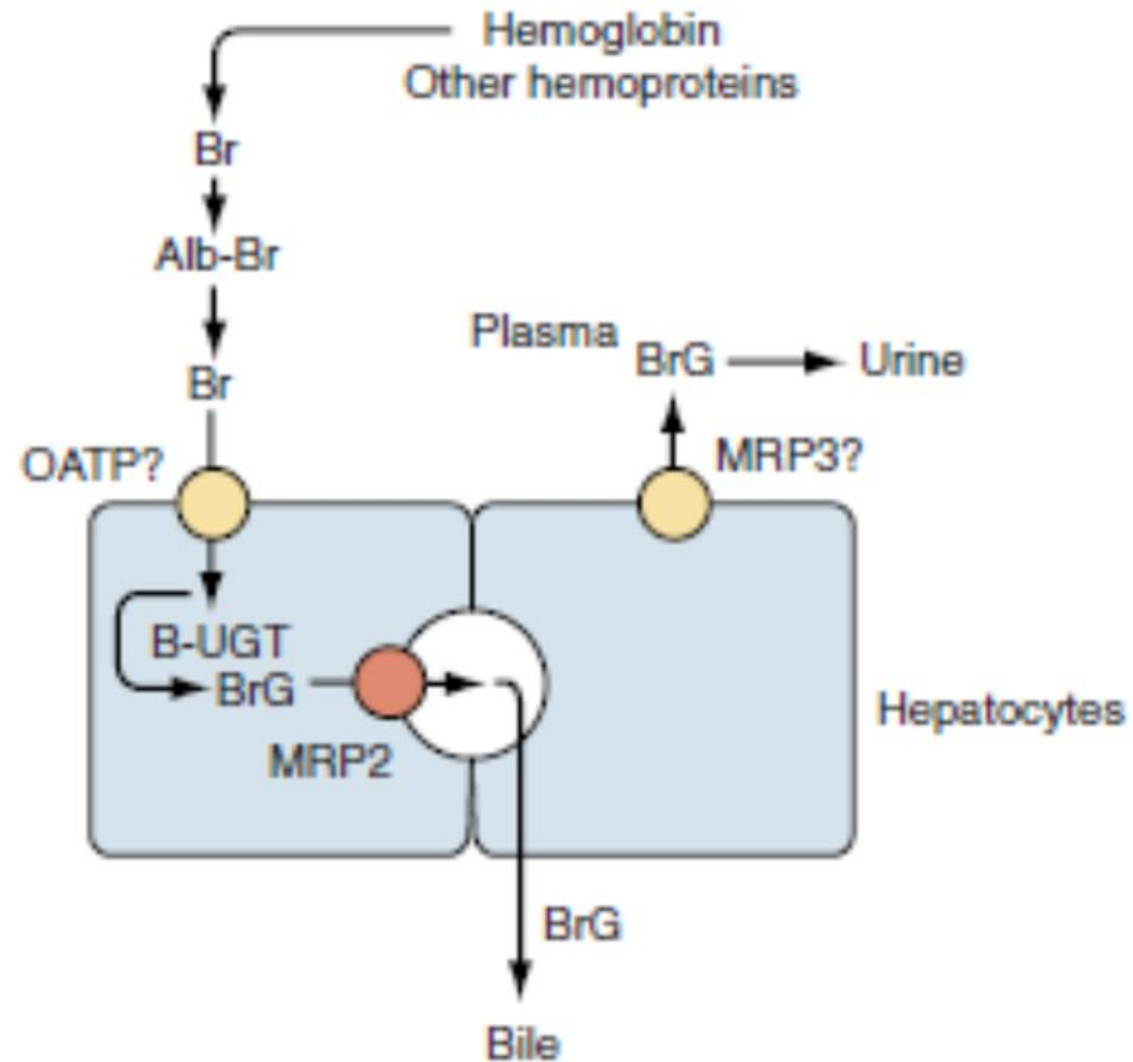


# Bilirubin metabolism, jaundice

Practical – experimentally induced obstructive jaundice

# Overview of bilirubin metabolism

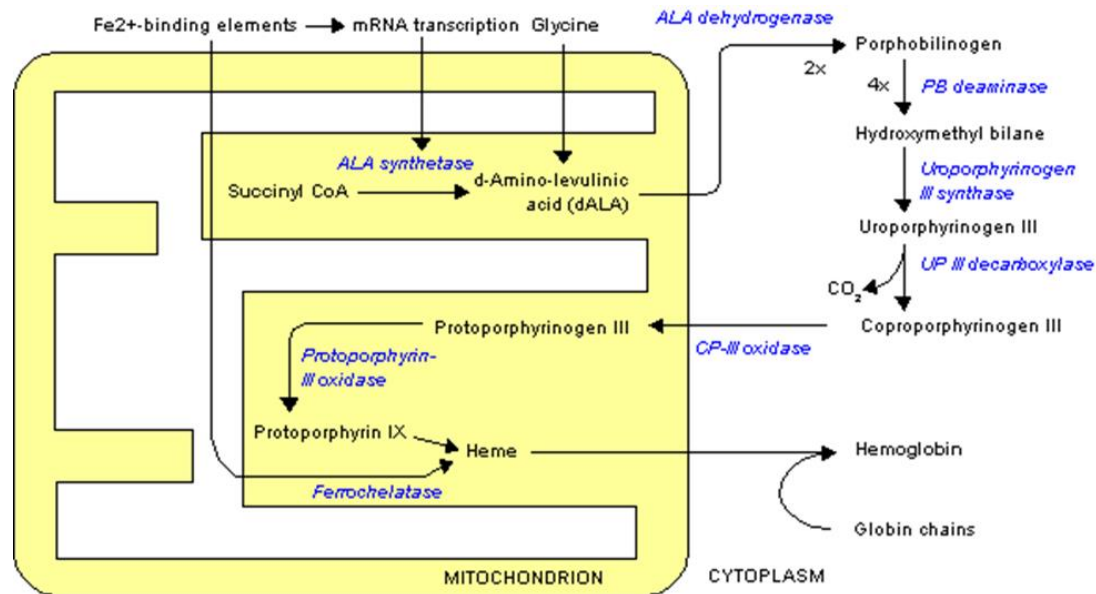


# Historical aspects of bilirubin metabolism

- 1916 – van den Bergh
    - 2 different types of bilirubin
  - 1933 – Hans Fischer
    - bilirubin structure
  - 1956 – Edmund Talafant
    - bilirubin transformation in the liver
  - 1968 – Tenhunen
    - description of heme oxygenase
  - 1987 – Stocker
    - antioxidant properties of bilirubin
- ikterus
    - a yellow bird (Greek)
      - Baltimore oriole (*Icterus galbula*)



# Heme synthesis



- localization

- bone marrow – hemoglobin production - erythropoiesis
- liver - cytochrome P450 – metabolism of drugs and toxins

