The metabolic functions of the liver

Catabolism of haemoglobin, bilirubin

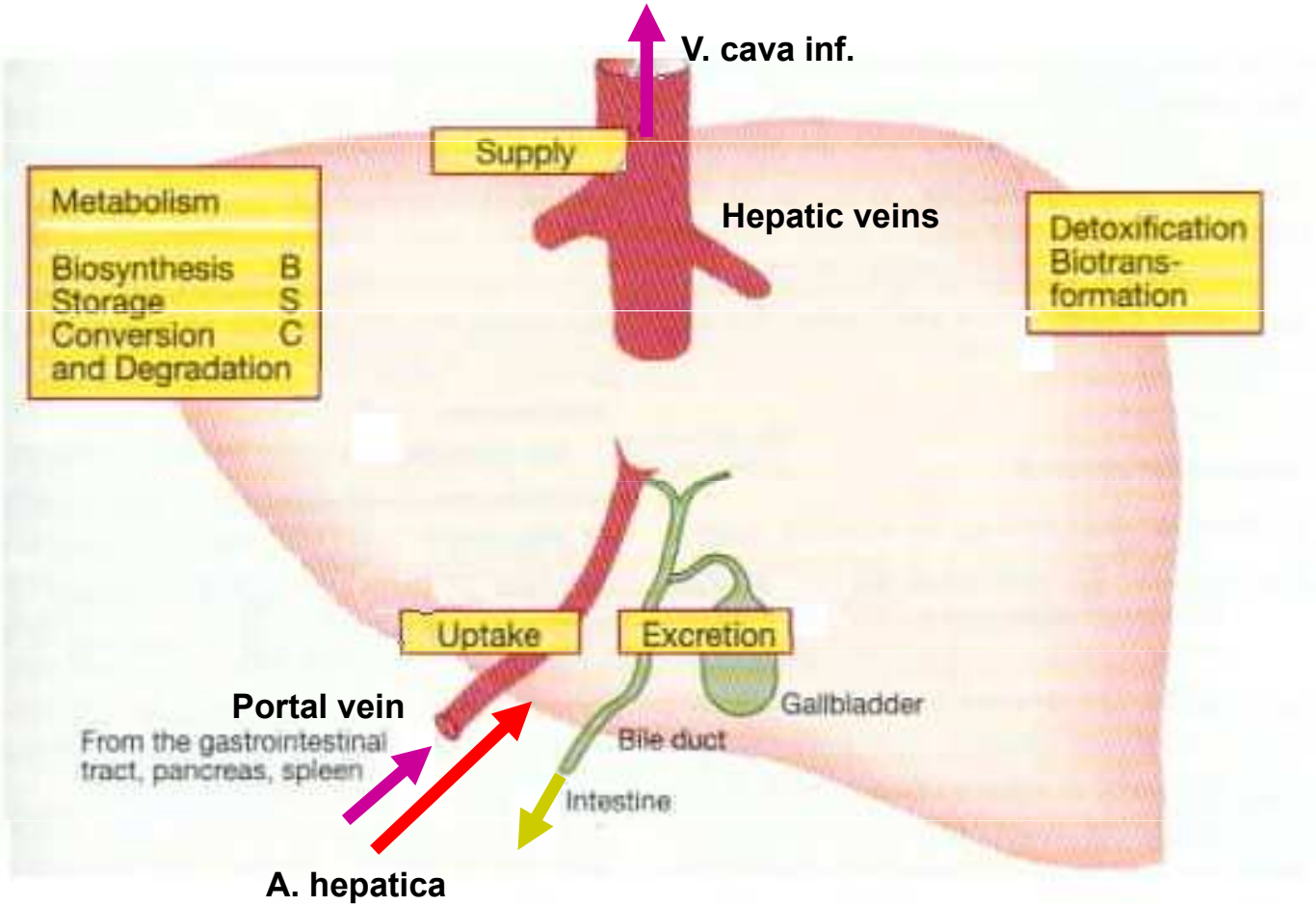
Metabolism of iron

Biochemistry II
Lecture 4

2008 (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).



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.

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

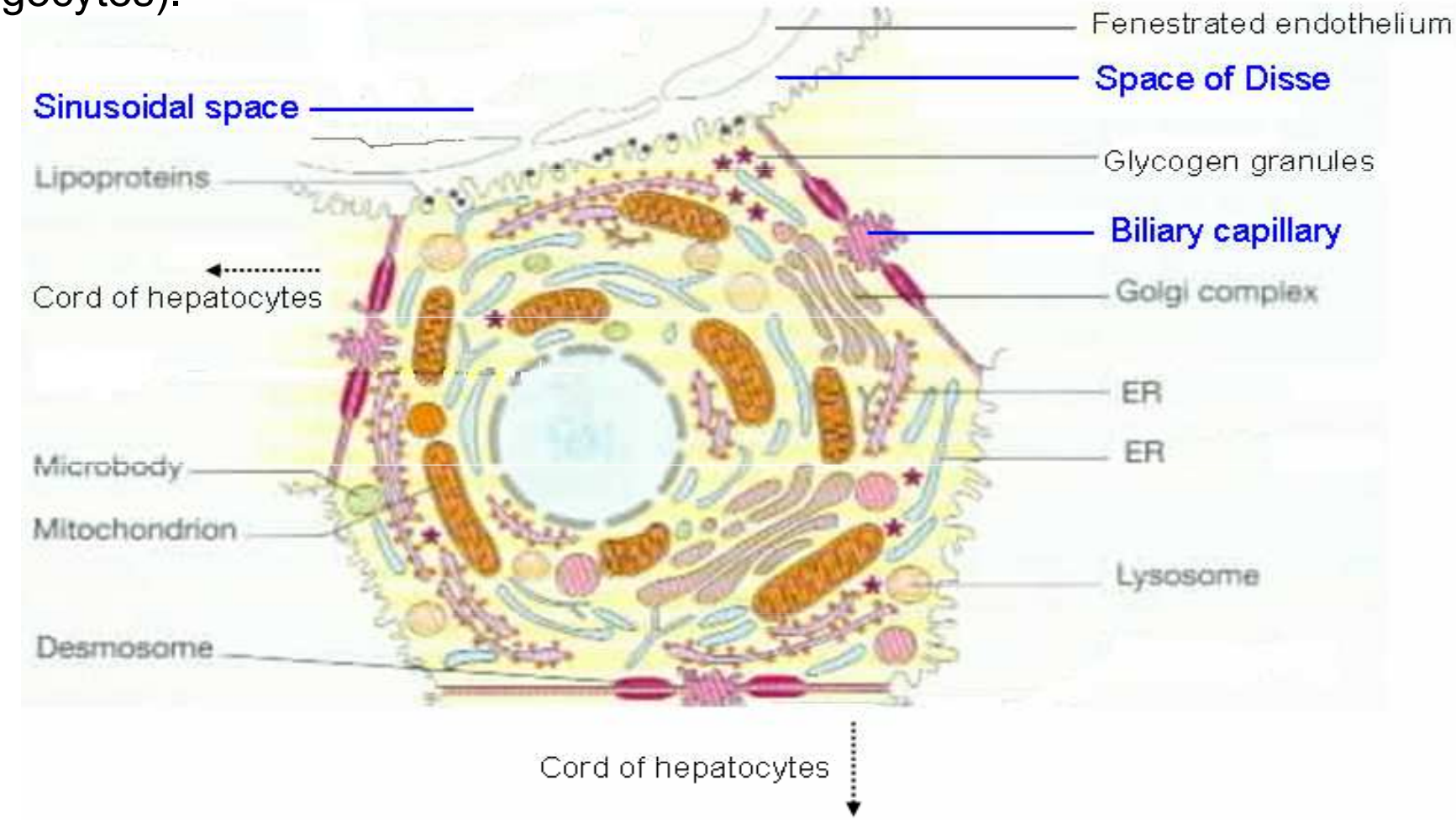
– Hydroxylation of calciols to calcidiols, splitting of β-carotene to retinol.
– The liver represent a store of retinol esters and cobalamin (B_{12}).

Iron and copper metabolism

– Synthesis of transferrin, coeruloplasmin, ferritin stores, excretion of copper.

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).

