinogens**
are removed in the
liver (oxidation ?)

Small amounts of **urobilinogens**
not removed by the liver

Conjugated bilirubin

Portal
urobilinogens

A. renalis

Urobilinogens
and **dipyrromethenes**

Urine:
urobilinogens < 5 mg / d

Feces:
urobilinoids and **bilifuscins ~ 200 mg / d**

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In the **absence of intestinal microflora** (before colonization in newborns or during treatment with broad-spectrum antibiotics):

Uptake of bilirubin and its **conjugation**

Excretion into the bile

Plasma: normal bilirubin concentration

Conjugated bilirubin

Intestinal flora is lacking or inefficient (speedy passage in diarrhoea)

Urine: urobilinogens are **absent**

Feces: BILIRUBIN (**golden-yellow colour** that turns green on the air), urobilins and bilifuscins are absent

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Major types of hyperbilirubinaemias

Hyperbilirubinaemia – serum bilirubin > 20 – 22 μmol / l

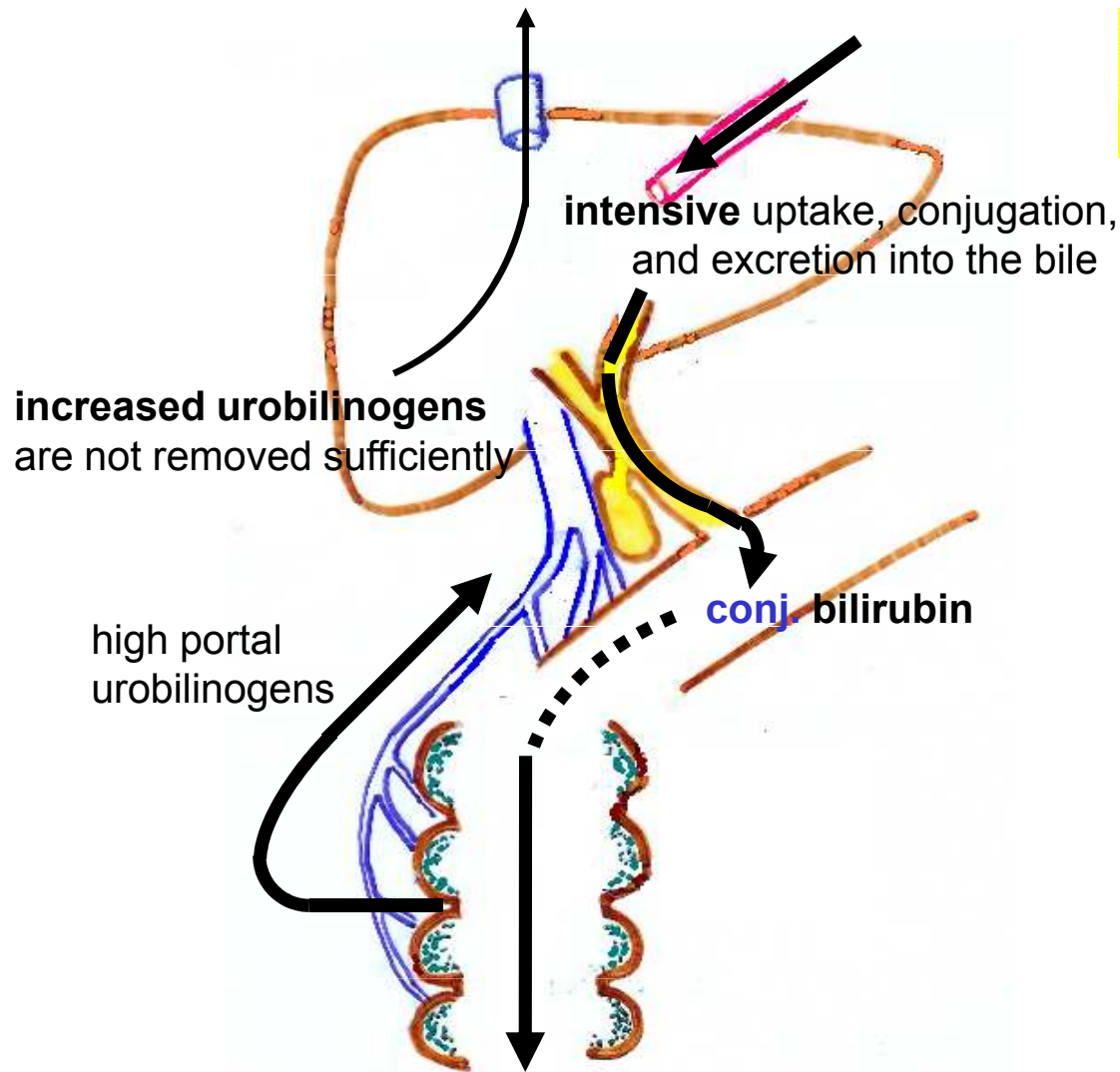**Icterus** (jaundice) – yellowish colouring of scleras and skin,
serum bilirubin usually more than 30 – 35 μmol / l

The causes of hyperbilirubinaemia are conventionally classified as

– **prehepatic** (haemolytic) – increased production of bilirubin,

– **hepatocellular** due to inflammatory disease (infectious hepatitis), hepatotoxic compounds (e.g. ethanol, acetaminophen), or autoimmune disease; chronic hepatitis can result in liver cirrhosis – fibrosis of hepatic lobules,