- occurs partly in mitochondria and partly in the cytosol – 8 reactions

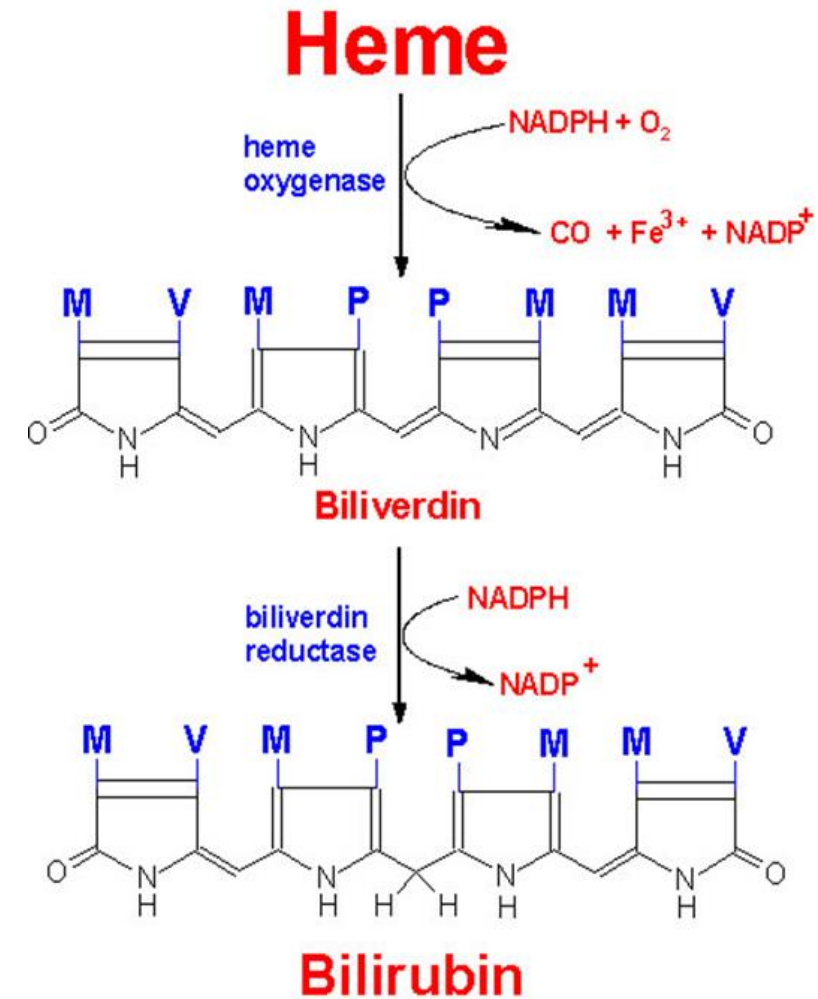
- initial substances are succinyl-CoA (from citric acid cycle ) and glycine
- rate limiting enzyme ALA syntetase

- lead poisoning inhibits 3 enzymes of heme synthesis and leads to

- insufficient heme synthesis and anemia
- accumulation of byproducts
  - toxic

# Bilirubin metabolism

- bilirubin is the final product of heme degradation
  - 85% from hemoglobin
  - 15% from myoglobin, cytochrome and premature destruction of RBC
- in reticuloendothelial cells
  - spleen, liver, bone marrow
- enzyme heme oxygenase
  - induced by raised heme level
- biliverdin reductase
  - cytosolic
- this type of bilirubin (=unconjugated, indirect)
  - insoluble in water
- in plasma bilirubin binds to albumin

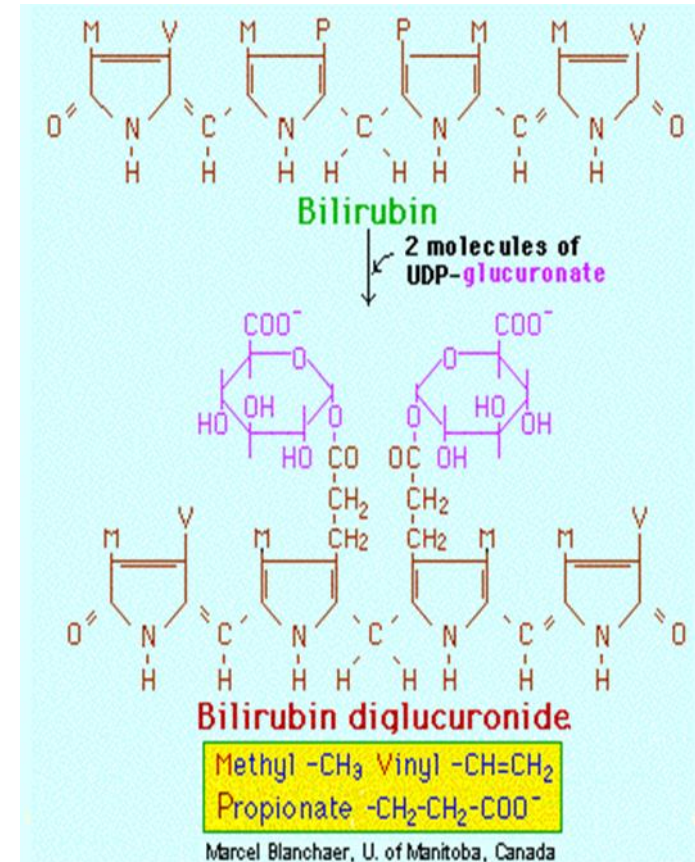


# Bilirubin metabolism

- bilirubin is carried bound to albumin
  - competition with certain medicaments and fatty acids
- albumin-free anion fraction
  - diffusion into tissues - injury
- in physiologic conditions low bilirubin plasmatic concentration
  - can be replaced by some substances (e.g. salicylates)
    - important in nursing
- in the liver
  - free bilirubin is released from the albumin and moves into hepatocytes
    - process with great capacity
- in hepatocyte bilirubin undergoes conjugation
  - conversion into soluble conjugate which can be secreted into the bile