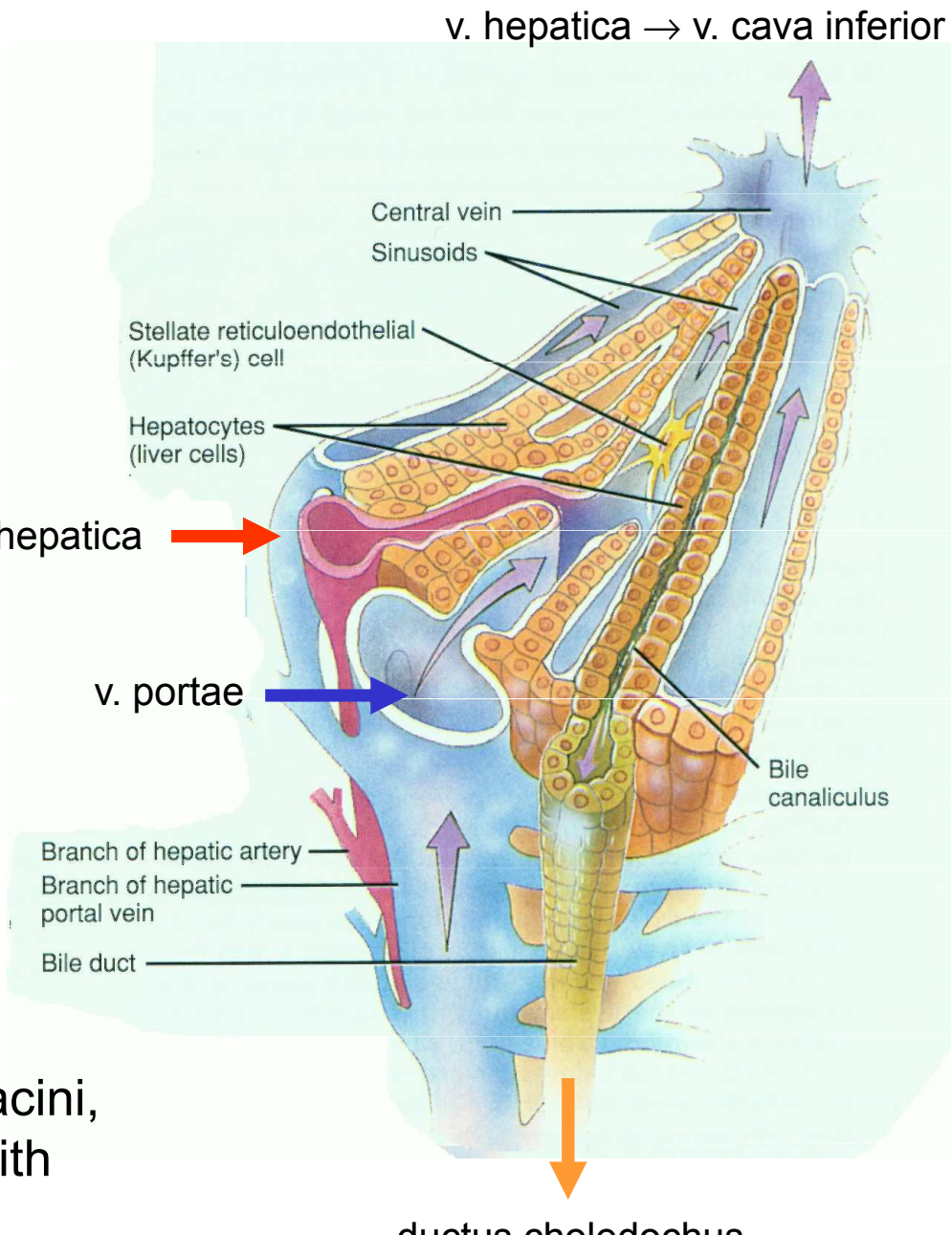


Plasmatic membrane directed to the space of Disse – the "blood" pole, the "bile" pole – lateral parts of the membrane with gap junctions and parts forming bile capillaries.

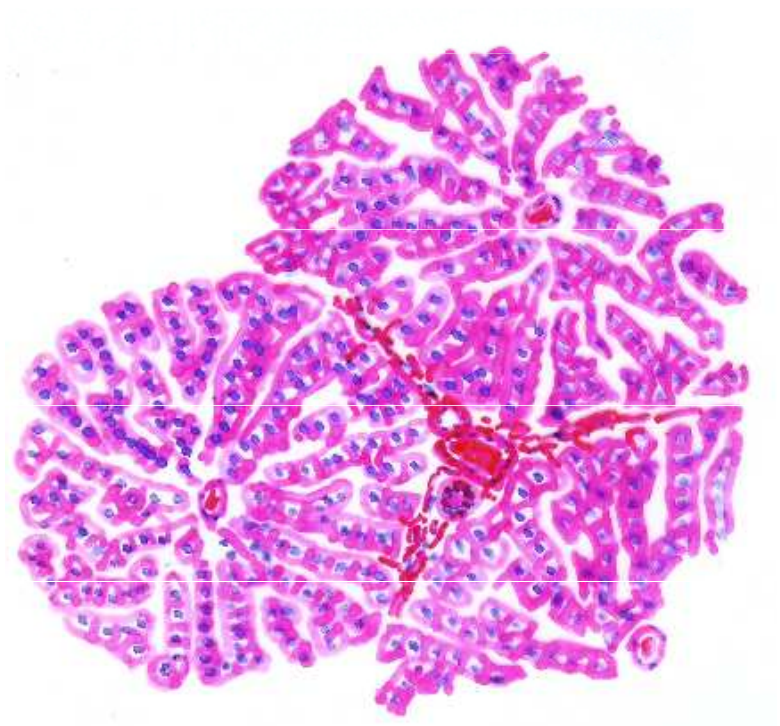
The liver receives venous blood from the intestine. Thus all the products of digestion, in addition to ingested drugs and other xenobiotics, perfuse the liver before entering the systemic circulation.

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.

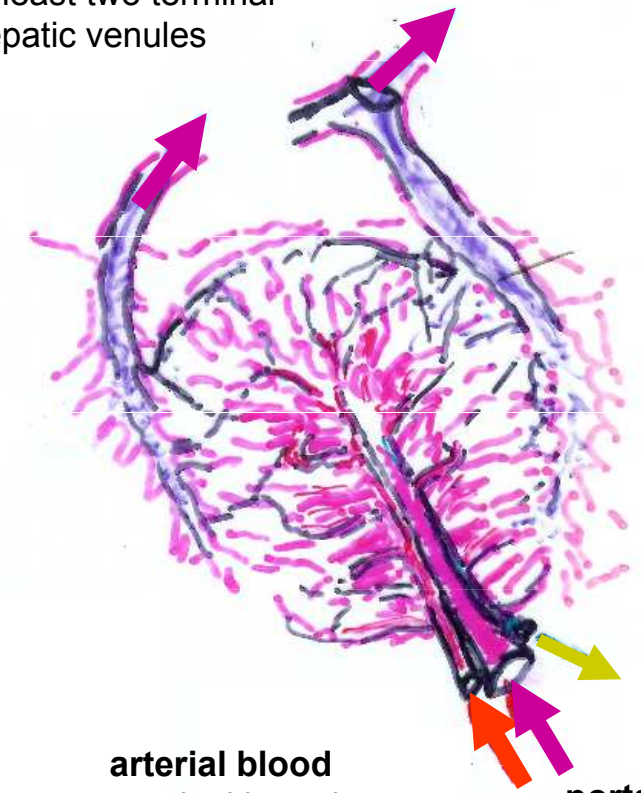


Hexagonal hepatic lobules
round terminal hepatic venules
are not functional units



