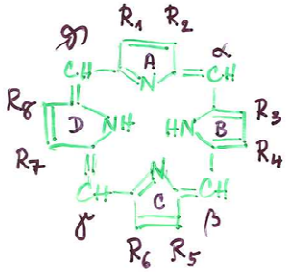
PORPHYRINS and BILE PIGMENTS

JIŘÍ JONÁK

PORPHYRINS – cyclic compounds formed by the linkage of four pyrrole rings through methenyl bridges (=CH–)



They form complexes with metal ions bonded to nitrogen atom of the pyrrole rings:

METALLOPORPHYRINS:

+ Fe^{2+} → iron porphyrins → **HEME**

+ Mg^{2+} → magnesium → **CHLOROPHYL** (photosynthetic pigment of plants)

HEME + PROTEINS = HEMOPROTEINS:

1) HEME + GLOBIN → **MYOGLOBIN** (in muscle cells; monomer 1+1; 4+Cl)
the globin moiety is almost identical with the β subunit of hemoglobinA (153 aa res).
STORAGE OF O_2 in mitochondria

4 HEME$_S$ + 4 GLOBIN$_S$ → **HEMOGLOBIN** (in the blood, tetramer)
the ordinary human (adult) HbA is composed of 2α (141 aa) + 2β (146 aa) globin subunits

only Fe^{2+} ± O_2

$Fe^{2+} \rightarrow Fe^{3+}$: met-Hb, met-Mb } do not bind O_2 !

↑ reductase

BINDS O_2 REVERSIBLY

2) CYTOCHROMES → a, b, c carrier of e^- in the ox-red chain in mt

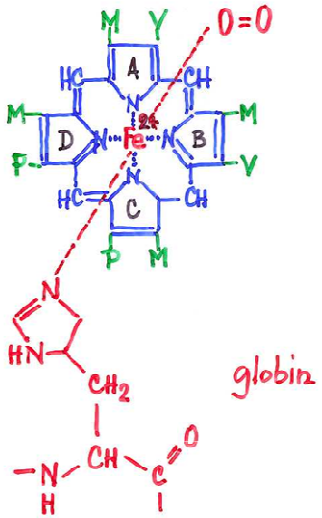
$Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$

P-450 cytochromes (monooxygenases) in the liver – "detoxification" hemoprotein: modification of xenobiotics, steroids etc. by HYDROXYLATION

3) CATALASES: $H_2O_2 \rightarrow H_2O + 1/2\,O_2$ (peroxisomes)

4) TRYPTOPHANEPYROLASE: Trp → N-formylkynurenin (Trp degradation)
= tryptophane-2,3-dioxygenase

Oxygenated HEME



M : $-CH_3$

V : $-CH=CH_2$ (vinyl)

P : $-CH_2-CH_2-COOH$ (propionate)

ferroprotoporfyrin IX (III)

(Hb, Myogl ; Fe^{2+})

HEME BIOSYNTHESIS: ~85% in erythroid precursor cells in the bone marrow
~15% in hepatocytes

CLINIC

Abnormalities, disorders in the HEME biosynthesis pathway ⇒

PORPHYRIAS

diseases not prevalent but important in
dermatology
hepatology - gastroenterology
psychiatry

Abnormalities, disorders in the pathway of production or elimination of HEME DEGRADATION PRODUCTS =
= BILE PIGMENTS ⇒

JAUNDICE, ICTERUS

condition rather frequent; due to elevation of bilirubin in the plasma

1) overproduction of bilirubin
2) failure of bilirubin excretion

It is seen in numerous diseases, ranging from hemolytic anemias to viral hepatitis and cancer of pancreas.

