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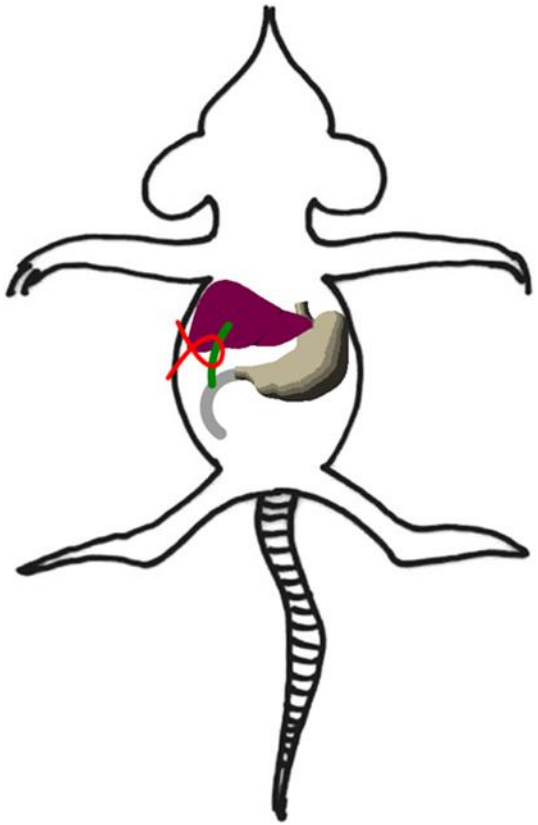
Experimentally induced jaundice

Experimental induction of hyperbilirubinemia

- general anaesthesia
  - additional dose during experiment
- laparotomy
- ductus choledochus
  - separation from vena portae
  - ligature
- suture



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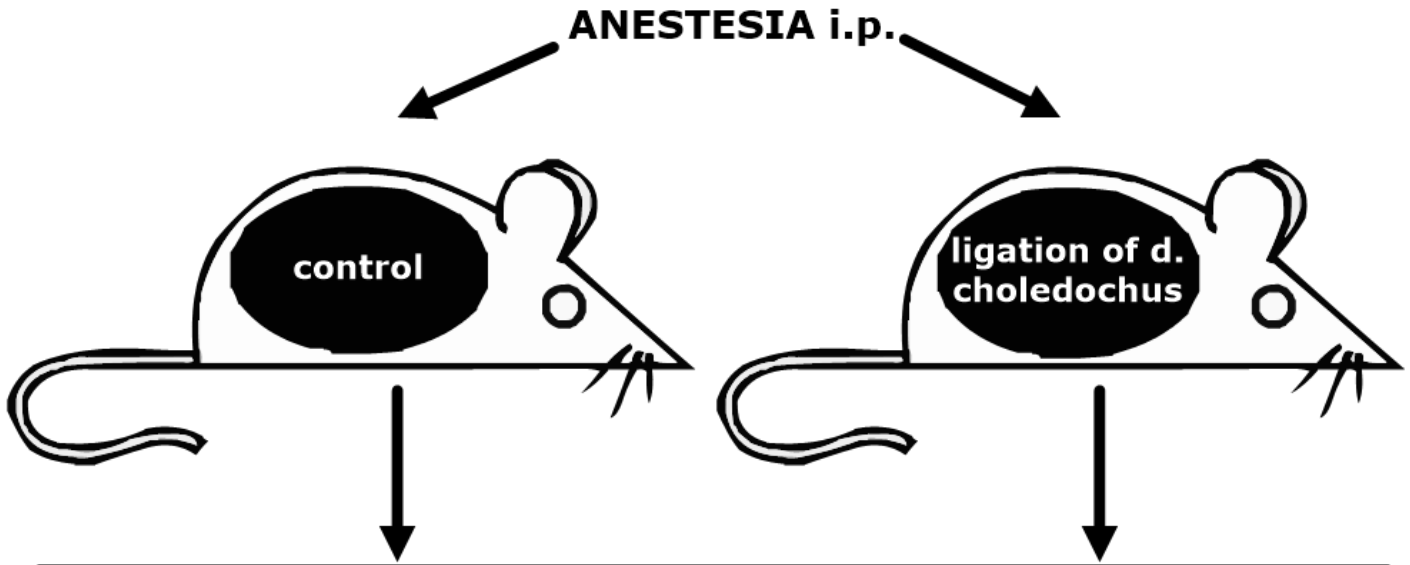
Practicals II - hyperbilirubinemia