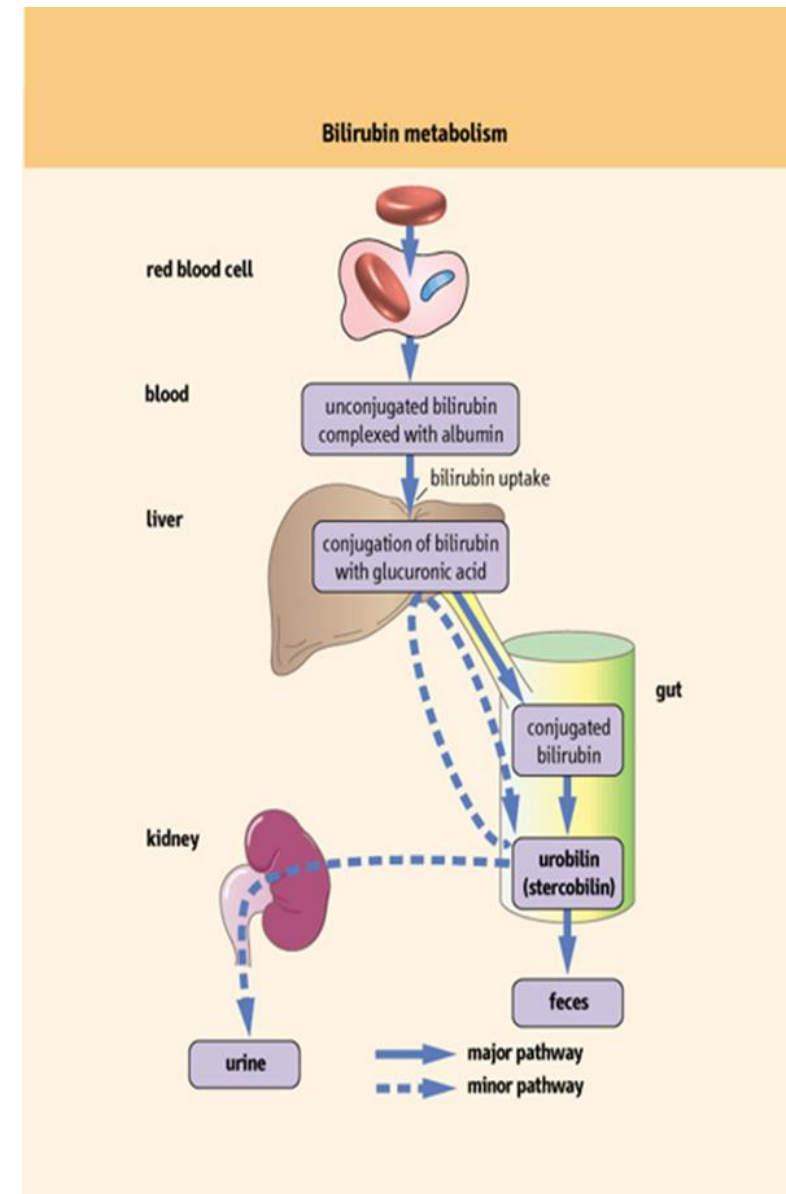
# Bilirubin metabolism in the liver

- in hepatocytes
  - proteins Y and Z
- enzyme uridine diphosphate-glucuronyl-transferase (UGT1A1)
  - family of conjugating enzymes
    - steroid hormones, drugs
- conjugation of bilirubin with glucuronic acid in endoplasmic reticulum generates mono- and diglucuronides
  - **conjugated bilirubin**
- specific transporter (cMOAT=MRP2) for release of conjugated bilirubin from hepatocyte
  - rate-limiting step
- conjugated bilirubin is secreted via the bile to the small intestine
  - highly efficient process



# Bilirubin metabolism

- bilirubin passes through the bile ducts into the small intestine
  - deconjugation by bacterial enzyme  $\beta$ -glucuronidase
    - production of urobilinogen (colourless)
  - urobilinogen is
    - re-absorbed (enterohepatic circulation)
    - or degraded into coloured urobilins and excreted in the feces
  - most of the absorbed urobilinogen is returned to the liver to be re-excreted into the bile
    - small amount excreted in the urine



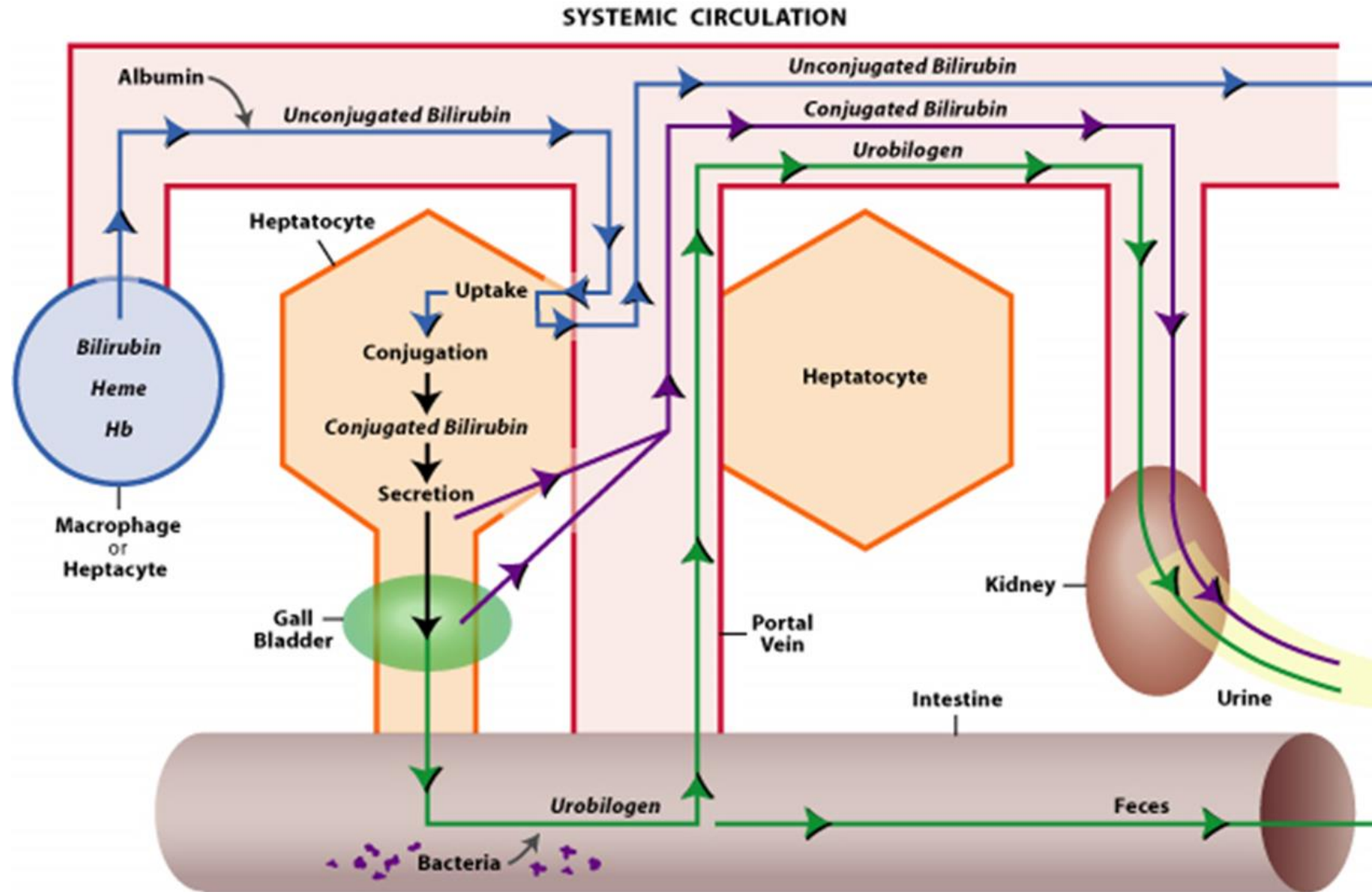
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# Alternative pathways of bilirubin metabolism

- cytochrome P-448
  - low expression in the liver
  - increased in hyperbilirubinemia
  - can be induced
    - indol-3-carbinol
- direct secretion
  - unconjugated bilirubin
  - through the gut wall
  - passive diffusion
- enzyme bilirubinoxidase
  - low importance in humans
  - substitution?