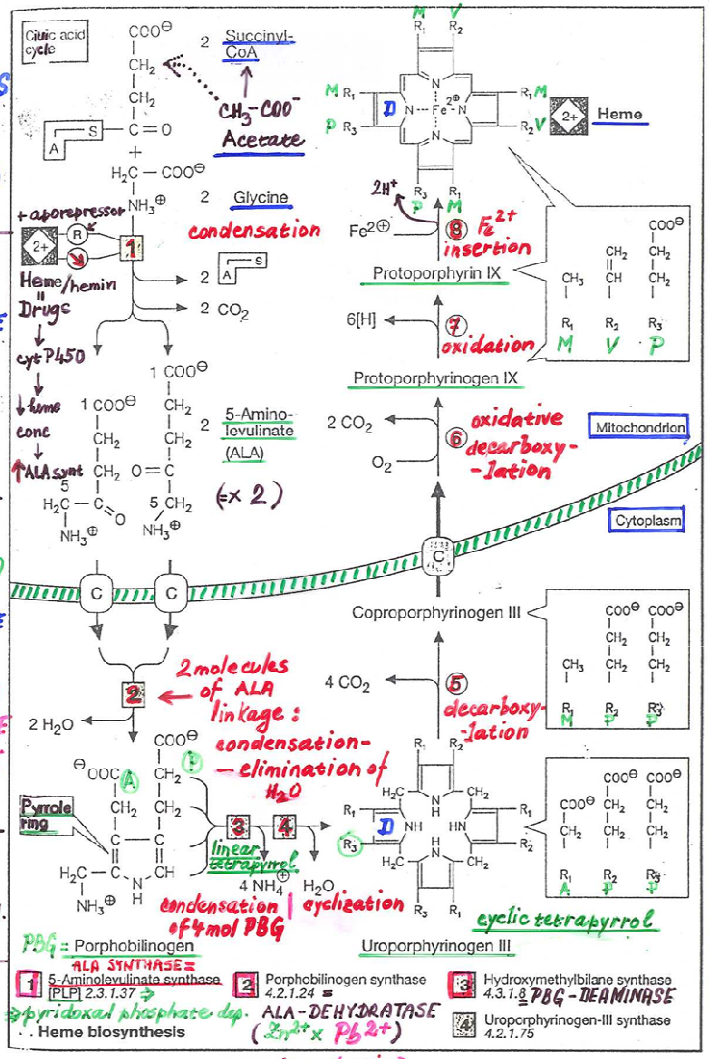
HEME BIOSYNTHESIS

(GLYCINE METABOLISM)

HEME BIOSYNTHESIS REGULATION:

1) IN LIVER: THE MAIN CONTROL TARGET IS: ALA-SYNTHASE [1]
↓↑ HEME
↓
↑↓ ALA-SYNTHASE
cytochrome P450 (detoxification enzyme)

2) IN ERYTHROID CELLS: THE MAIN CONTROL TARGET IS: [8] FERROCHELATASE
PBG-DEAMINASE [3]
↓↑ HEME + IRON-TRANSFER-RING COMPLEX
↓
↓↑ PROTEIN SYNTH. IN RETICULOCYTES



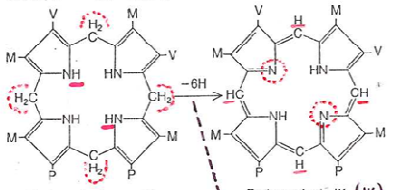
(psychosis)

⑧ FERROCHELATASE (Pb^{2+}!)
~SH

= xenobiotics; steroids

Barbiturates, griseofulvin induce ALA synthase [1]. They do it indirectly by inducing cytochrome P450 (monooxygenase, hydroxylation activity, metabolism of xenobiotics), which uses up heme and thus derepresses ALA-synthase → ATTACKS OF PORPHYRIA!

Fig. 52-25 Formation of protoporphyrin from protoporphyrinogen. In this conversion, a hydrogen atom is removed from each of the four methene bridge carbons, and two hydrogens are removed from pyrrolic nitrogens, as indicated. M = $-CH_3$; P = $-CH_2{\cdot}CH_2{\cdot}COOH$; V = $-CH{=}CH_2$.



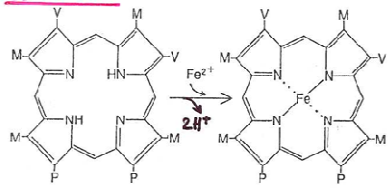
Protoporphyrinogen IX

= reduced protoporphyrin IX

colourless

Protoporphyrin IX (III)

colourfull (red)



Protoporphyrin IX

Heme

Fig. 52-27 Formation of heme from protoporphyrin and iron. M = $-CH_3$; P = $-CH_2{\cdot}CH_2{\cdot}COOH$; V = $-CH{=}CH_2$.

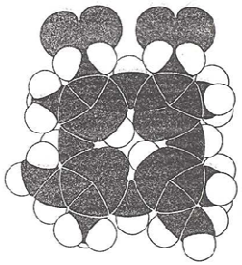


Figure 28-30
Model of protoporphyrin IX, the immediate precursor of heme.

OXIDATION :

1) enzymatic (protoporphyrinogen oxidase)

2) autooxidation (autonomous)

PORPHYRINOGENS + PORPHYRINES = HAVE SIDE CHAINS

PORPHYRINOGENS = COLOURLESS (methylene bridges)

PORPHYRINES = COLOURFULL AND FLUORESCE (400nm) RED ↓ in UV light

PHOTOSENSITISATION: (Photodynamic properties)

1) methenyl (methin) bridges

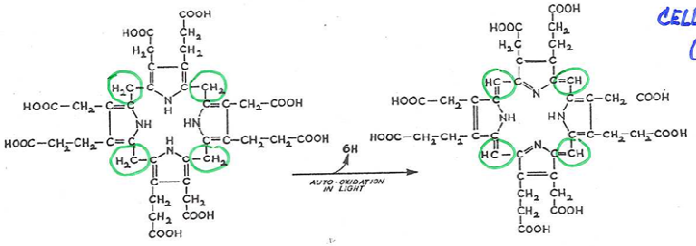
2) conjugated double bonds

CANCER PHOTOTHERAPY

PORPHYRIAS (porphyra = purple)

PRODUCTION OF ROS

CELL DAMAGE (lysosomes)



Uroporphyrinogen III

COLOURLESS

Uroporphyrin III

COLOURFULL

Figure 14–11. Oxidation of uroporphyrinogen to uroporphyrin.