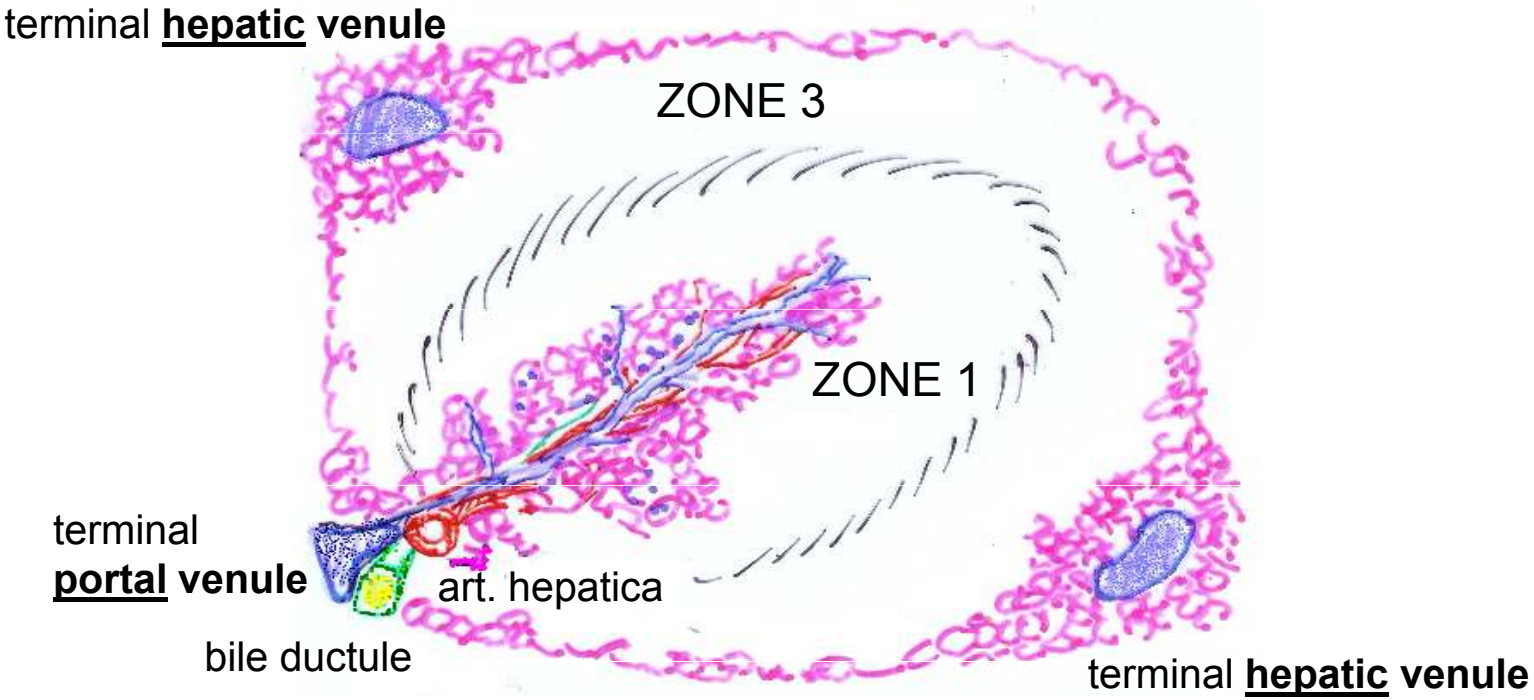
simple liver ACINUS – a functional unit

efferent vessels
at least two terminal
hepatic venules



portal field with afferent vessels

Metabolic areas in the acini



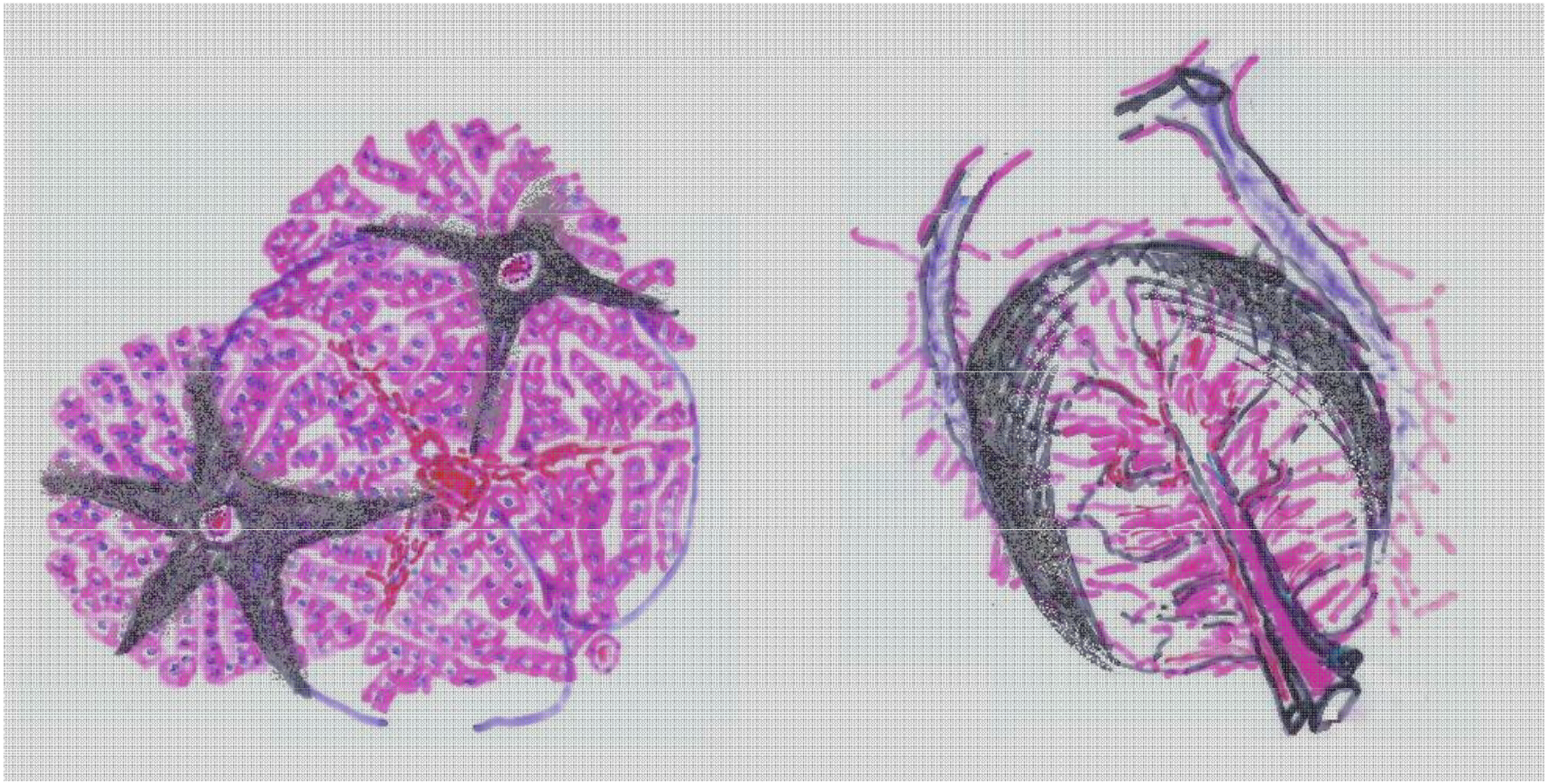
Zone 1 – **periportal area**

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

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).

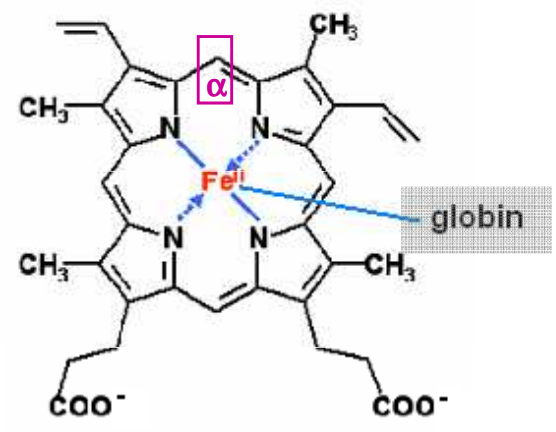
Excretion of cholesterol and bile acids is the major way of removing cholesterol from the body. Bile also removes hydrophobic metabolites, drugs, toxins and metals (e.g. copper, zinc, mercury).

Neutralization of the acid chyme in conjunction with HCO_3^- from pancreatic secretion.

Degradation of haemoglobin to bilirubin– bile pigments

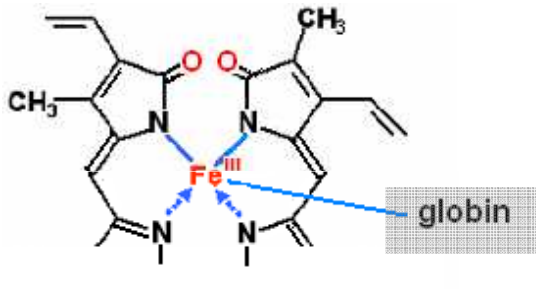
Erythrocytes are taken up by the **reticuloendothelial cells** (cells of the spleen, bone marrow, and Kupffer cells in the liver) by phagocytosis.