– **posthepatic** (obstructive) – insufficient drainage of intrahepatic or extrahepatic bile ducts (cholestasis).
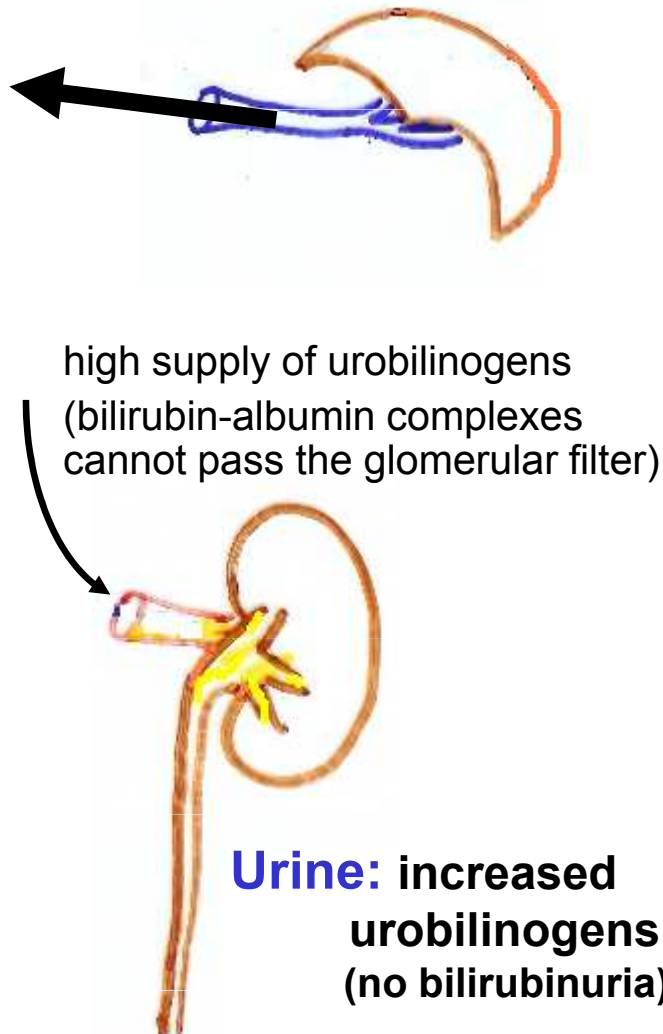
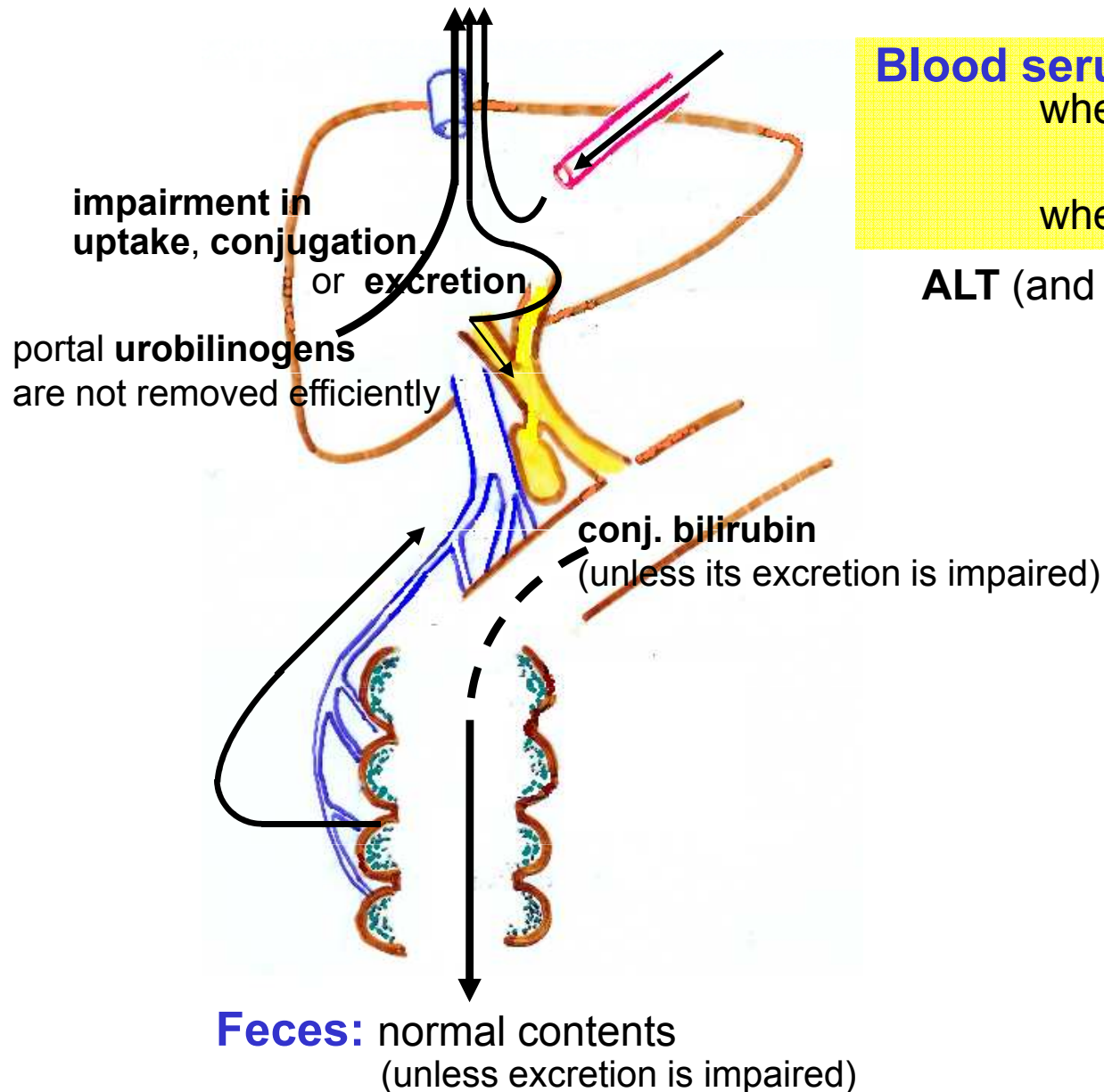# Prehepatic (haemolytic) hyperbilirubinaemia
– excessive erythrocyte breakdown