ABNORMALITIES IN HEME BIOSYNTHESIS : PORPHYRIAS

genetic = gene(s) mutation 3
acquired = Pb^{2+}, Hg^{2+}, polyhalogenated hydrocarb.

Accumulation of 1) ALA, PBG: are toxic for abdominal nerves + CNS (abdominal pain ① + neuropsychiatric symptoms ②)
2) Porphyrinogens ox. → Porphyrins* cause photosensitivity ③

⇒ DIFFERENTIAL DIAGNOSIS

Table 52-1 Classification of the Major Human Porphyrias (on the basis of the organs/cells most affected = the organs/cells with a particularly active synthesis of HEME)

Classification	Deficient enzyme	Inheritance	Principal symptomatology	Increased erythrocyte porphyrins*	Excess excretion of ALA, PBG, porphyrins* (DIAGNOSIS)	
					Urine	Stool
Hb : Erythropoietic (bone marrow) ①						
• Congenital erythropoietic porphyria	Uroporphyrinogen III cosynthase	Autosomal recessive	Photosensitivity (hair growth: face, extremities (werewolf legends); skin: ulcers, scars; teeth: brown fluorescence; nocturnal activities)	Uroporphyrin, coproporphyrin	Uroporphyrin,‡ coproporphyrin‡ (red urine)	Coproporphyrin‡
• Erythropoietic protoporphyria	Ferrochelatase	Autosomal dominant	Photosensitivity	Protoporphyrin	Absent	Protoporphyrin
cytochrom P450: Hepatic (liver) ②						
ALA dehydratase deficiency porphyria	ALA dehydratase (90% patient clinically unaffected)	Autosomal recessive	Neurovisceral	Protoporphyrin	ALA	—
• Acute intermittent porphyria	PBG deaminase (latent before puberty)	Autosomal dominant	Neurovisceral (madness, abdominal pain)	Absent	ALA, PBG (urine may become red, port wine)	—
Hereditary coproporphyria	Coproporphyrinogen oxidase	Autosomal dominant	Neurovisceral ± photosensitivity	Absent	ALA, PBG, coproporphyrin	King of England George III ↓ coproporphyrin, protoporphyrin
Variegate porphyria	Protoporphyrinogen oxidase	Autosomal dominant	Neurovisceral ± photosensitivity	Absent	ALA, PBG, coproporphyrin	
MOST COMMON • Porphyria cutanea tarda (middle/late adult life)	Uroporphyrinogen decarboxylase (genetic/acquired)	Variable†	Photosensitivity (mild / severe) + liver disease	Absent	Uroporphyrin, 7-carboxylate porphyrin	Isocoproporphyrin
Hepatoerythropoietic porphyria (HEP)	Uroporphyrinogen decarboxylase (= homozygous deficiency)	Autosomal recessive	Photosensitivity ± neurovisceral	Protoporphyrin	Uroporphyrin, 7-carboxylate porphyrin	Isocoproporphyrin

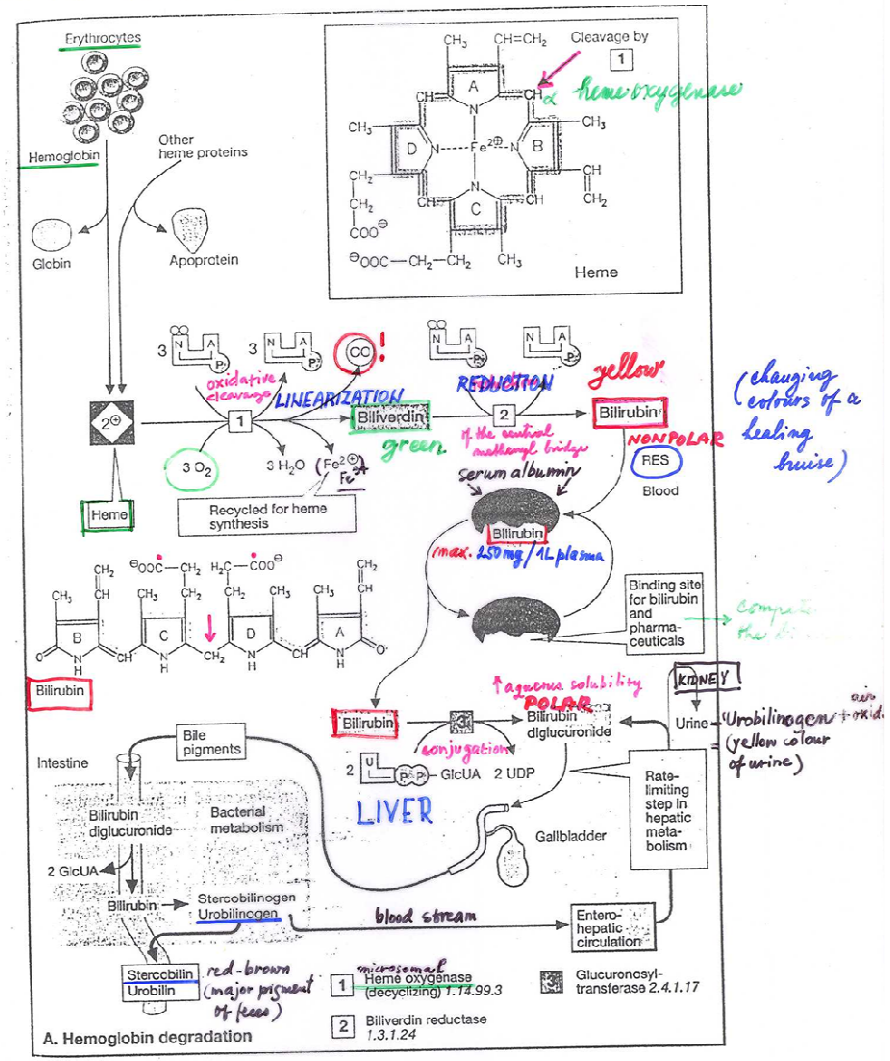
*Only major diagnostic findings are listed.
†Autosomal dominant inheritance has been documented in some families but not in others.
‡Type I isomers.
NOTE: ALA = δ-aminolevulinic acid; PBG = porphobilinogen.