# Summary



# Icterus/jaundice

- yellow discoloration of skin, mucose membranes and sclera
  - occurs when bilirubin concentration  $> 30 - 50 \text{ umol/l}$
  - deposition of bilirubin in tissues rich in elastin
- normal plasma level  $< 17 \text{ umol/l}$
- subicterus – small increase (35 - 40 umol/l)
  - low-grade icteric condition, does not have to be obvious
- hyperbilirubinemia
  - increased plasmatic bilirubin level
- objective symptom
  - alert to presence of other problem
- marked icterus in conjugated hyperbilirubinemia

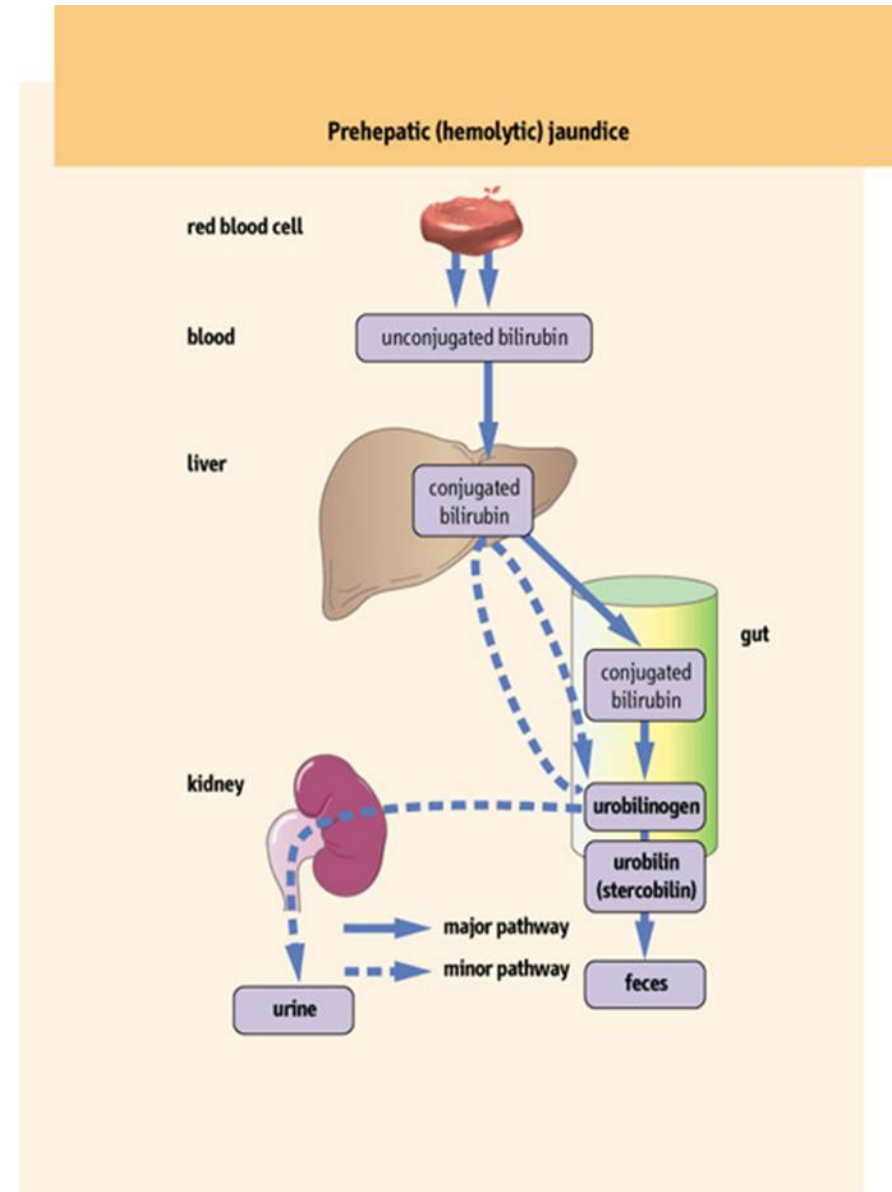


## The causes of jaundice

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 $\alpha_1$ 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 biliary cirrhosis cholangitis	common uncommon common
	extrahepatic bile ducts	gall stones pancreatic tumor cholangiocarcinoma	very common uncommon rare

# Prehepatic jaundice

- excessive load of bilirubin
- increased supply
  - increased amount in the gut
- mostly due to excessive destruction of red blood cells
- mild jaundice
- exceeding of conjugating capacity
  - unconjugated bilirubin is elevated
- urobilinogen in urine
- bilirubin is absent in urine
- hypercholic stool



# (Intra)hepatic jaundice

- caused by disorders that affect liver
  - disturbed ability of the liver to remove bilirubin from the blood or conjugate it
  - disintegration of hepatocytes and release of bilirubin into the circulation
- conjugated and/or unconjugated levels may be elevated
  - depends on type of disorder
- urobilinogen and bilirubin are both in urine
- liver damage
  - hepatitis, cirrhosis, drugs, chemicals
- hereditary hyperbilirubinemias
- damage of liver architecture
  - communication between liver and bile capillaries
- hypocholeic stool
  - lower production of bilirubin

# Hereditary disorders of bilirubin metabolism

PARAMETER	Syndrome				
	GILBERT'S	CRIGLER-NAJJAR TYPE I	CRIGLER-NAJJAR TYPE II	DUBIN-JOHNSON	ROTOR'S
Incidence	6%-12%	Very rare	Uncommon	Uncommon	Rare
Gene affected	UGT1A1	UGT1A1	UGT1A1	MRP2	Unknown
Metabolic defect	↓Bilirubin conjugation	No bilirubin conjugation	↓↓Bilirubin conjugation	Impaired canalicular export of conjugated bilirubin	Impaired canalicular export of conjugated bilirubin
Plasma bilirubin (mg/dL)	≤3 in absence of fasting or hemolysis, almost all unconjugated	Usually >20 (range, 17-50), all unconjugated	Usually <20 (range, 6-45), almost all unconjugated	Usually <7, about half conjugated	Usually <7, about half conjugated
Liver histology	Usually normal, occasional ↑lipofuscin	Normal	Normal	Coarse pigment in centrilobular hepatocytes	Normal
Other distinguishing features	↓Bilirubin concentration with phenobarbital	No response to phenobarbital	↓Bilirubin concentration with phenobarbital	↑Bilirubin concentration with estrogens; ↑↑urinary coproporphyrin I/III ratio; slow BSP elimination kinetics with secondary rise	Mild ↑urinary coproporphyrin I/III ratio; very slow BSP* elimination kinetics without secondary rise
Prognosis	Normal	Death in infancy if untreated	Usually normal	Normal	Normal
Treatment	None	Phototherapy as a bridge to liver transplantation	Phenobarbital for ↑↑bilirubin concentration	Avoid estrogens	None available