Haemoglobin



3 O_2

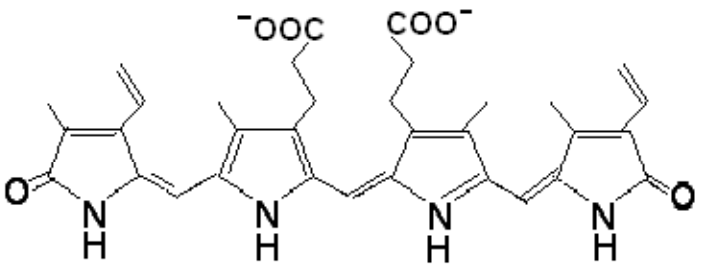
CO



Verdoglobin IX-α

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

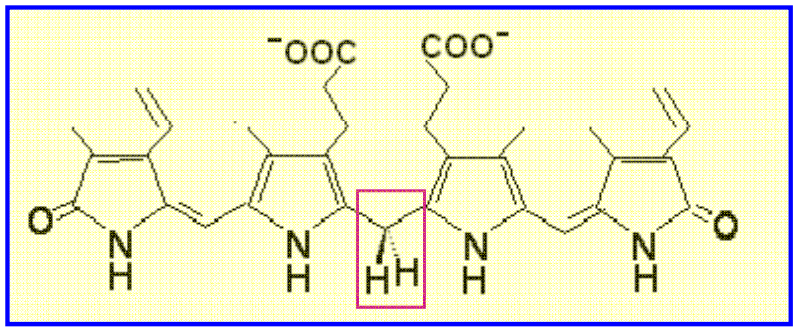
Fe^{3+}
globin



Biliverdin IX-α

NADPH

Biliverdin reductase

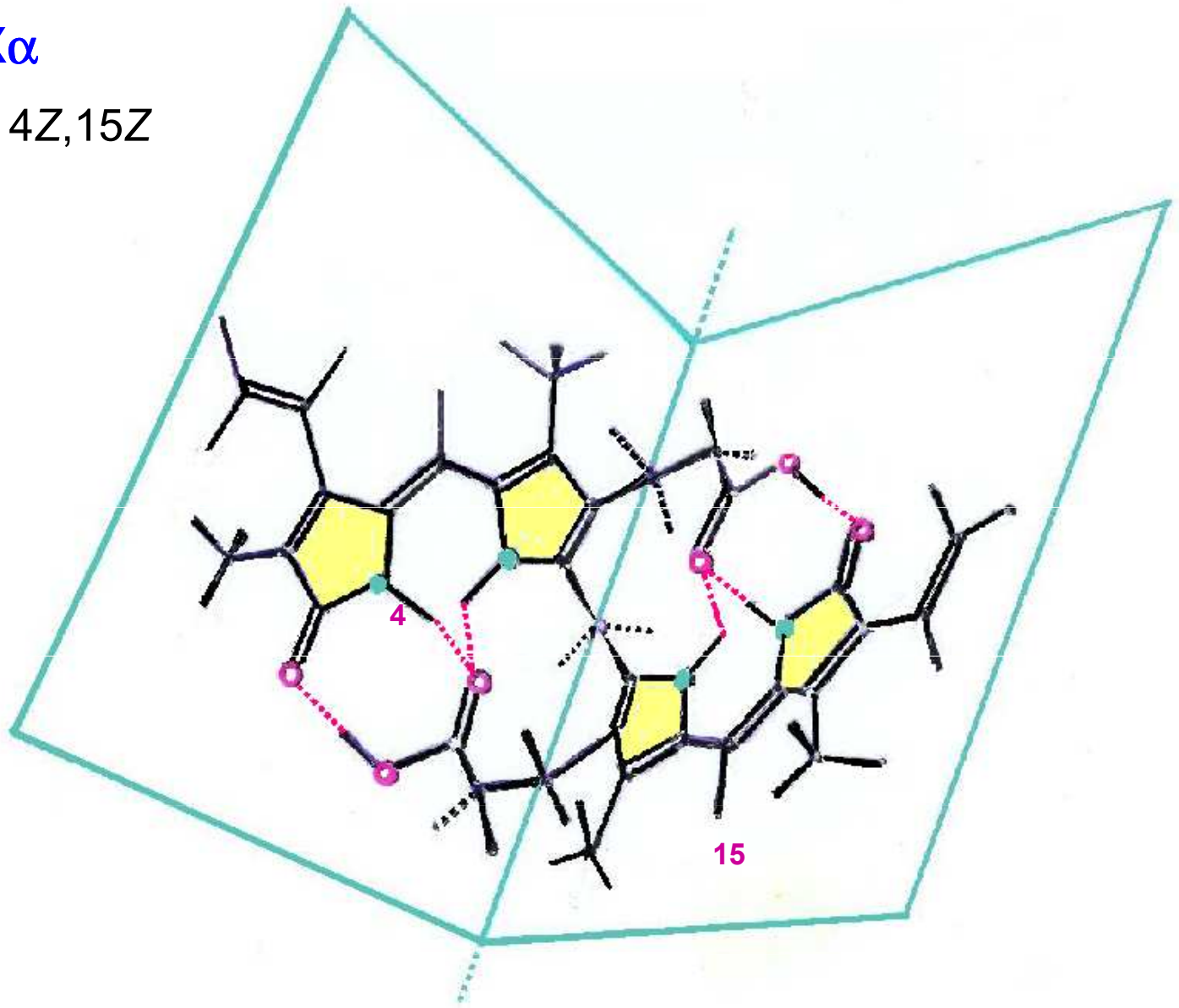


Bilirubin IX-α

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

Bilirubin IXα

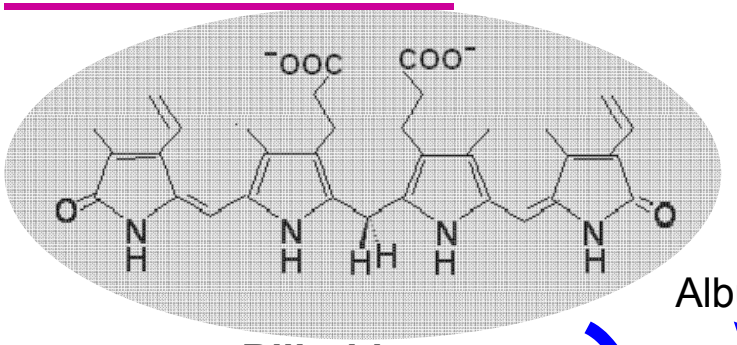
configuration 4*Z*,15*Z*



Polarity of the two carboxyl groups of unconjugated bilirubin is masked by formation of hydrogen bonds between the carboxyl groups and the electronegative atoms within the opposite halves of bilirubin molecules.

The hepatic uptake, conjugation, and excretion of bilirubin

UNCONJUGATED bilirubin (bilirubin-albumin complex)
in hepatic sinusoids



Albumin

Bilirubin receptor
(bilitranslocase)

the amount that can leak from excretion

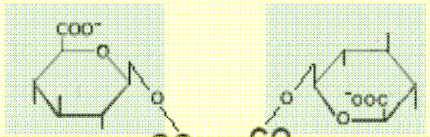
plasma membrane of hepatocytes

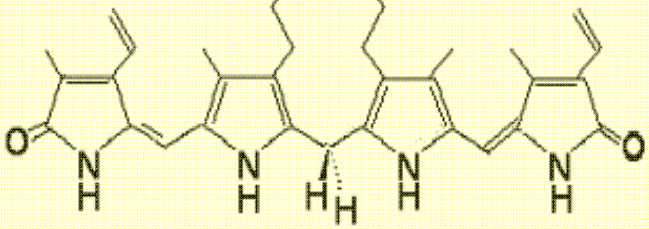
Ligandin
(protein Y)

UDP-glucuronate

UDP

glucosyluronate transferase
on ER membranes





bilirubin monoglucosiduronate
bilirubin bisglucosiduronate

terminal bile ductule

CONJUGATED bilirubin

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

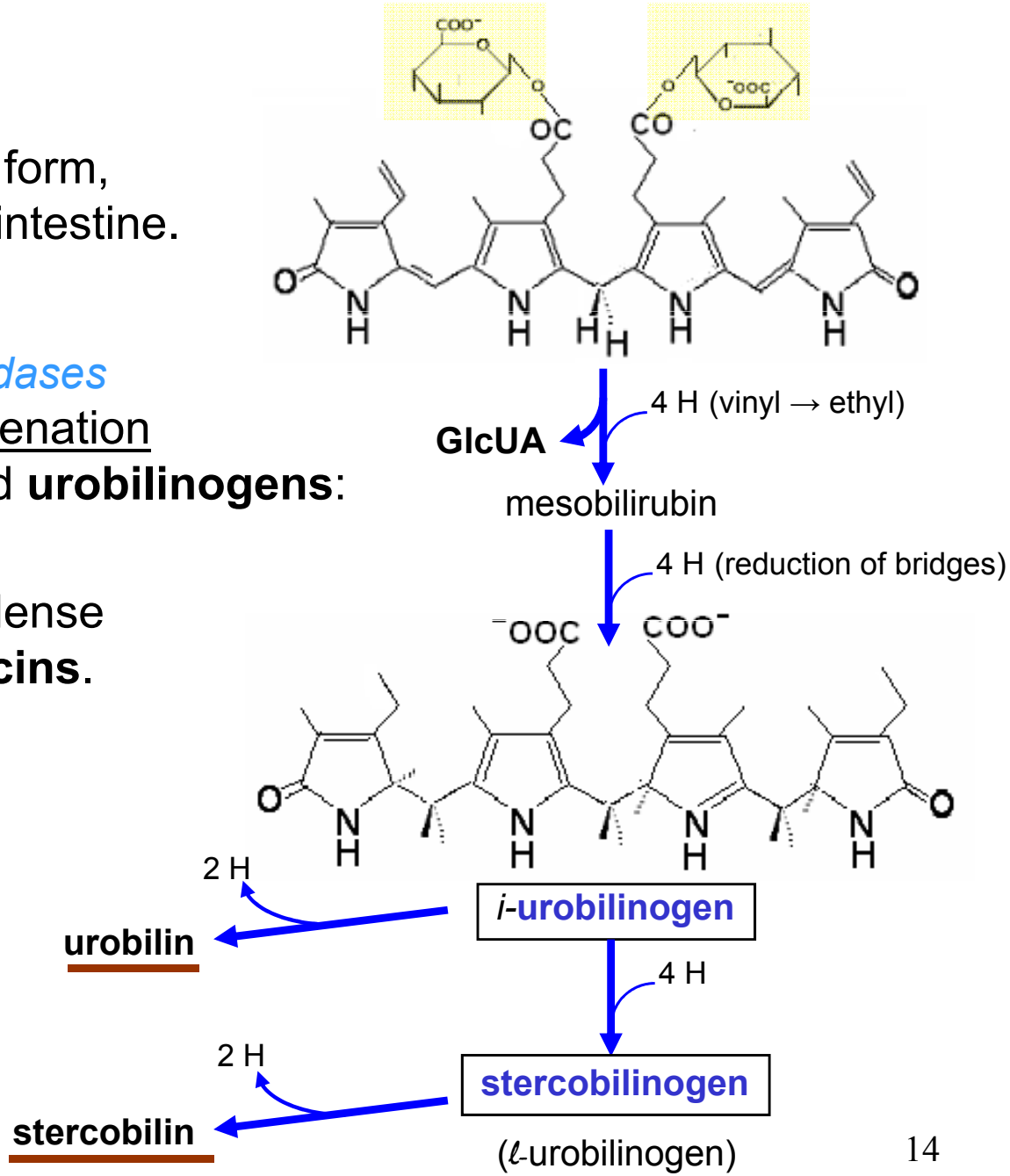
The formation of urobilinoids by the intestinal microflora

Conjugated bilirubin
is secreted into the bile. As far as bilirubin remains in the conjugated form, it cannot be absorbed in the small intestine.