* Photosensitivity: a reaction to visible light of abt 400nm: porphyrin excitation → porphyrins* react with molecular oxygen → oxygen radicals (ROS) → lysosome damage → release of degradative enzymes → skin damage (incl. scarring). × β carotene (antioxidant)

HEMOGLOBIN DEGRADATION

~ 6g / day Hb (from ~200 milion of erythrocytes) → globin, iron, porphyrin

LIVER, BONE MARROW, SPLEEN (macrophages): 1g Hb → 35mg bilirubin → (250–350 mg/day) in total



A. Hemoglobin degradation

(changing colors of a healing bruise)

HEME DEGRADATION

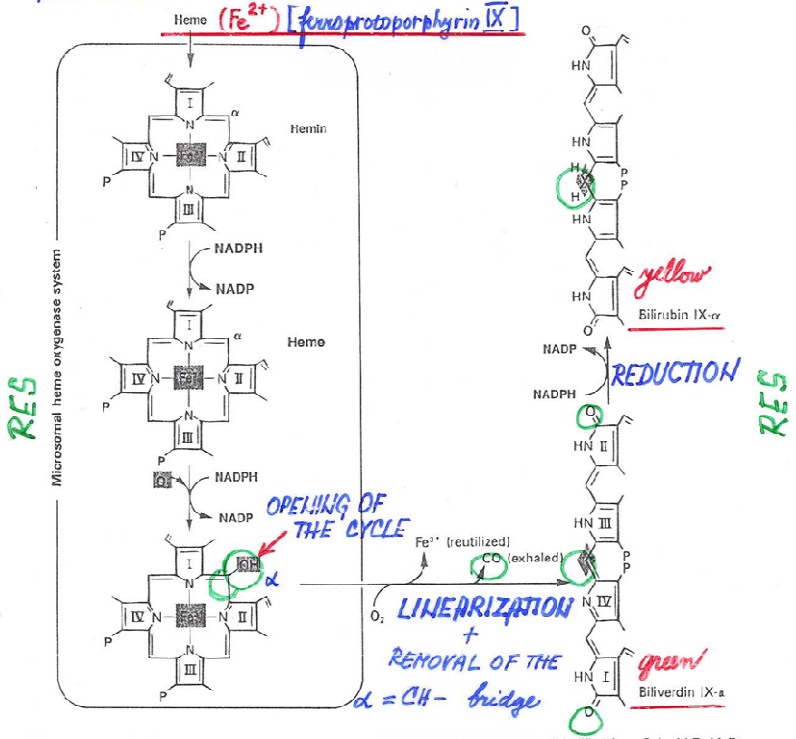


Figure 34–12. Schematic representation of the microsomal heme oxygenase system. (Modified from Schmid R, McDonough AF in: *The Porphyrins*. Dolphin D [editor]. Academic Press, 1978.)

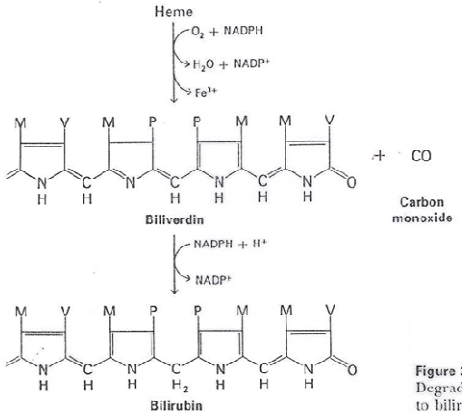


Figure 28-31
Degradation of heme to bilirubin.