intensive uptake, conjugation, and excretion into the bile

Blood serum:
unconjugated bilirubin elevated

increased urobilinogens
are not removed sufficiently

conj. bilirubin

high portal
urobilinogens

high supply of urobilinogens
(bilirubin-albumin complexes
cannot pass the glomerular filter)

Urine: increased
urobilinogens
(no bilirubinuria)

Feces: polycholic (high amounts
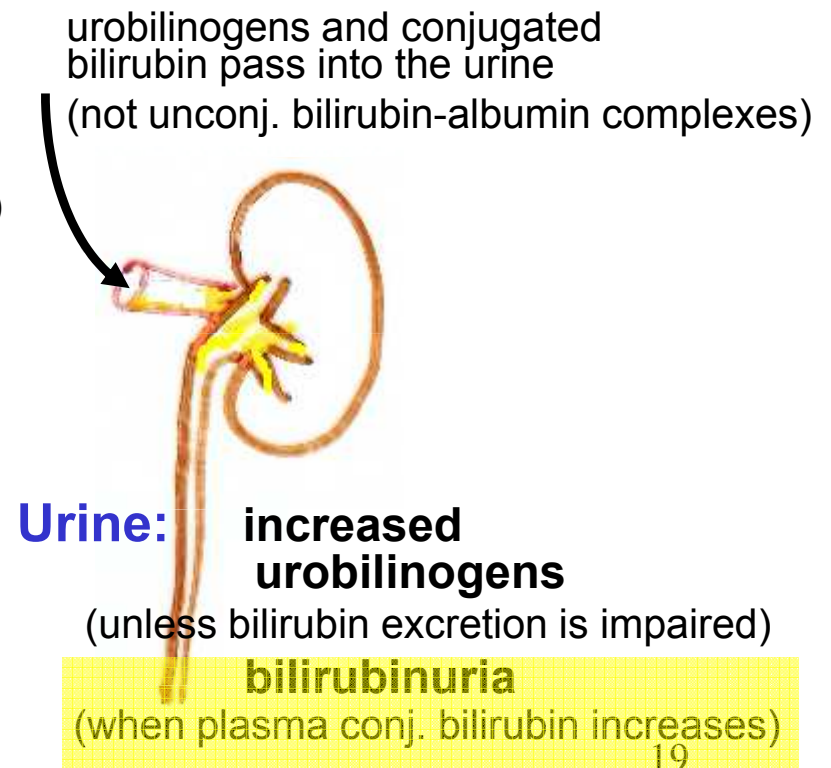of urobilinoids and bilifuscins)

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Hepatocellular hyperbilirubinaemia

The results of biochemical test depend on whether an impairment of hepatic **uptake**, **conjugation**, or **excretion** of bilirubin predominates.
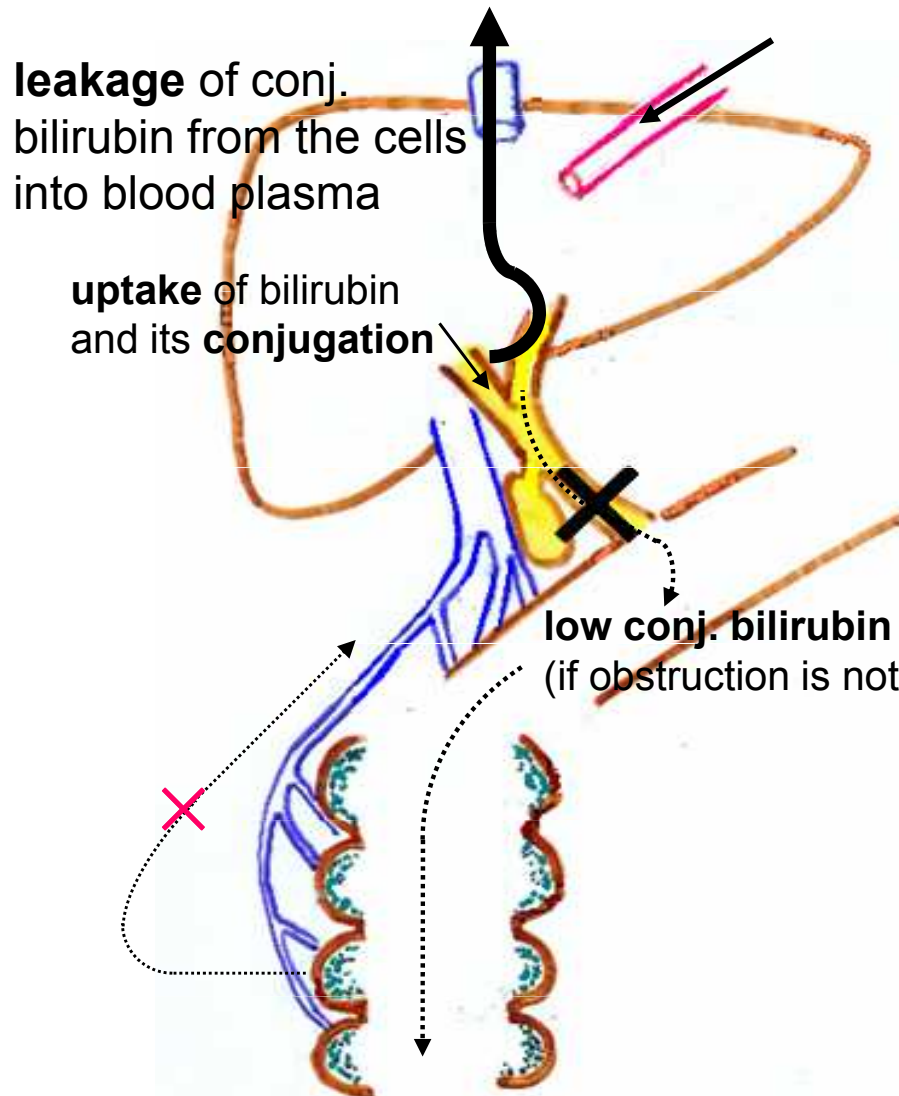
impairment in uptake, **conjugation**, or **excretion**