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

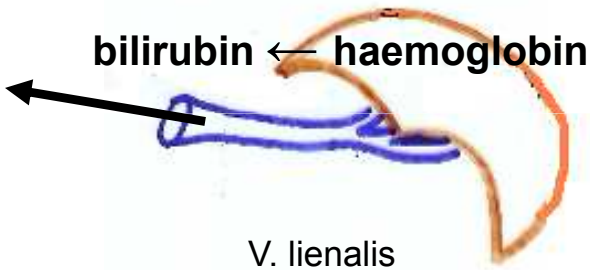
A part of urobilinogens is split to **dipyrromethenes**, which can condense to give intensively coloured **bilifuscins**.

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**.



Healthy individuals:

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



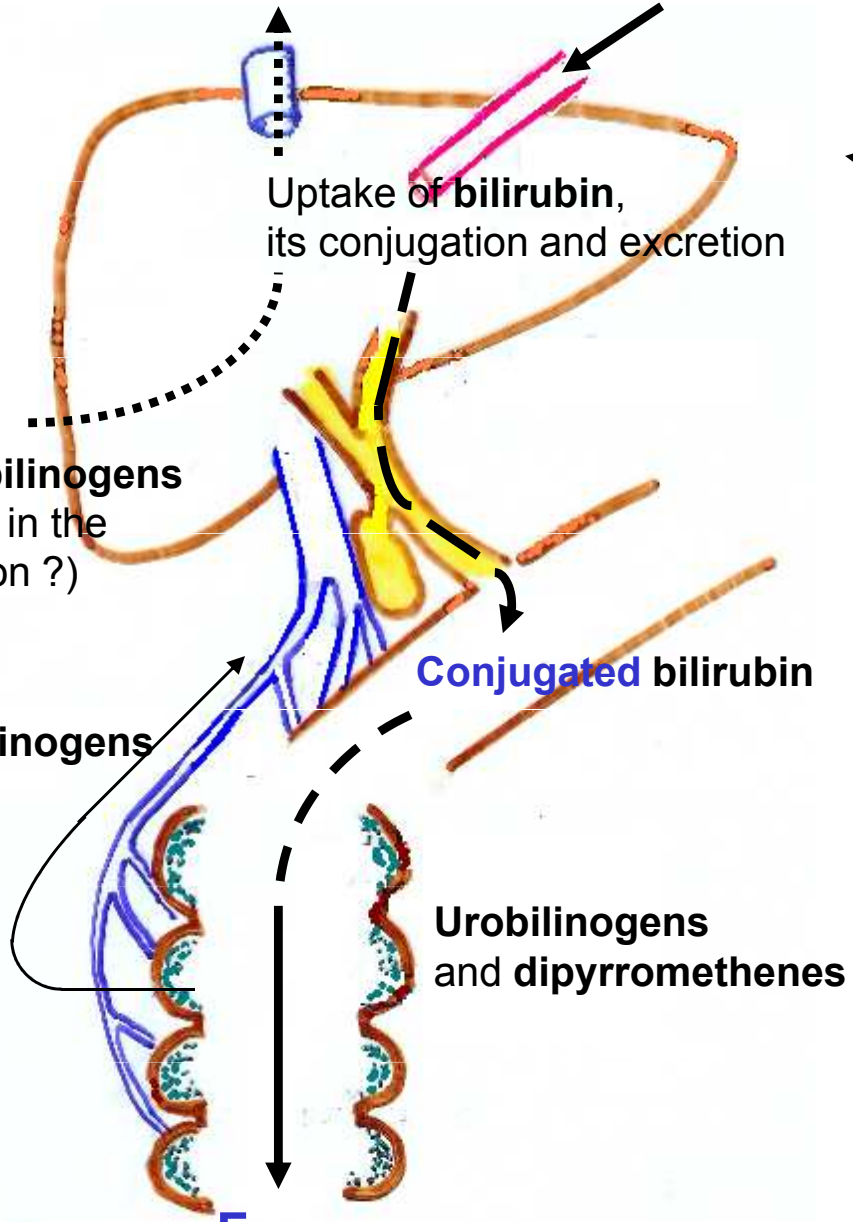
bilirubin ← haemoglobin

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

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

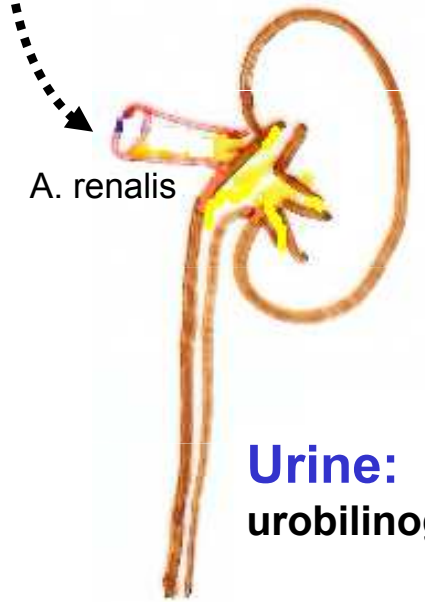
Most of **urobilinogens**
are removed in the
liver (oxidation ?)