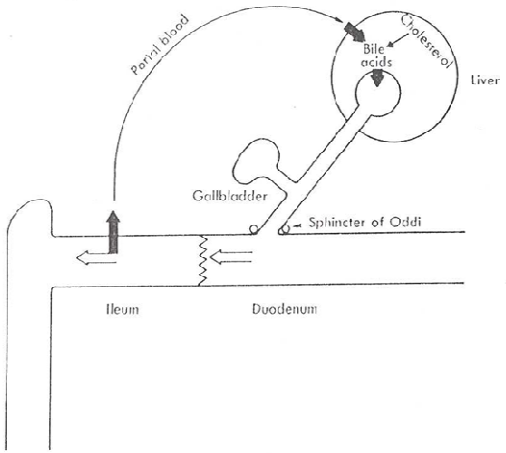


Fig. 10-2. Enterohepatic circulation of bile acids. These acids are actively secreted by the liver. Once in the intestine they participate in the digestion and absorption of lipids. As they are propelled toward the distal small bowel, some of the "primary" acids are altered, becoming "secondary" acids. These, along with the "primary" ones, are absorbed actively from the terminal ileum. However, a minor fraction of both types is not absorbed but is propelled into the colon. The absorbed bile acids are transported via the portal circulation to the liver, where they are extracted actively from the blood (almost 100%) and resecreted. Synthesis of new "primary" acids from cholesterol occurs at a rate to compensate for the acids lost from the bowel. *Solid arrows* denote active absorption, secretion, and synthesis; *open arrows*, propulsion of contents by contractions of the intestine.

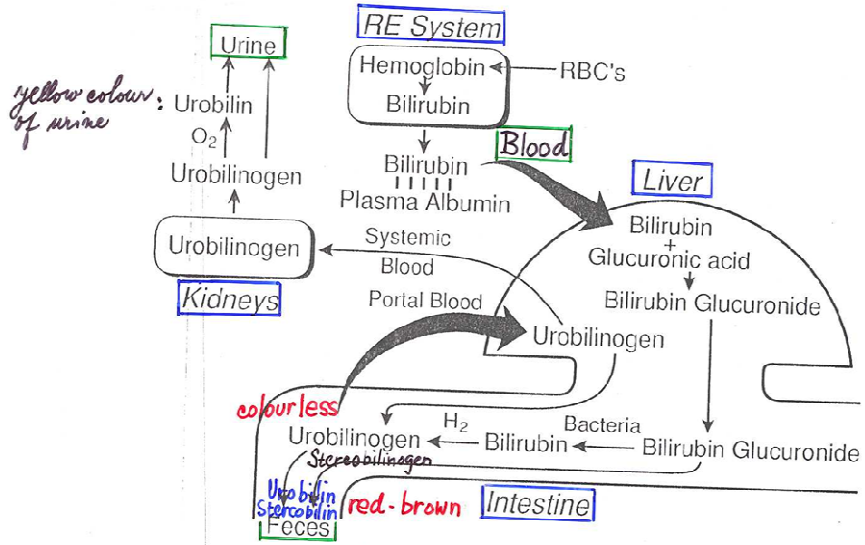


Fig. 10-3. Excretion of bile pigments. Bilirubin is produced by cells of the reticuloendothelial system from aged red blood cells. The unconjugated pigment is then carried, tightly bound to plasma albumin, to the liver. There it is actively taken up, conjugated with glucuronic acid, and secreted into the bile. The water-soluble conjugates are propelled along the intestine. In the distal small bowel and colon a portion of the conjugated pigment is acted upon by bacteria and becomes unconjugated bilirubin and other pigments. Some of these pigments are absorbed passively into the blood and either are returned to the liver and resecreted, or pass through the liver and are excreted by the kidneys. Most, however, pass through the colon and are excreted. *Bold arrows* indicate active absorption.

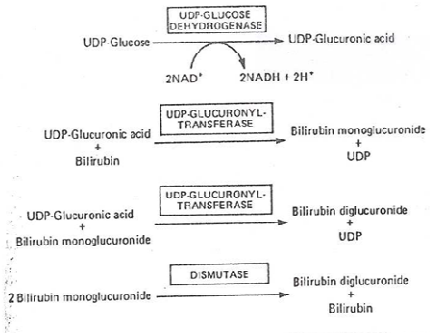
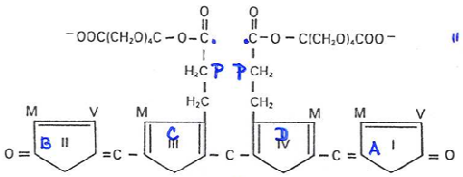
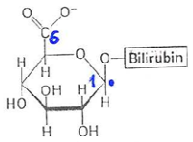


Figure 34–14. Conjugation of bilirubin with glucuronic acid. The glucuronate donor, UDP-glucuronic acid, is formed from UDP-glucose as depicted. At least two isoforms of glucuronosyltransferase that use bilirubin as a substrate are present in human liver.



CONJUGATED "direct-reacting" BILIRUBIN

Figure 34–13. Structure of bilirubin diglucuronide (conjugated, "direct-reacting" bilirubin). Glucuronic acid is attached via ester linkage to the two propionic acid groups of bilirubin to form an acylglucuronide.