# Gilbert's syndrome

- genetically determined disorder
  - autosomal recessive
  - mutations in the promoter of UDPGT gene
    - decrease of enzyme activity by 70 %
      - responds to phenobarbital
- common
  - 5 – 10 % prevalence
  - more common in males
- benign
  - lifelong hyperbilirubinemia
    - Up to 100  $\mu\text{mol/l}$
- without liver disease or hemolysis
- manifestation during puberty
- icterus commonly insignificant
  - may worsen with
    - stress
    - fasting
    - sleep deprivation
    - dehydration
    - illness (flu)
- requires no treatment

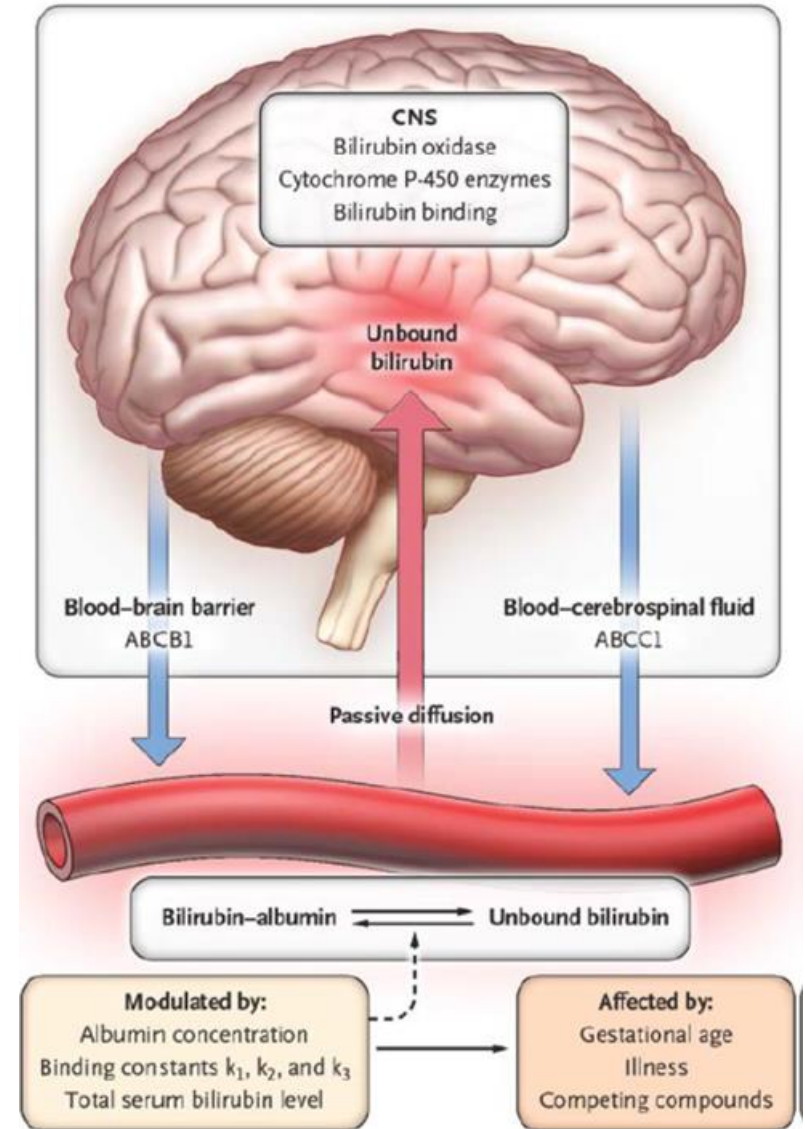


# Posthepatic (cholestatic) jaundice

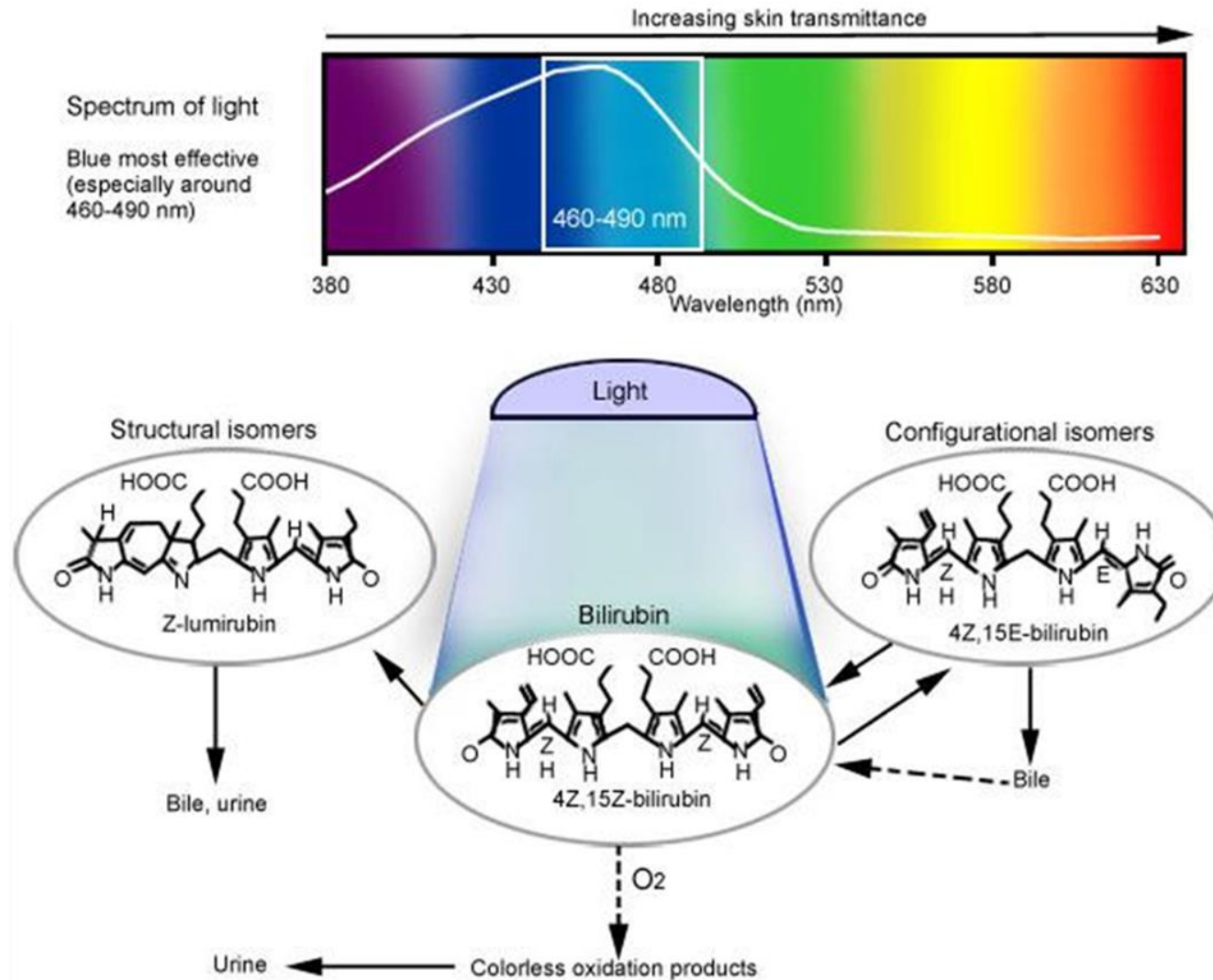
- bile flow is obstructed between the liver and the intestine
- cholestasis
  - intrahepatic – failure inside the liver
  - extrahepatic – obstruction of the large bile ducts
    - strictures of the bile ducts, gallstones, tumors of the bile duct
- increased conjugated bilirubin
- accumulation of bile pigment in the liver common to all types of cholestasis
- if the obstruction is complete
  - only bilirubin is found in urine
- acholic stool, urobilinogen is absent in urine