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



Portal
urobilinogens

Conjugated bilirubin



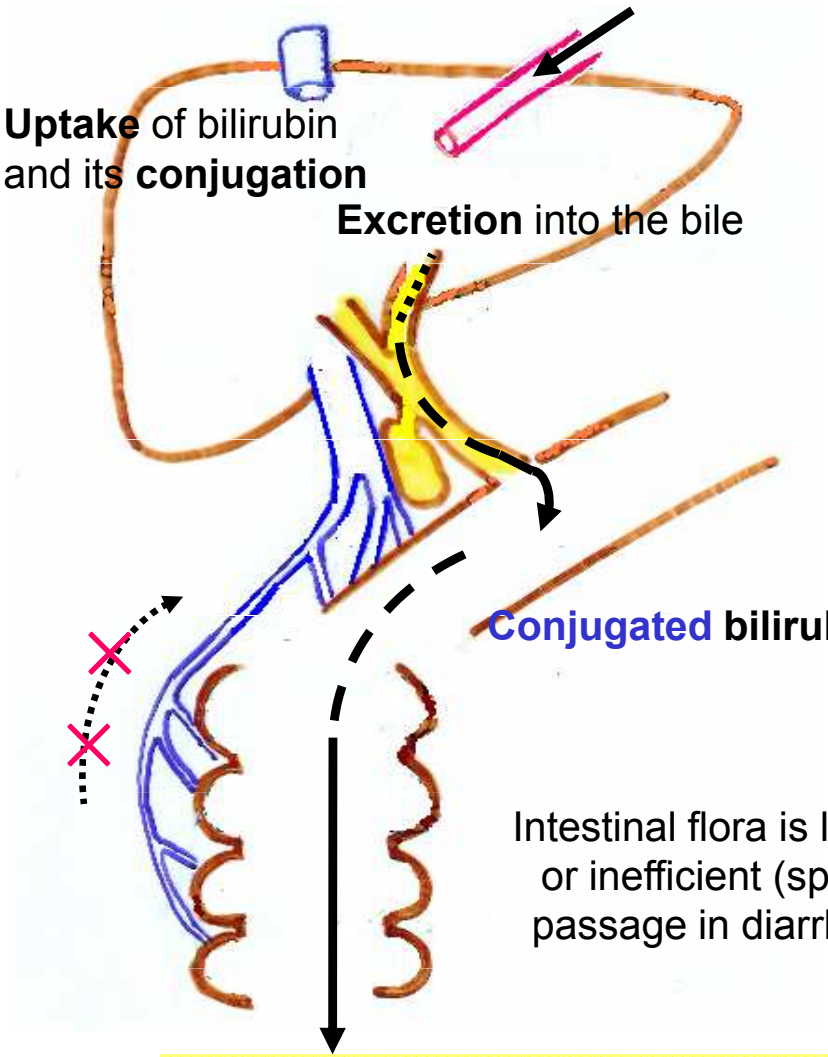
A. renalis

Urobilinogens
and **dipyrromethenes**

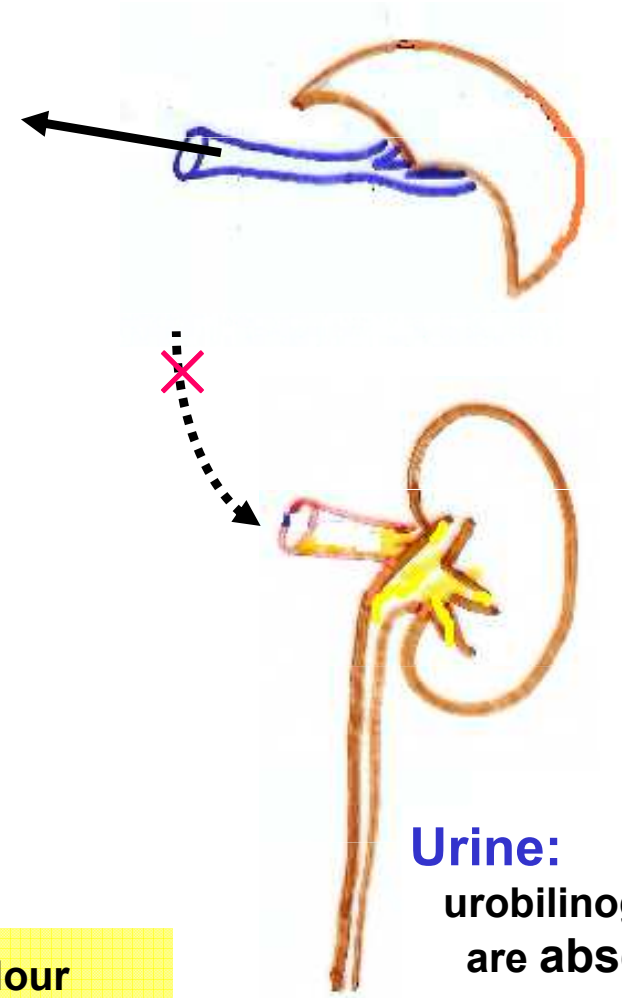
Urine:
urobilinogens < 5 mg / d

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

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



Plasma: **normal bilirubin concentration**



Urine:
urobilinogens are absent

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

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

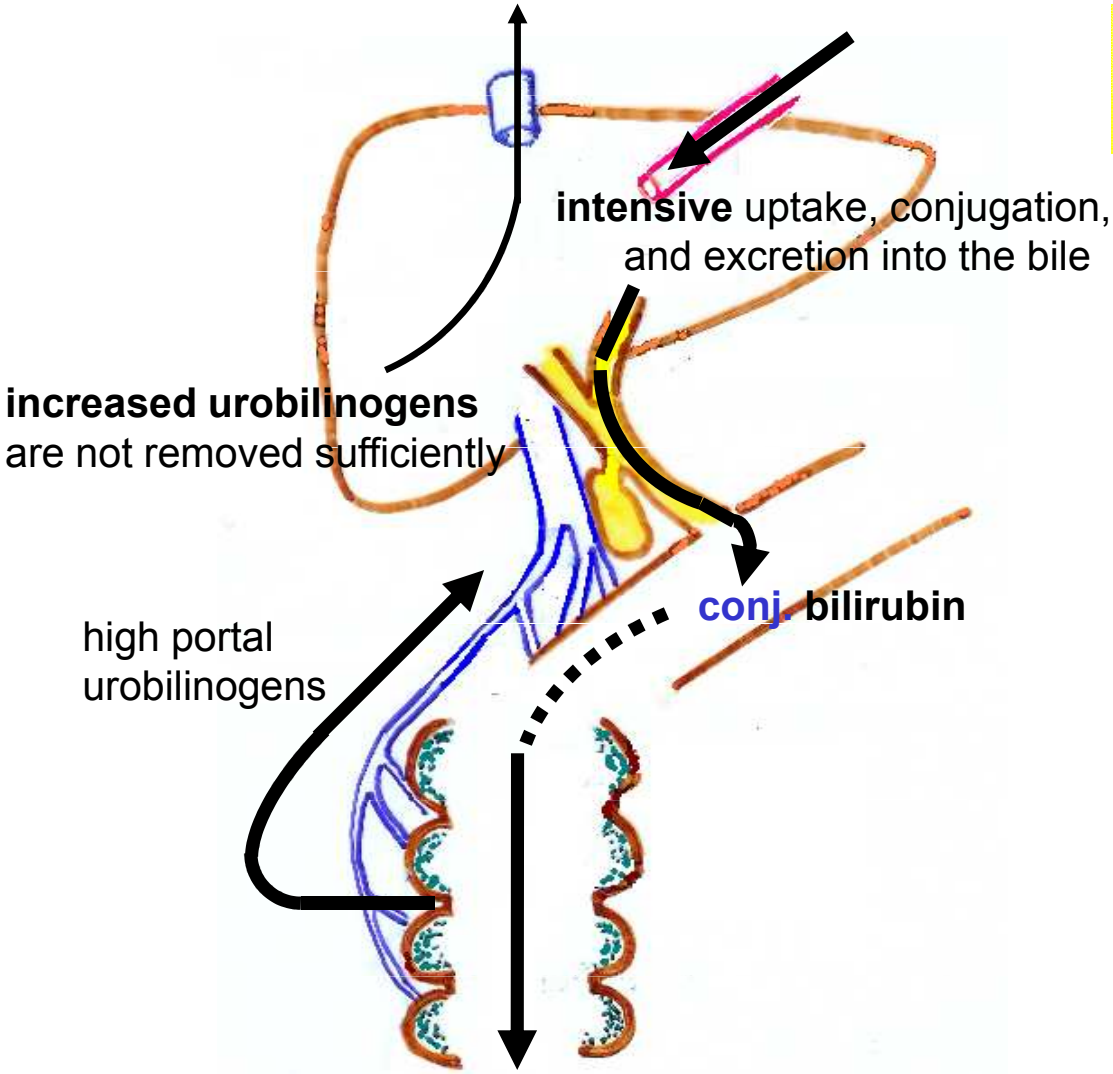
– **prehepatic** (haemolytic) – increased production of bilirubin,

– **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



Blood serum:
unconjugated bilirubin elevated

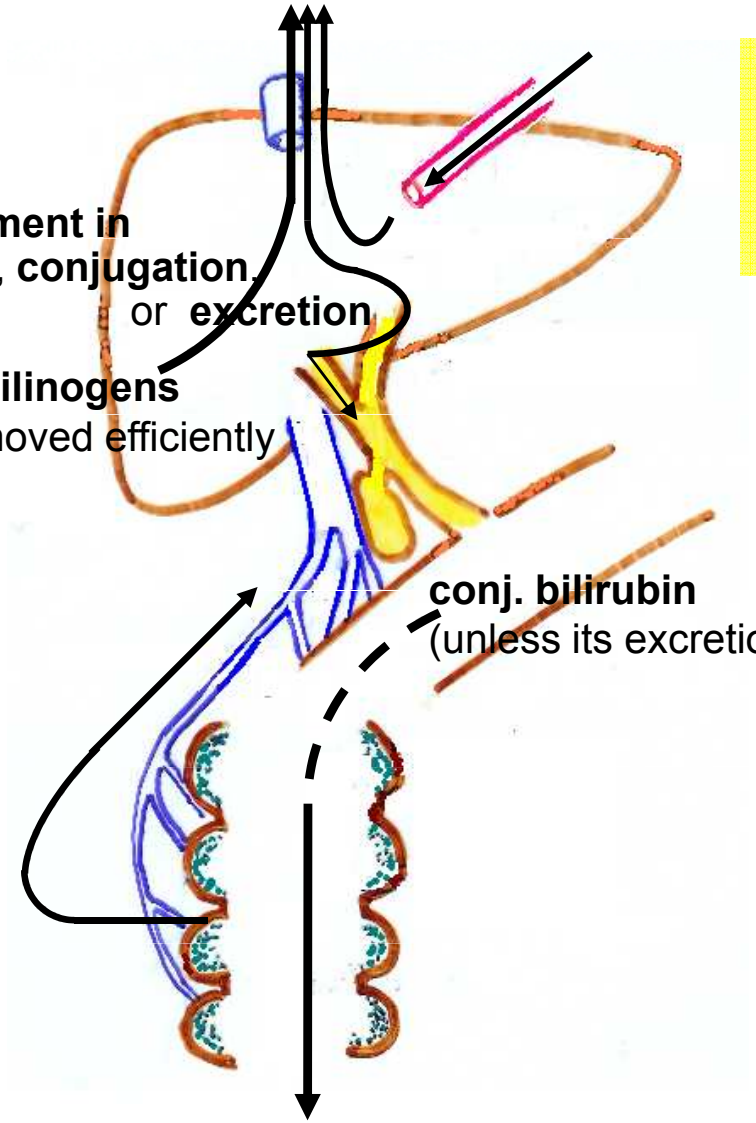
high supply of urobilinogens
(bilirubin-albumin complexes cannot pass the glomerular filter)

Urine: increased urobilinogens
(no bilirubinuria)

Feces: polycholic (high amounts of urobilinoids and bilifuscins)

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: **unconj. bilirubin** is elevated, when its uptake or conjugation is impaired