portal **urobilinogens** are not removed efficiently

conj. bilirubin (unless its excretion is impaired)

Feces: normal contents (unless excretion is impaired)

Blood serum: <u>**unconj. bilirubin**</u> is elevated, when its uptake or conjugation impaired

<u>**conj.**</u> **bilirubin** is elevated, when its excretion or drainage is impaired

ALT (and AST) catalytic concentrations increased

urobilinogens and conjugated bilirubin pass into the urine (not unconj. bilirubin-albumin complexes)

Urine: **increased urobilinogens** (unless bilirubin excretion is impaired)

bilirubinuria (when plasma conj. bilirubin increases)

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Obstructive (posthepatic) hyperbilirubinaemia

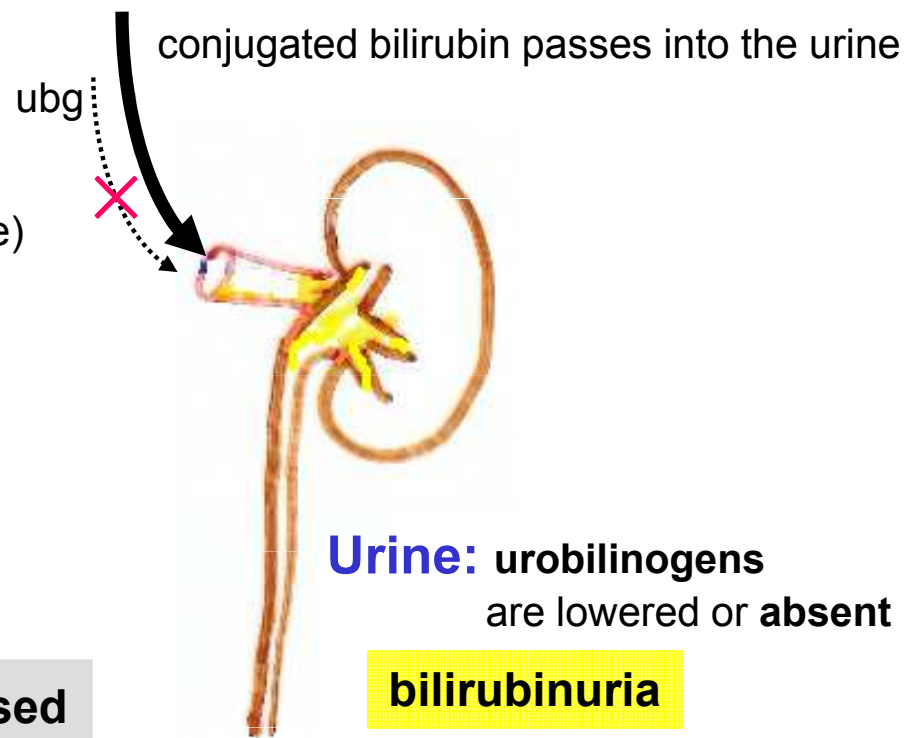**leakage** of conj. bilirubin from the cells into blood plasma

uptake of bilirubin and its **conjugation**

low conj. bilirubin (if obstruction is not complete)

Blood serum:
conjugated bilirubin elevated

bile acids concentration increased
catalytic concentration of **ALP** increased

conjugated bilirubin passes into the urine

ubg

Urine: urobilinogens are lowered or **absent**

bilirubinuria

Feces: urobilinoids and bilifuscins **decreased** or **absent** (grey, **acholic** feces)

Summary:

Type	Bilirubin		(derivatives)	Urobilinogens	
	Blood serum	Urine	Feces	Blood	Urine
PREHEPATIC (or haemolytic)	**increased** (unconjugated)	absent	**increased**	**increased**	**increased**
HEPATOCELLULAR	**increased** (unconj./conj.)	**present** (unconj.)	normal to decreased	increased or decreased	increased or decreased
OBSTRUCTIVE (posthepatic)	**increased** (conjugated)	**present** (unconj.)	**decreased** or absent	**decreased** or absent	**decreased** or absent

Laboratory tests
for detecting an impairment of liver functions ("liver tests")
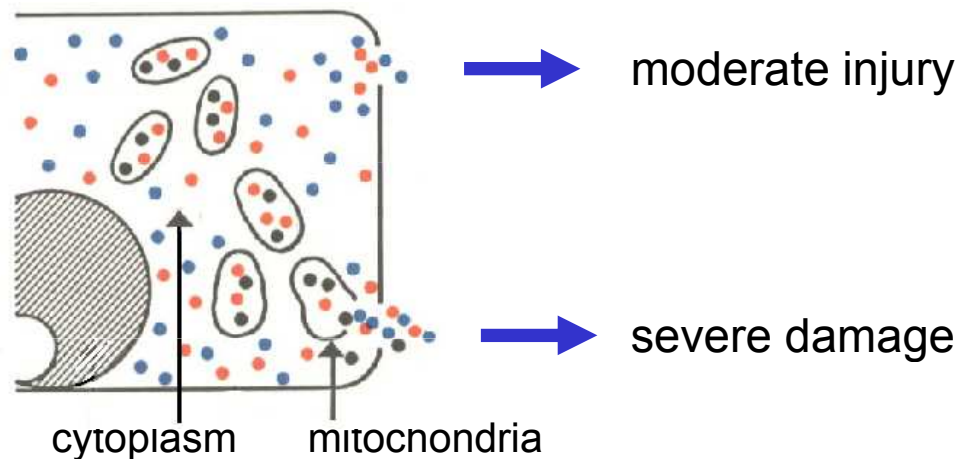
- **Plasma markers of hepatocyte membrane integrity**

  Catalytic concentrations of intracellular enzymes in blood serum increase:
  An assay for **alanine aminotransferase** (**ALT**) activity is the most sensitive one.
  In severe impairments, the activities of
  aspartate aminotransferase (**AST**) and
  glutamate dehydrogenase (**GD**) also increase.

Increase of catalytic concentrations:

moderate injury

severe damage

cytoplasm   mitochondria

ALT AST GD

- **Tests for decrease in liver proteosynthesis**

  Serum concentration of **albumin** (biological half-time about 20 days), **transthyretin** (prealbumin, biological half-time 2 days) and **transferrin**, **blood coagulation factors** (prothrombin time increases), activity of serum non-specific **choline esterase** (**ChE**).

- **Tests for the excretory function and cholestasis**

  Serum **bilirubin concentration**

  Serum catalytic concentration of **alkaline phosphatase** (**ALP**) and **γ-glutamyl transpeptidase** (**γGT**)

  Test for **urobilinogens** and **bilirubin** in urine

  Estimation of the excretion rate of **bromosulphophthalein** (**BSP test**) is applied to convalescents after acute liver diseases.

- **Tests of major metabolic functions** are not very decisive:

  Saccharide metabolism   low glucose tolerance (in oGT test)

  Lipid metabolism   increase in VLDL (triacylglycerols) and LDL (cholesterol)

  Protein catabolism   decreased urea, ammonium increase (in the final stage of liver failure, hepatic coma)

- **Special tests to specific disorders:** serological tests to viral hepatitis, serum α-foetoprotein (liver carcinoma), porphyrins in porphyrias, etc.

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Metabolism of iron

The body contains 4 – 4.5 g Fe:
In the form of haemoglobin   2.5 – 3.0 **g** Fe,
        tissue ferritin stores   up to 1.0 **g** Fe in men (0.3 – 0.5 g in women),
myoglobin and other haemoproteins 0.3 **g** Fe,
        circulating transferrin   3 – 4 <u>mg</u> Fe.

The **daily supply** of iron in mixed diet is about **10 – 20 mg**.
From that amount, not more than **only <u>1 – 2 mg are absorbed</u>**.
Iron metabolism is regulated by control of uptake, which have to replace the daily loss in iron and prevent an uptake of excess iron.

A healthy adult individual loses on average 1 – 2 mg Fe per day in desquamated cells (intestinal mucosa, epidermis) or blood (small bleeding, so that women are more at risk because of net iron loss in menstruation and pregnancy).

There is no natural mechanism for eliminating excess iron from the body.