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



(1) inspection (skin) - icterus??
(2) middle laparotomy
(3) punction of urine bladder - semi-quantitative test for Bi and uroBi by dip stick
(4) punction of heart ventricle (+ heparin) - blood for measurement of Bi in plasma
(3) excision of liver and weighing

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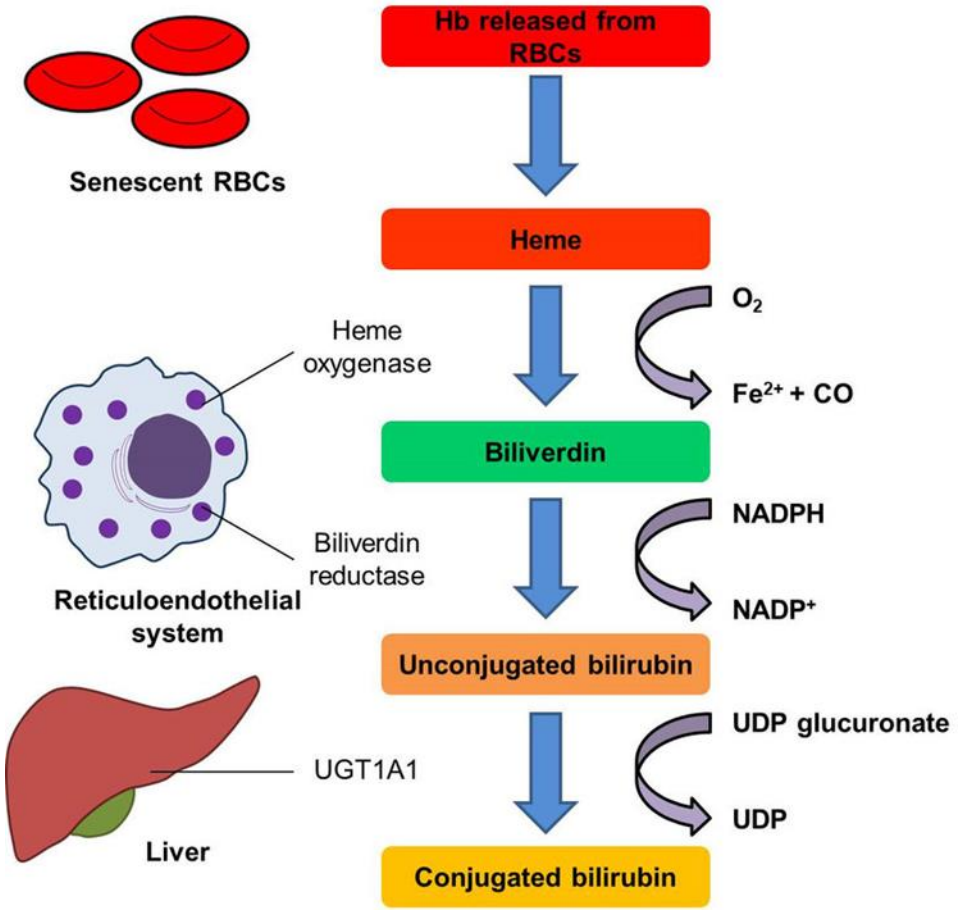
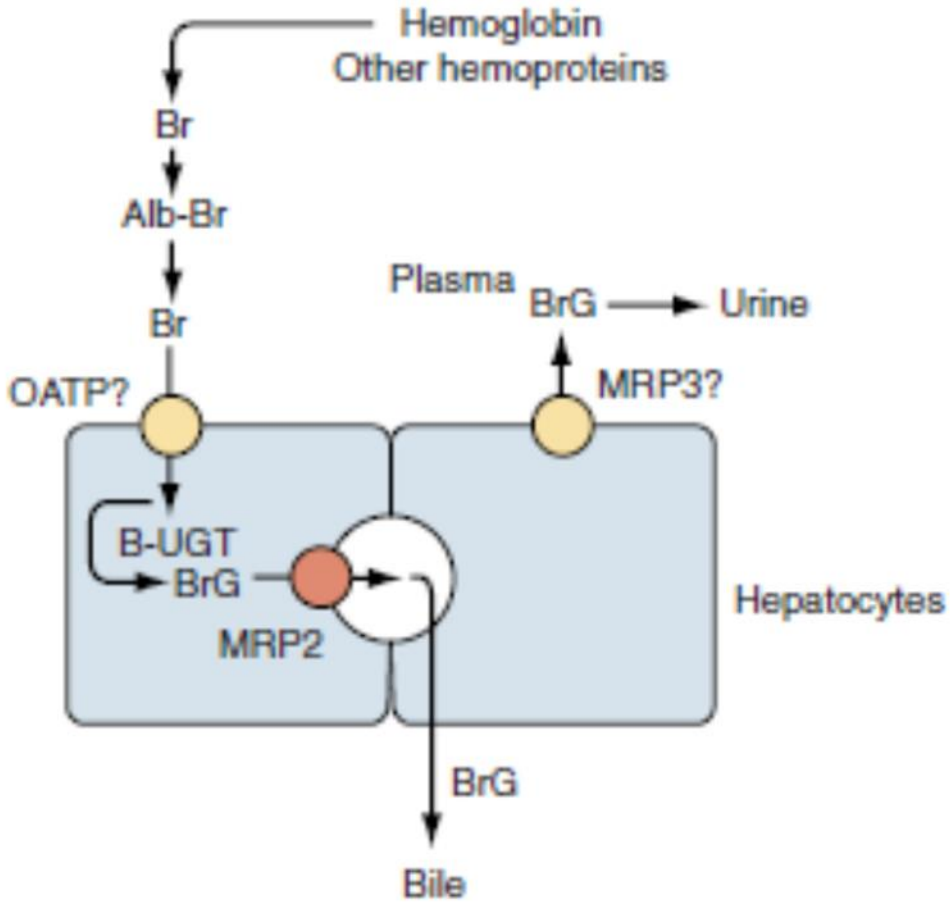
Overview of bilirubin metabolism