conj. bilirubin is elevated, when its excretion or drainage is impaired

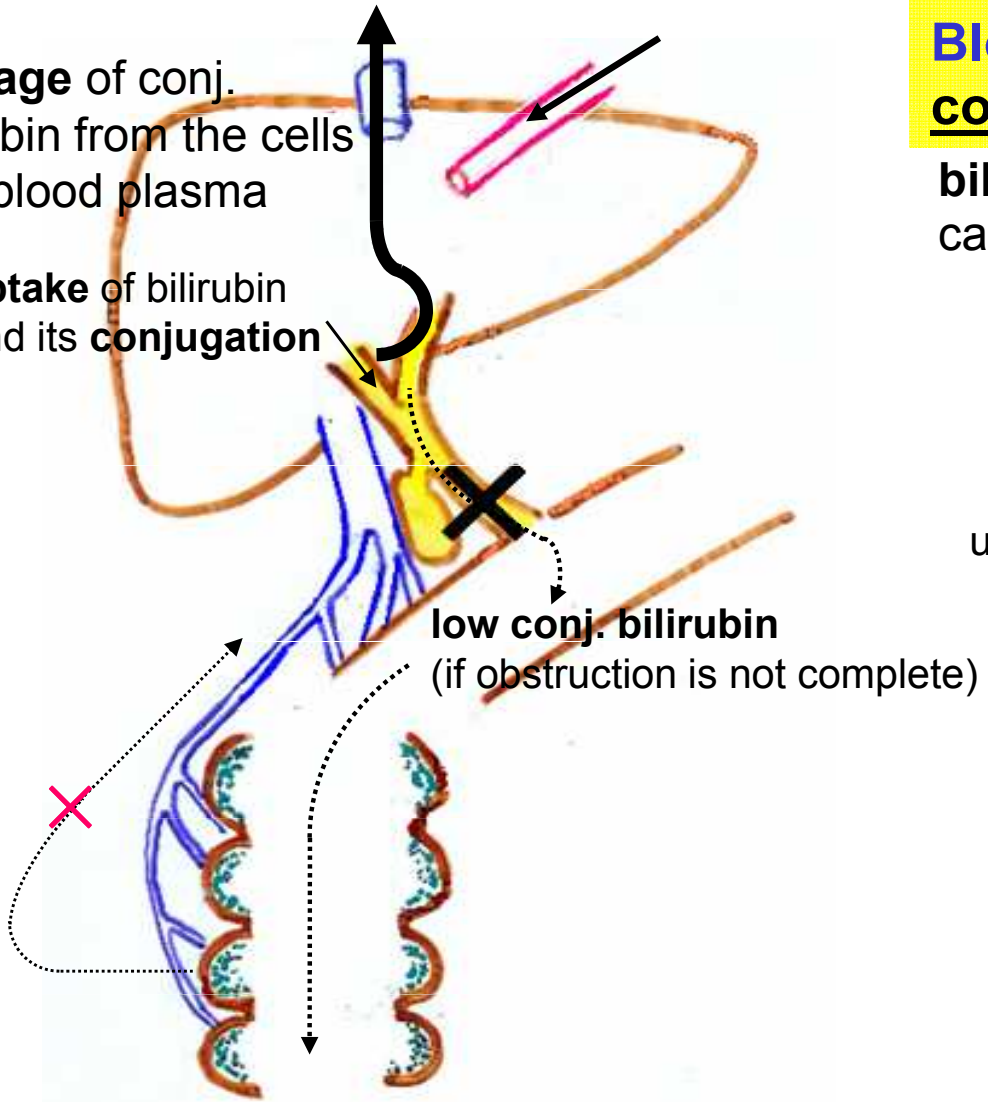
ALT (and AST) catalytic concentrations increased

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

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

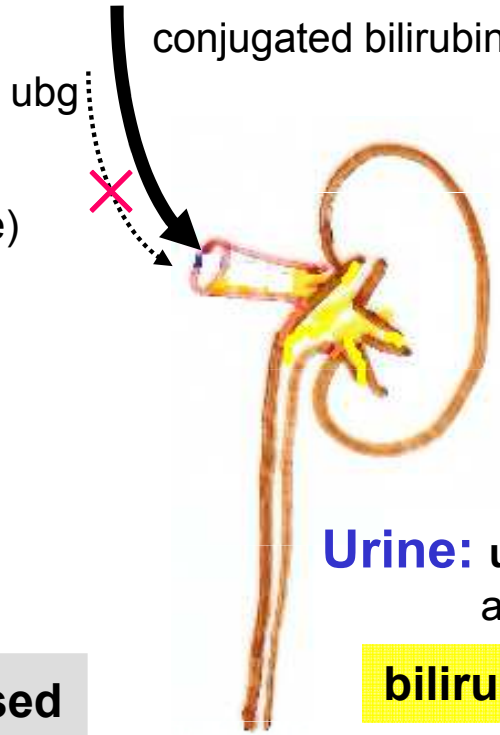
bilirubinuria (when plasma conj. bilirubin increases)

Obstructive (posthepatic) hyperbilirubinaemia



Blood serum:
conjugated bilirubin elevated

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



Urine: **urobilinogens** are lowered or **absent**

bilirubinuria

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

Summary:

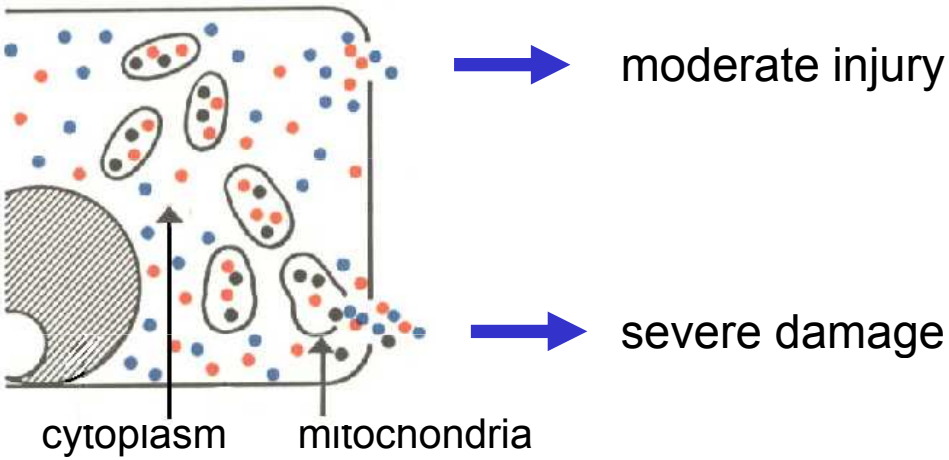
Type	**Bilirubin** Blood serum	Urine	(derivatives) Feces	**Urobilinogens** Blood	Urine
PREHEPATIC (or haemolytic)	**increased** (unconjugated)	absent	**increased**	**increased**	**increased**
HEPATOCELLULAR	**increased** (unconj./conj.)	**present** (unconj.)	normal to decreased	increased or decreased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

- **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.

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 mg Fe.

The **daily supply** of iron in mixed diet is about **10 – 20 mg**.
From that amount, not more than **only l – 2 mg are absorbed**.
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.

Absorption of iron in duodenum and jejunum

10 – 20 mf Fe

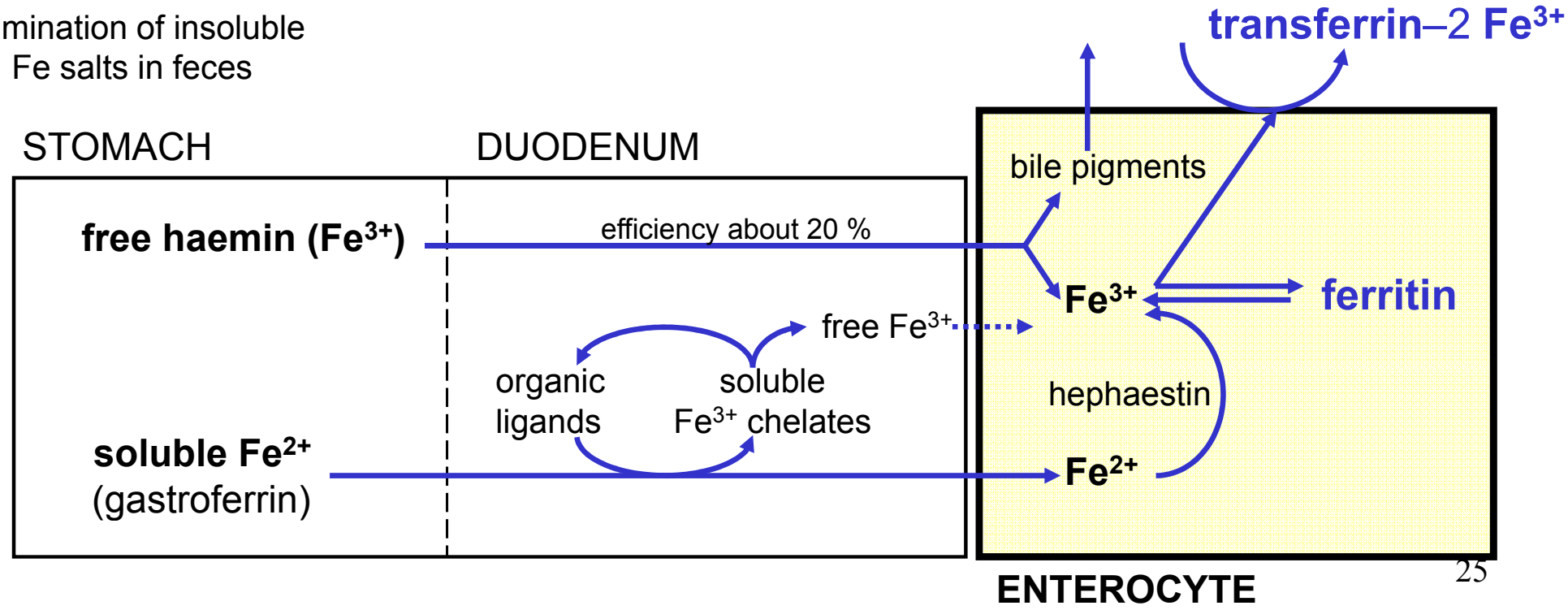
8 – 19 mg Fe

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 prevents its oxidation to Fe^{3+}, from which insoluble iron salts are formed.



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.

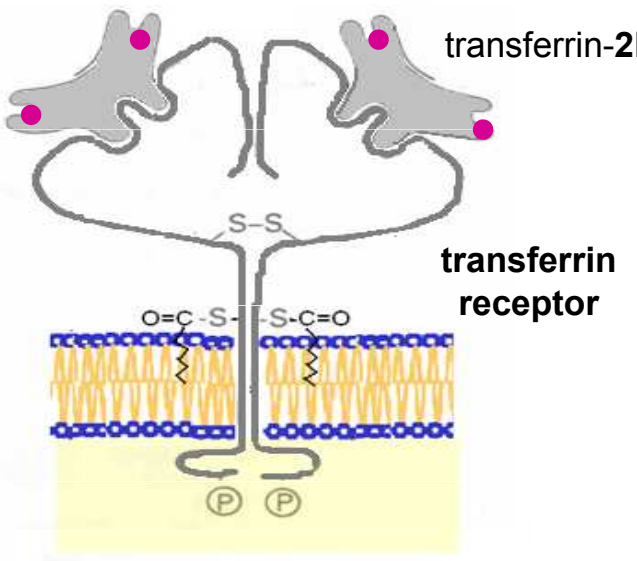
Circadian rhythm exists, the morning concentrations are higher by 10 - 30 % than those at night..