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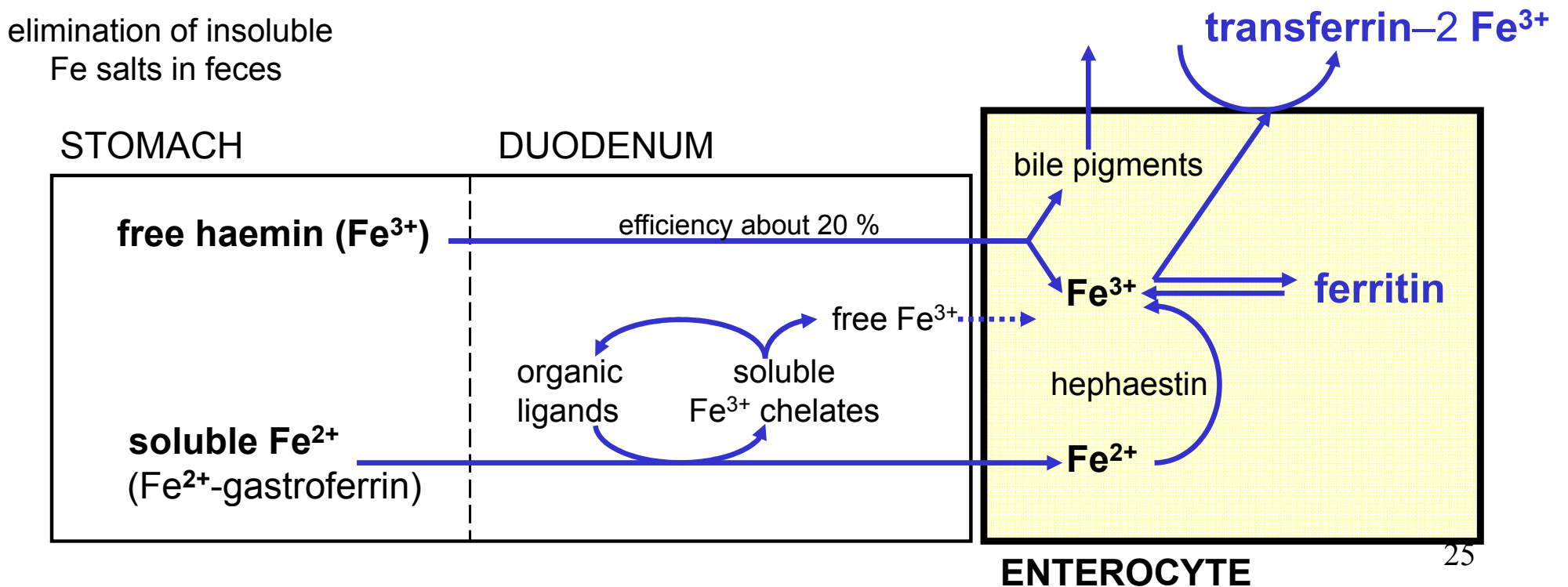# **Absorption of iron** in duodenum and jejunum

10 – 20 mf Fe

8 – 19 mg Fe

elimination of insoluble Fe salts in feces

Phosphates, **oxalate**, and **phytate** (*myo*-inositol hexakis(dihydrogen phosphate), present in vegetable food) form insoluble Fe^{3+} complexes and disable absorption.

Fe^{2+} is absorbed much easier than Fe^{3+}. Reductants such as **ascorbate** or **fructose** promote absorption, as well as Cu^{2+}.

Gastroferrin, a component of gastric secretion, is a glycoprotein that binds Fe^{2+} maintaining it soluble and preventing its oxidation to Fe^{3+}, from which insoluble iron salts are formed.

transferrin–2 Fe^{3+}

STOMACH   DUODENUM

bile pigments

free haemin (Fe^{3+})   efficiency about 20 %

Fe^{3+} **ferritin**

free Fe^{3+}

organic ligands   soluble Fe^{3+} chelates

hephaestin

soluble Fe^{2+} (Fe^{2+}-gastroferrin) Fe^{2+}

ENTEROCYTE

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Transferrin (Trf)

is a plasma glycoprotein (a major component of β_1-globulin fraction), M_r 79 600.

Plasma (serum) **transferrin concentration 2.5 – 4 g / l** (30 – 50 μmol / l)

Transferrin molecules have two binding sites for Fe ions,
total iron binding capacity (TIBC) for Fe ions is higher than **60 μmol / l**.

Serum Fe^{3+} (i.e. transferrin-Fe^{3+}) concentration is about **10 – 20 μmol / l**,
                14 – 26 μmol / l in men,
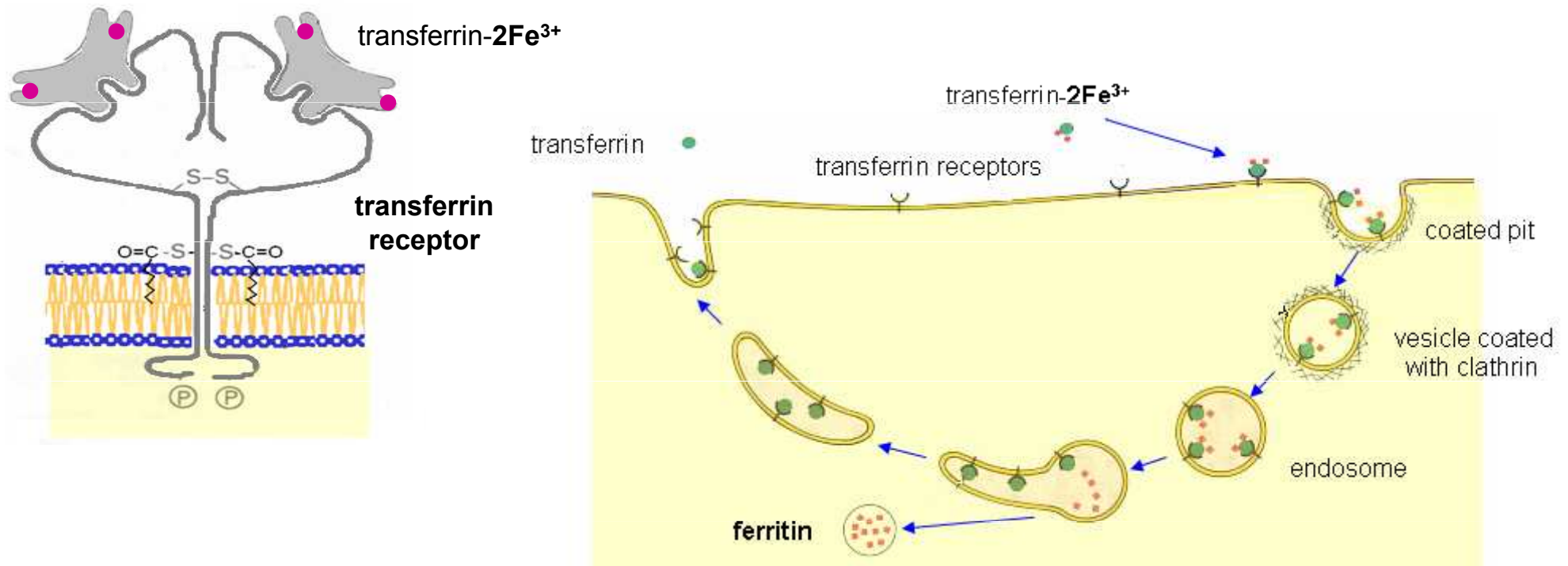   11 – 22 μmol / l in women.