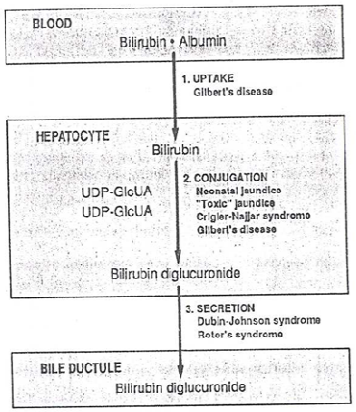


Figure 34–15. Diagrammatic representation of the three major processes (uptake, conjugation, and secretion) involved in the transfer of bilirubin from blood to bile. Certain intracellular proteins of hepatocytes, such as ligandin and Y protein, are involved in the uptake of bilirubin by these cells. The process affected in a number of conditions causing jaundice is also shown.

BILIRUBIN = conjugated*

1. "direct" - in the Van den Bergh assay with the Ehrlich diazo-reagent

2. "indirect" - free, unconjugated, insoluble in water. Requires addition (caffeine, sodium benzoate) of MeOH for the solubilization and detection by the above reagents.

CROSSES THE BLOOD-BRAIN BARRIER → encephalopathy - KERNICTERUS

HYPERBILIRUBINEMIA and ICTERUS

Serum bilirubin total > 17 μmol/L (10 mg/L) : HYPERBILIRUBINEMIA
> 35 μmol/L (20 mg/L) : JAUNDICE, ICTERUS → yellow colour of the eye white and the skin

THREE MAIN TYPES:

- **unconjugated: prehepatal (hemolytic) icterus**
  - increased bilirubin production, e.g. hemolytic anemia
  - decreased uptake and/or conjugation in the liver, e.g. jaundice of the newborn, some inborn errors such as Gilbert's disease, Criegler-Najjar's syndrome
- **conjugated: posthepatal (obstructive) icterus**
  - biliary obstruction, e.g. due to cholelithiasis (stones in gall bladder or biliary ducts) or tumours of biliary tract or pancreas
  - impairment of the secretion of conjugated bilirubin, e.g. some inborn errors such as Dubin-Johnson's and Rotor's syndromes
- **mixed: hepatocellular (hepatal) icterus**
  - e.g. viral hepatitis, toxic liver damage (chloroform, CCl_4, cirhosis, Am. phaloides)

Table 1: Characteristic findings of the examination of bile pigments in serum and urine in different kinds of icterus:

Type of icterus	*Findings in serum*	*Bilirubin in urine*	*Urobilinogen in urine*	*Colour of urine*	*Colour of stool*
Prehepatal hemolytic	Indirect bilirubin increased	Negative	Positive (higher than physiologic)	Normal light yellow	Dark
Posthepatal obstructive	Direct bilirubin increased[2]	Positive* conjug.	Negative (in complete obstruction)	Dark – brown	Destained (greyish)
Hepatocellular hepatal	Both direct and indirect bilirubin increased	Positive* conjug.	Positive (higher than physiologic)	Dark	Light
Normal physiologic	Indirect bilirubin to 12 μmol/L Direct bil. to 5 μmol/L Total bil.: ≤ 17 μmol/L	Negative	0-7 μmol / 24h	Light yellow	Brown

[2] Longer lasting posthepatal icterus secondarily dameges function of hepatocytes. The laboratory findings then consist of combination of posthepatal and hepatocellular icterus.

* Serum levels of conjugated bilirubin exceed the renal threshold for bilirubin (30-34 μmol/L, ~20 mg/L)

SUBICTERUS : yellow colour brought about by bilirubin is first visible only on mucosal membranes and on the parts of sclera covered by eyelids → (bilirubin is protected here from the degradation by light)

Table 29-1 Features of jaundice

	Usual significance
Abrupt onset	Viral hepatitis or biliary obstruction
Intermittent, mild	Hemolysis, Gilbert's or hepatocellular disease
Very pronounced	Cholestasis
Insidious onset	Chronic hepatocellular disease or medical cholestatic disease

Table 29-2 Associated symptoms

	Usual Significance
Pruritus	Cholestasis
Dark urine	Bilirubinuria
Pale stools	Cholestasis, carcinoma of pancreas
Abdominal pain	Choledocholithiasis (intermittent, severe)
Fever	Viral hepatitis, cholangitis (episodic)
Anorexia, nausea, vomiting	Cholangitis, viral hepatitis
Considerable weight loss	Carcinoma of pancreas, ampulla, bile ducts

TOTAL BILIRUBIN > 10mg/l (17μM) = HYPERBILIRUBINEMIA in blood

~> 25mg/l (43μM) = JAUNDICE (ICTERUS)
~> 20 mg/l (35μM)

yellow colours of the eye white and the skin

SUBICTERUS

yellow colour first visible on parts of sclera covered by eyelids (protected from light); mucoses

BILIRUBIN OVERPRODUCTION

- Extravascular red blood cell destruction
  - Trauma, hematoma resorption
  - Burns
  - Pulmonary infarction
- Intravascular hemolysis
  - Stored or mismatched blood transfusion
  - Defective heart valves
  - Drugs, e.g., α-methyldopa
  - Bacterial infections, e.g., *Mycoplasma pneumoniae*
  - Wilson's disease
  - Red blood cell defects, e.g., sickle cell disease