# Neonatal hyperbilirubinemia

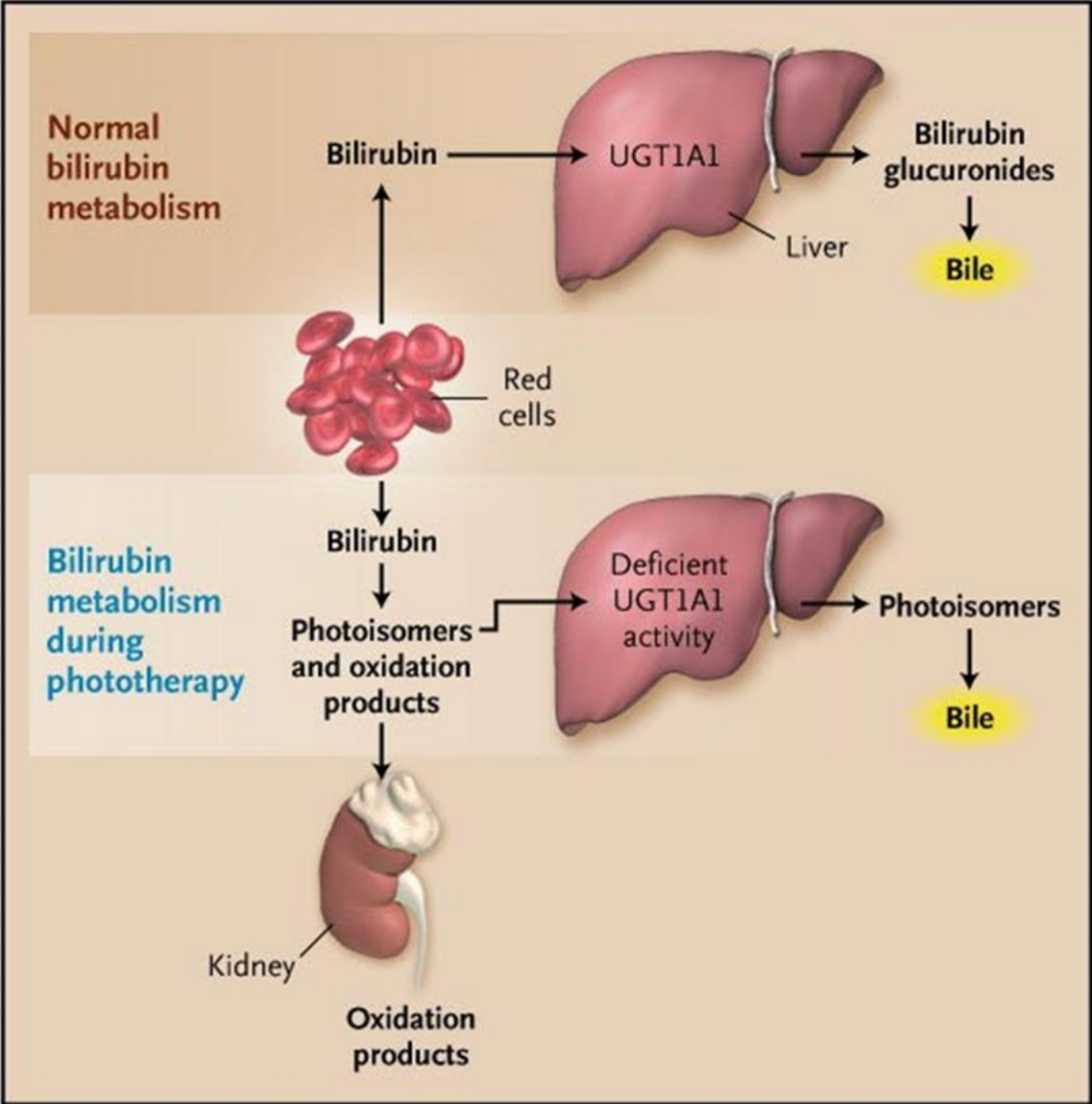
- physiological neonatal hyperbilirubinemia (icterus neonatorum)
  - increased erythrocytes destruction
  - immature liver conjugation and transport systems
  - increased bilirubin absorption and its lowered binding to albumin
  - unconjugated hyperbilirubinemia
  - peak in first five days, in half newborns
- kernicterus
  - in preterm infants, with hemolytic anemia and with neonatal hepatitis
  - considerably increased plasmatic bilirubin level
    - passes through the hematoencephalic barrier, deposits and damages basal ganglia