                    Circadian rhythm exists, the morning concentrations are
                    higher by 10 - 30 % than those at night..

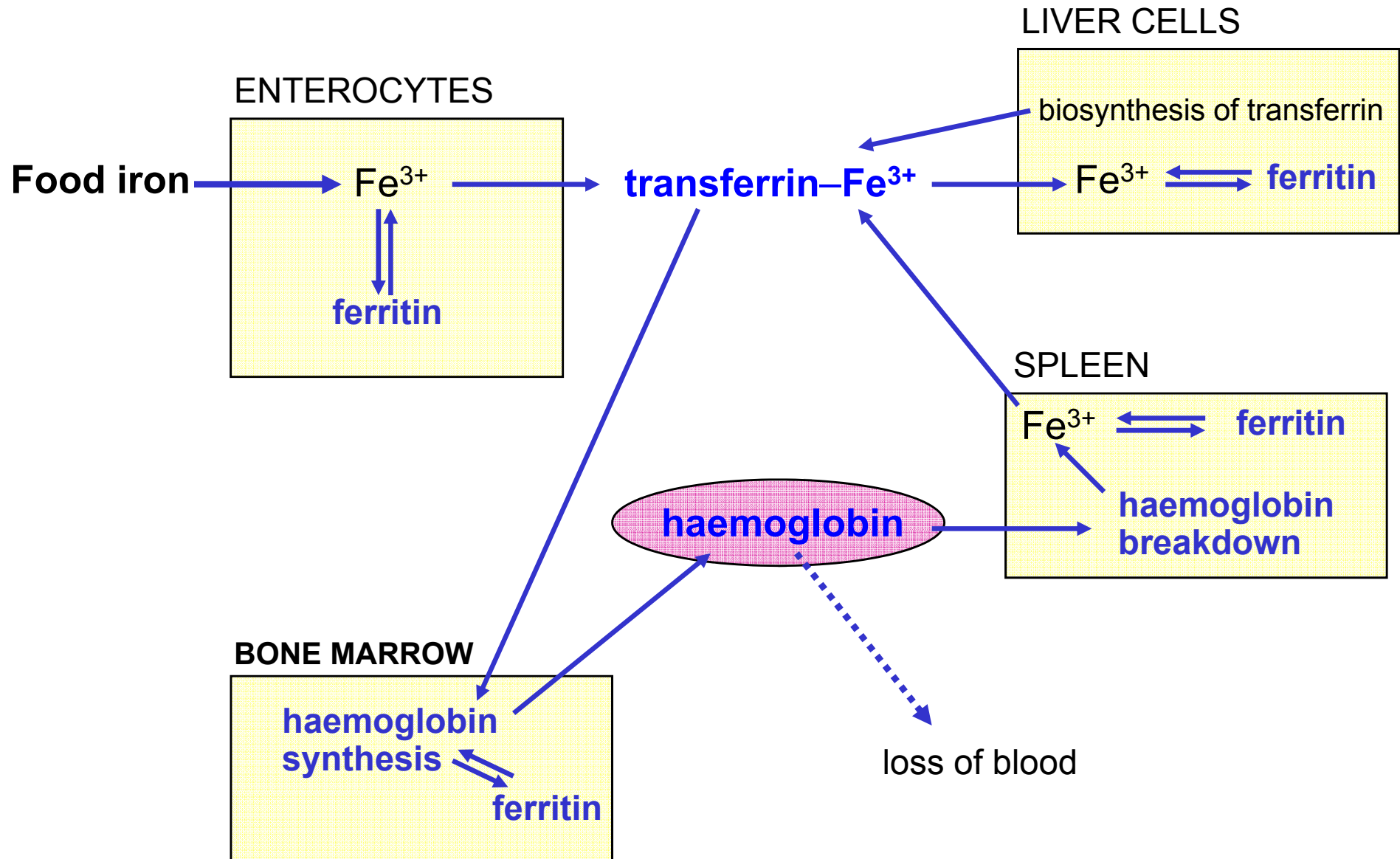**Saturation of transferrin** with Fe^{3+} equals usually **about 1/3**.