Table 29-3 Investigation of isolated mild jaundice

	Anticipated results	
	Gilbert's disease	Hemolysis
Unconjugated bilirubin	Elevated	Elevated
Conjugated	Normal	Elevated
Hb Hct	Normal	Normal or low
Reticulocyte count	Normal	Elevated
Blood Smear	Normal	Abnormal
Urine for bilirubin	Negative	Negative
Transaminases, alkaline phosphatase	Normal	Normal

1) increased production of bilirubin
2) decreased (degradation) in the liver (uptake, conjugating, excretion)
3) obstruction of liver efferent ducts

→ damage (HEPATITIS)
→ immature (KERNICTERUS in newborns) : > 250mg bilirubin/l
→ PHYSIOLOGIC JAUNDICE

Table 34–3. Laboratory results in normal patients and patients with three different causes of jaundice.

Condition	Serum Bilirubin	Urine Urobilinogen	Urine Bilirubin (direct)	Fecal Urobilinogen
Normal	Direct: 0.1–0.4 mg/dL Indirect: 0.2–0.7 mg/dL	0–4 mg/24 h	Absent	40–280 mg/24 h
Hemolytic anemia	Elevation of indirect	Increased	Absent	Increased
Hepatitis	Elevations of direct and indirect	Decreased	Present if microobstruction occurs	Decreased
Obstructive jaundice[1]	Elevation of direct	Absent	Present direct	Trace to absent

[1]The commonest causes of obstructive (posthepatic) jaundice are cancer of the head of the pancreas and a gallstone lodged in the common bile duct. The presence of bilirubin in the urine is sometimes referred to as choluria; hence, hepatitis and obstruction of the common bile duct cause choluric jaundice, whereas the jaundice of hemolytic anemia is referred to as acholuric.

BILIRUBIN IN SERUM

1) unconjugated

hydrophobic, nonpolar
insoluble in water
easily passes through biomembranes → cells → toxic if > ~350 μmol/L

⇒ bound noncovalently to albumin (max. cap. 250 mg b./L plasma)
→ transported to liver for conjugation and excretion into bile;
due to the binding with albumin reacts slowly with diazo reagents
= slow Van den Berg reaction with diazotized sulfanilic acid (Ehrlich reagent): "indirect bilirubin" – indirect Van den Berg reaction
→ for COLOR DEVELOPMENT the addition of an accelerator (methanol, urea; sodium benzoate, caffeine) is required = to break down bonds with albumin

Hemolytic disease of newborns (HDN) (– from 2nd pregnancy; "abortus")
mother Rh negative (d), foetus Rh positive (D):
foetal erythrocytes sensitize mother → produces IgG antibodies
→ pass through placenta and destruct Rh positive erythrocytes of foetus → hemolytic anemia.
In severe cases: hydrops foetalis – death
In less severe cases: jaundice → if the concentration of unconjugated bilirubin exceeds the capacity of albumin to bind it and the capacity of immature liver to conjugate it, then this b. passes through the hemato-encephalic barrier into the newborn brain and causes kernicterus →
→ brain damage
→ mental retardation

→ Monitoring of titre of anti-D antibodies in the blood of mother in the course of pregnancy. Monitoring of bilirubin level in amniotic fluid by direct spectrometry at 450 nm.

THERAPY:
1) blue light – phototherapy
2) intrauterine transfusion
3) premature delivery

BILIRUBIN IN SERUM (cont.)

2) **conjugated** = with glucuronic acid C1 (via -COOH of propionyl group in bilirubin – OH ⇒ acyl-glucuronide)
soluble in water
polar, hydrophilic
→ reacts directly and rapidly (without accelerator) with diazotized sulfanilic acid (Ehrlich r.) = quick Van den Berg r. =
= **"direct bilirubin"** - direct Van den Berg reaction

+ Dr - bilirubin = conjugated b. covalently bound to albumin (or other serum protein) by a peptide bond

(bilirubin-(-CH_2-CH_2-COOH + NH_2-$(CH_2)_4$-CH(CO-NH)} albumin)

propionyl- Lysin

3) **total**: unconjugated + conjugated + Dr - bilirubin

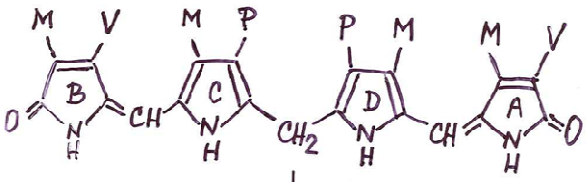
TOTAL BILIRUBIN ESTIMATION
IN SERUM/BLOOD