# Phototherapy

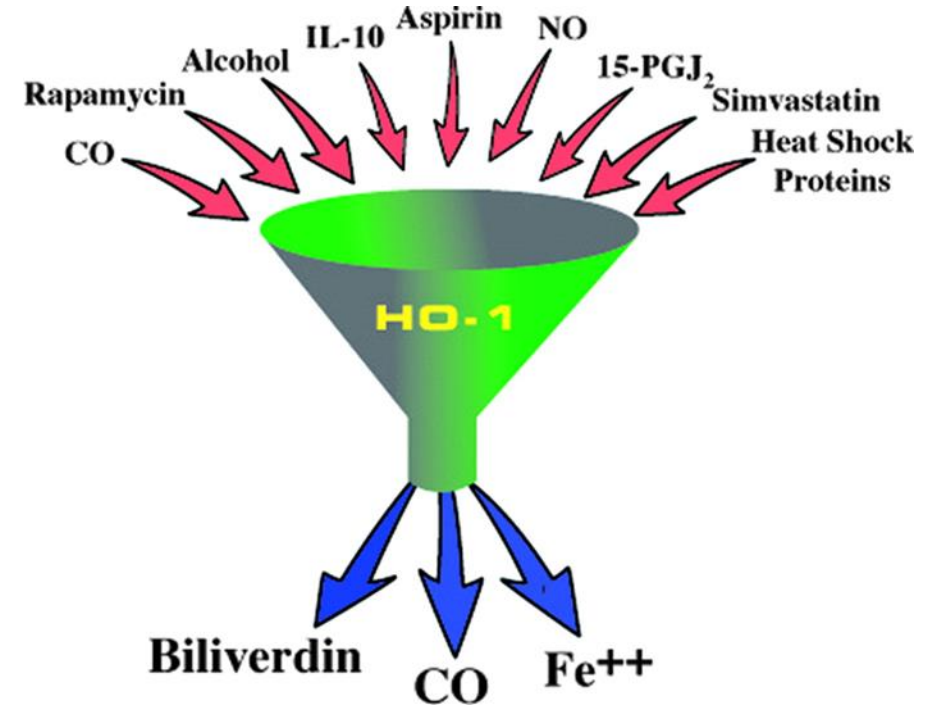


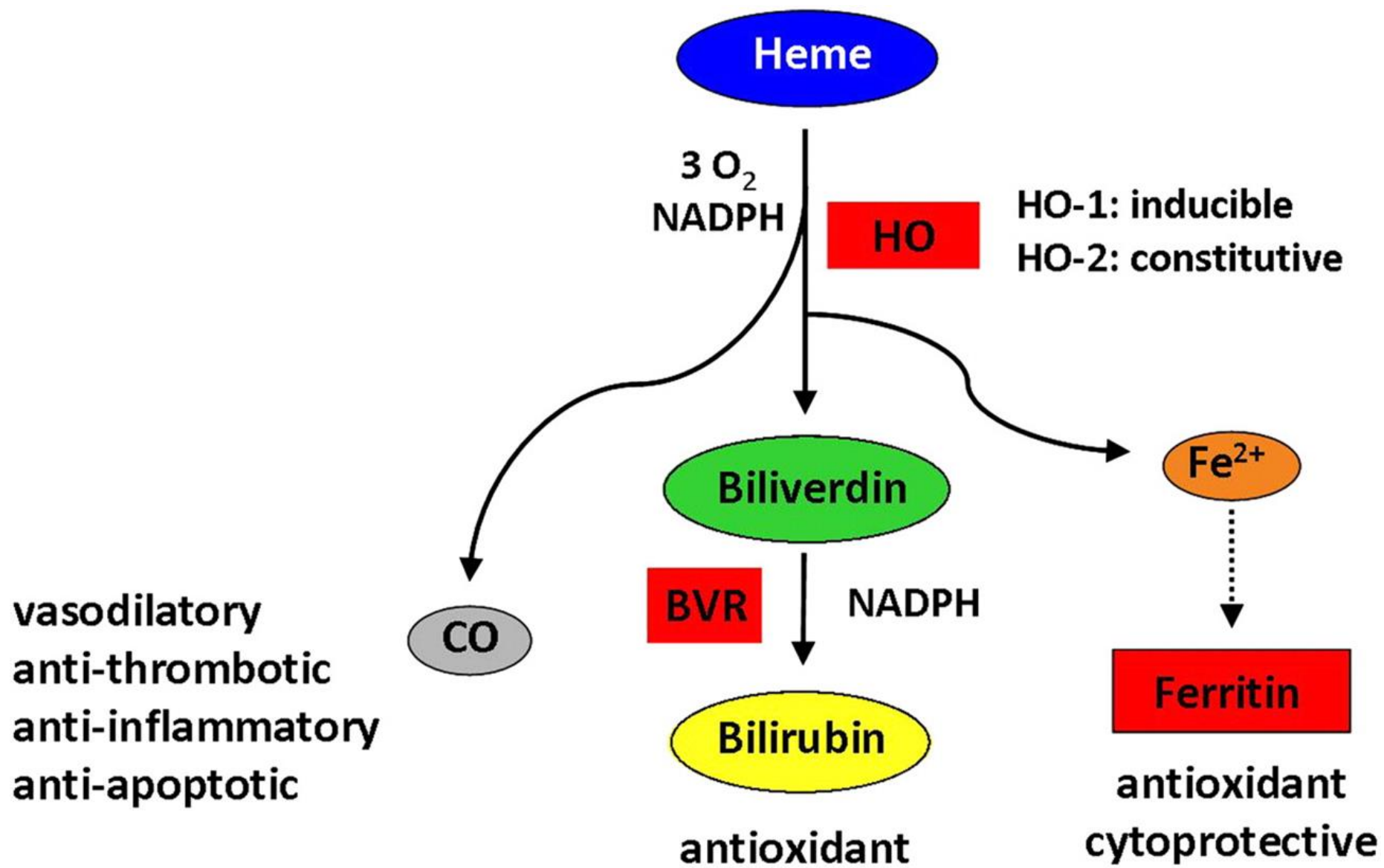
# Bilirubin metabolism during phototherapy