Because the biosynthesis of transferrin is stimulated during iron deficiency (and plasma iron concentration decreases), the decrease in saturation of transferrin is observed.

Iron is taken up by the cells
through a **specific receptor-mediated endocytosis**.

transferrin-**2Fe^{3+}**

transferrin receptor

transferrin-**2Fe^{3+}**

transferrin

transferrin receptors

coated pit

vesicle coated with clathrin

endosome

ferritin

Some receptors are released from the plasmatic membranes. Increase in serum concentration of those **soluble transferrin receptors** is the earliest marker of iron deficiency.

ENTEROCYTES

LIVER CELLS

Food iron $\rightarrow$ Fe^{3+} $\rightarrow$ transferrin–Fe^{3+}

Fe^{3+} $\rightleftharpoons$ ferritin

biosynthesis of transferrin

Fe^{3+} $\rightleftharpoons$ ferritin

ferritin

SPLEEN

Fe^{3+} $\rightleftharpoons$ ferritin

haemoglobin breakdown

haemoglobin

BONE MARROW

haemoglobin synthesis $\rightleftharpoons$ ferritin

loss of blood

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Ferritin

Ferritin occurs in most tissues (especially in the liver, spleen, bone-marrow, and enterocytes).

The protein apoferritin is a ball-shaped homopolymer of 24 subunits that surrounds the core of hydrated iron(III) hydroxide. One molecule can bind few **thousands of Fe^{3+} ions**, which make up to 23 % of the weight of ferritin.

apoferritin
(colourless)

Iron(III)
hydroxide hydrate
core

ferritin
(brown)

Minute amounts of ferritin are released into the blood plasma from the extinct cells. **Plasma ferritin concentration** 25 – 300 µg/l is proportional to the ferritin stored in the cells, unless the liver is impaired (increased ferritin release from the hepatocytes).
If the loading of ferritin is excessive, ferritin aggregate into its degraded form, **haemosiderin**, in which the mass fraction of Fe^{3+} can reach 35 %.

Ferritin was discovered by V. Laufberger, professor at Masaryk university, Brno, in 1934.

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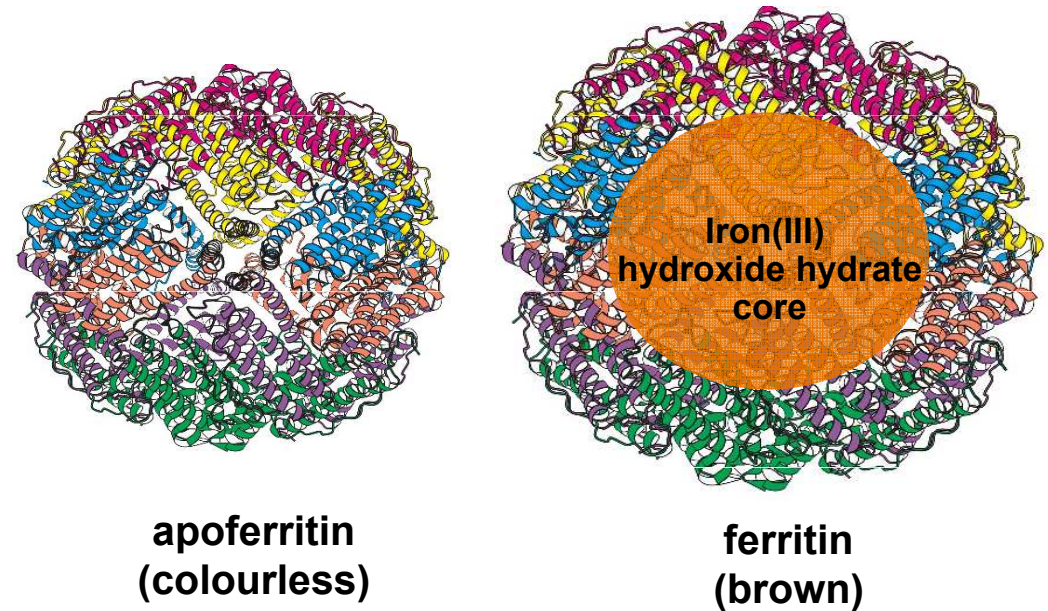
Hepcidin

is a polypeptide (M_r ~ 2000, 25 amino acid residues, from which 8 are Cys), discovered as the liver-expressed antimicrobial peptide, LEAP-1, in 2000.

It is produced by the liver (to some extent in myocard and pancreas, too) as a **hormone that limits the accessibility of iron** and also exhibits certain antimicrobial and antifungal activity.

The **biosynthesis of hepcidin is stimulated in iron overload** and **in inflammations** (hepcidin belongs to acute phase proteins type 2), and is supressed during iron deficiency .

Notice the fact that the same two factors stimulating hepcidin synthesis inhibit the biosynthesis of transferrin.

Effects of hepcidin: It – **reduces Fe^{2+} absorption** in the duodenum,
– **prevents the release of recyclable Fe from macrophages**,
– inhibits Fe transport across the placenta,
– diminishes the accessibility of Fe for invading pathogens.

Hepcidin is filtered in renal glomeruli and not reabsorbed in the renal tubules. So the amount of hepcidin excreted into the urine corresponds with the amount synthesized in the body. There is a positive correlation between this amount of hepcidin and the concentration of ferritin in blood plasma.

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