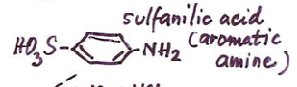
① COLORIMETRY

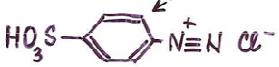
Albumin – bilirubin + ACCELERATOR

Bilirubin mono-, diglucuronate

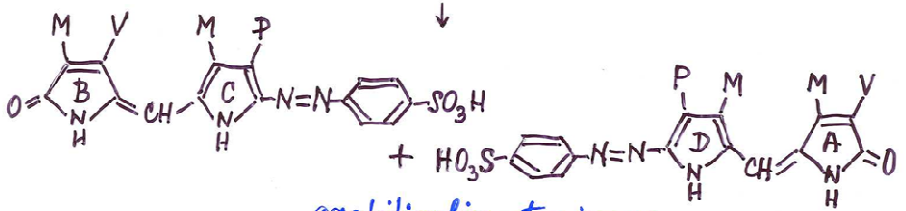
D bilirubin







diazotized salt of the sulfanilic acid = Ehrlich r. (diazonium chloride = arene = $Ar_1-N\equiv N$)



azobilirubin – two isomers (azo dye): blue – red (pH)

ACCELERATOR : caffeine + benzoate Na^+
pH ≫ 7 (alkaline tartrate) ⇒ BLUE COLOR
= JENDRASSIK-GROF MODIFICATION
photometry at 546nm

② DIRECT SPECTROMETRY
at 454nm (450nm)
amniotic fluid, CSF, blood of newborns

③ TRANSCUTANEOUS ICTEROMETRY
(neonatology)

BILIRUBIN IN SERUM - estimation

Bilirubin is strongly photosensitive, therefore, samples must be **kept in the dark** and processed as soon as possible.

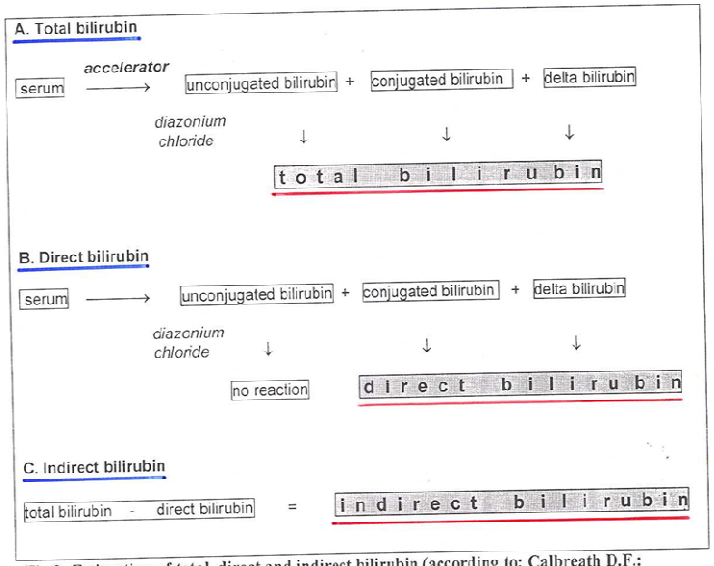


Fig.2: Estimation of total, direct and indirect bilirubin (according to: Calbreath D.F.: Clinical Chemistry. A Fundamentals Textbook. W.B. Saunders Company 1992, str. 228)

Reference values= physiological values :

fS-Total bilirubin: up to 17 µmol/L (10 mg/L)

fS-Direct bilirubin: up to 5 µmol/L (3 mg/L)

fS Indirect bilirubin : up to 12 µmol/L (7 mg/L)

ESTIMATION OF BILIRUBIN IN URINE

! PROTECTION FROM DIRECT SUNLIGHT

PHYSIOLOGICALLY: **NO BILIRUBIN**

PATHOLOGY: conjugated bilirubin ONLY

level of conj. bilirubin in serum exceeds the renal threshold = 30-34 µmol/L (~20mg/L)

URINE becomes DARK BROWN

ESTIMATION: ① DIAGNOSTIC STRIPS (azo-coupling ^.)

PINK or RED COLOUR DYE

+UROBILINOGEN → ORANGE

X ASCORBIC ACID !

② oxidation with HNO_3 → GREEN BILIVERDIN

③ —"— with IODINE → BLUE BILICYANIN

UROBILINOGEN IN URINE

ORIGIN ⇒ bacterial action on conjugated bilirubin in the intestine: deglucuronisation + reduction

+ absorption from the intestine into blood → liver → kidneys

NORMAL: ~ 0 – 17 μmol/L (0 – 10 mg/L/24 hr)

INCREASED AMOUNT

~ overload of the liver funct. capacity

a) prehepatal icterus (hemolytic)

b) hepatocellular icterus

ABSENCE OF UROBILINOGEN in urine:

1) complete blockade of biliary ducts
   the stool is whitish – grey
   (absence of pigments + undigested fat)
2) very severe liver damage
3) absence of intestinal microflora
   a) physiologically in newborns
      bilirubin not modified and/or → biliverdin
      - green stool
   b) antibiotic therapy
      — " —

DETECTION: (diano r.)

diagnostic strips

→ PINK or RED colour

WEAK PINK ~ physiologic excretion

+ bilirubin → YELLOW e. 1 min → BLUE, GREEN