# Protective effect of hemoxygenase

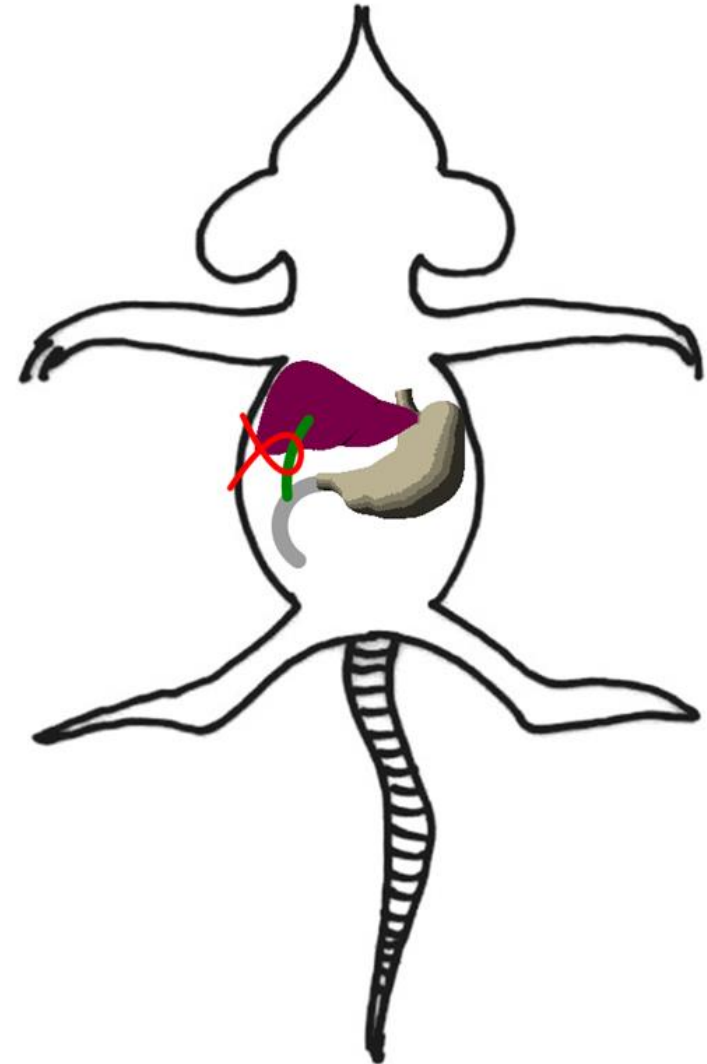
- cardiovascular system
  - inhibition of thrombocytes aggregation
  - smooth muscle relaxation
- inflammation
  - $\uparrow$  IL-10
  - $\downarrow$  TNF- $\alpha$  a IL-1 $\beta$
- cell proliferation
  - cyclins
  - direct anti-apoptotic effect
- metabolism
  - $\uparrow$  insulin secretion
- smokers
  - higher CO in the blood






# Animal experiment – part I

- the aim of the practical is to
  - prepare a model of obstructive jaundice
    - **part I**
  - observe changes of bilirubin metabolism
    - **part II**
- procedure – part I
  - general anesthesia
  - laparotomy
  - ligation of ductus choledochus

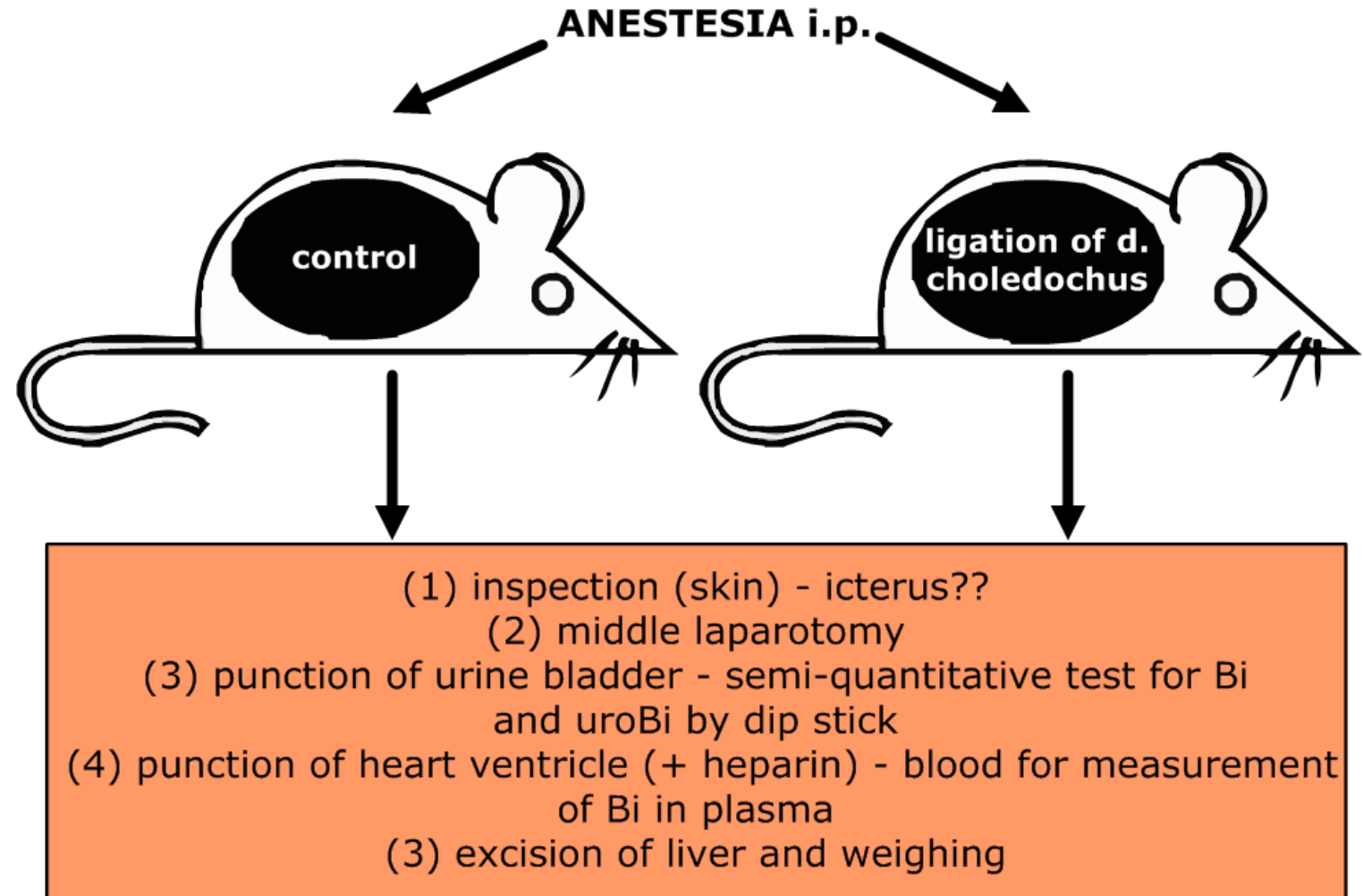


# Practical part II



**RESULTS**

- (1) comparison of Bi and uroBi concentration in the urine of control and expe. animals
- (2) comparison of Bi concentration (total, conj. and un-conj.) in plasma
- (3) comparison of liver weight





# Practical part II - procedure

- one week after ligation of ductus choledochus
- general anaesthesia, weighing of the animal
- observation of changes in animal coloration
- laparotomy
- urinary bladder puncture
  - test strips – presence of bilirubin and urobilinogen
- heart puncture, taking of blood and liver
  - measurement of bilirubin concentration in the serum
  - weighing of the liver

# Bilirubin measurement

- van den Bergh reaction
  - bilirubin reacts with sulphanilic acid to produce purple coloured azo bilirubin
  - photometric measurement
- conjugated bilirubin reacts fast
- unconjugated reacts slowly
  - acceleration by methanol
    - releases bilirubin from albumin
- (pre)analytic phase
  - prevent hemolysis
  - protect from sunlight