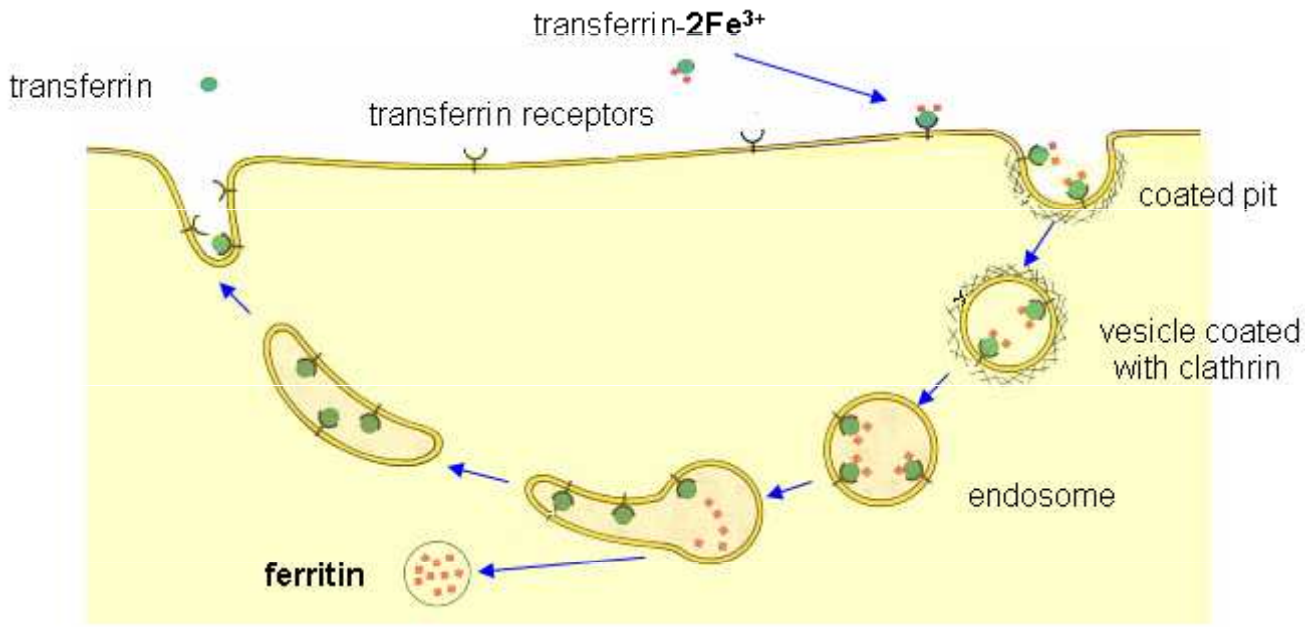
Saturation of transferrin with Fe^{3+} equals usually **about 1/3**.

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

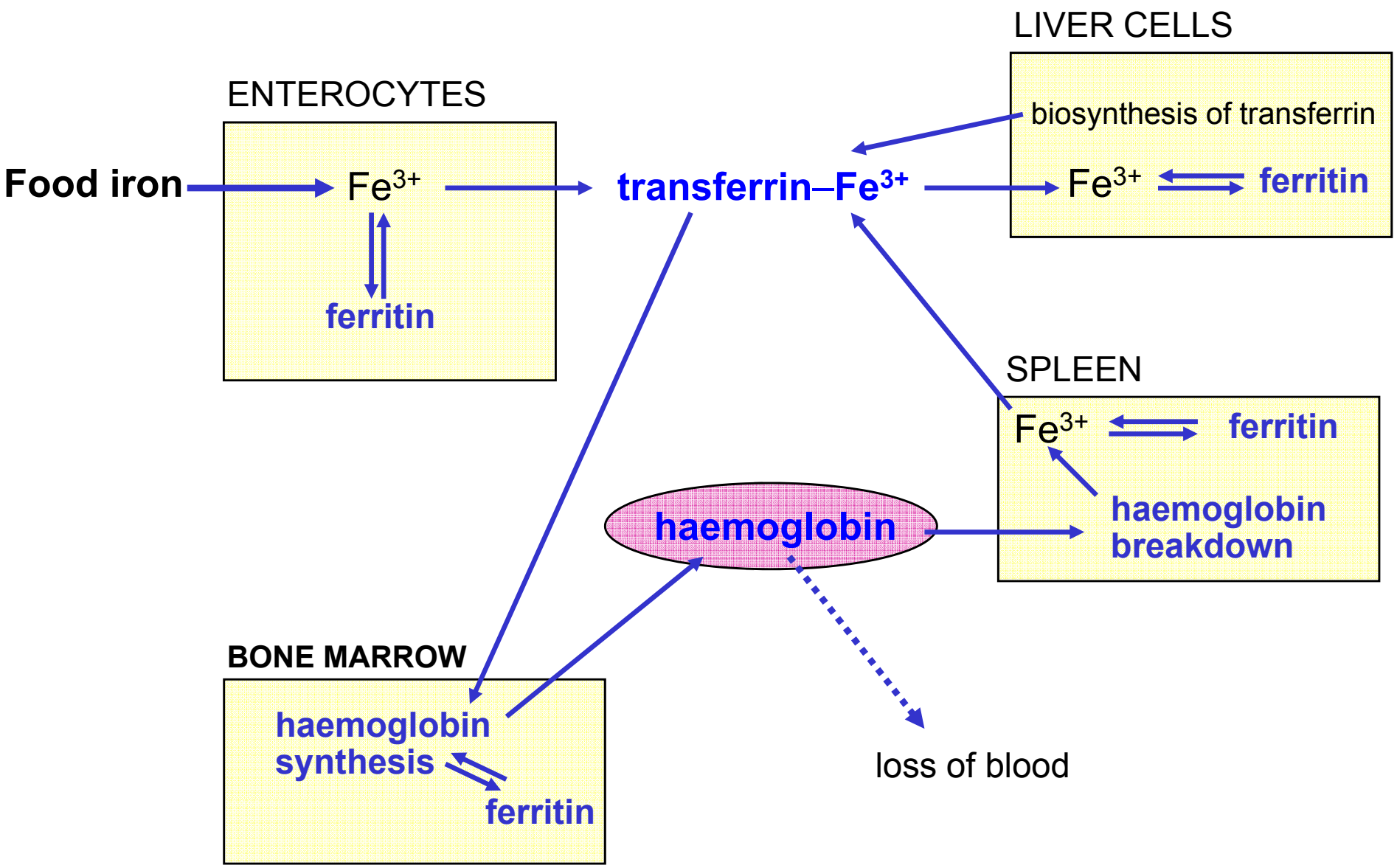
Iron is taken up by the cells

through a **specific receptor-mediated endocytosis**.





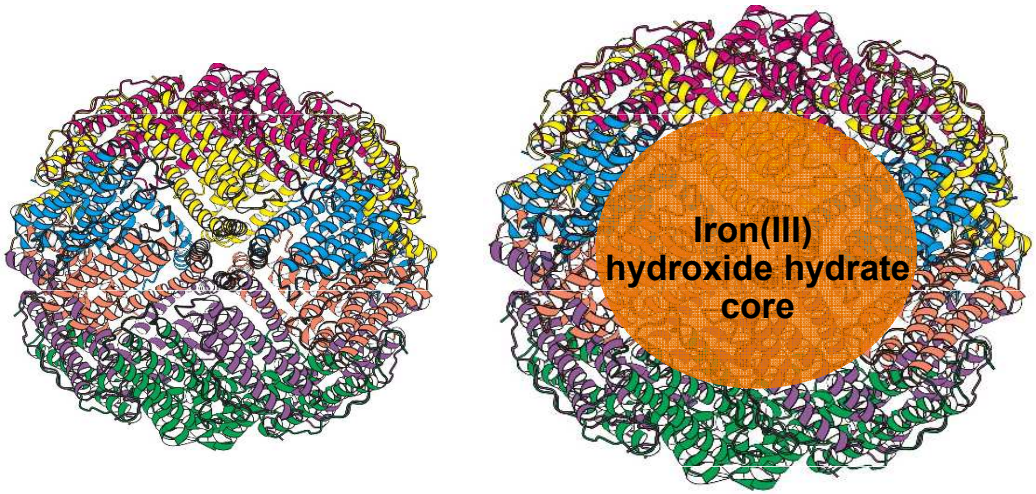
Some receptors are released from the plasmatic membranes. Increase in serum concentration of those **soluble transferrin receptors** is the earliest marker of iron deficiency.


LIVER CELLS
biosynthesis of transferrin
Fe3+ ferritin
ENTEROCYTES
Food iron
Fe3+
ferritin
transferrin–Fe3+
SPLEEN
Fe3+ ferritin
haemoglobin breakdown
haemoglobin
BONE MARROW
haemoglobin synthesis
ferritin
loss of blood

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)

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.

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.