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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
  - yellow bird (Greek)

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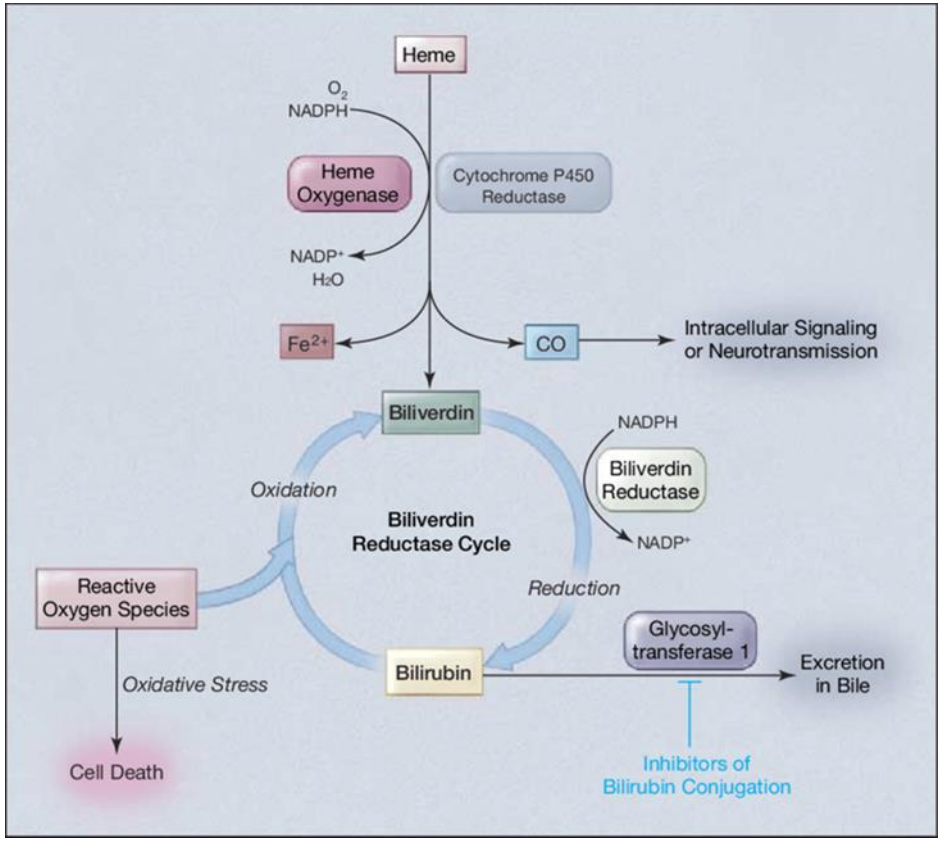
Bilirubin metabolism

- bilirubin is the final product of heme degradation
  - hydrophobic, potentially toxic
  - 70-80 % from hemoglobin (senescent RBC)
  - 20-30 % from myoglobin, cytochrome and premature destruction of RBC
- catabolism of RBC-derived Hb to bilirubin in reticuloendothelial cells
  - spleen, liver, bone marrow
- intravascular hemolysis
  - haptoglobin, hemopexin
  - free hemoglobin and Hp-Hb catabolized predominantly in the liver
- 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



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Bilirubin transport to the liver

- 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

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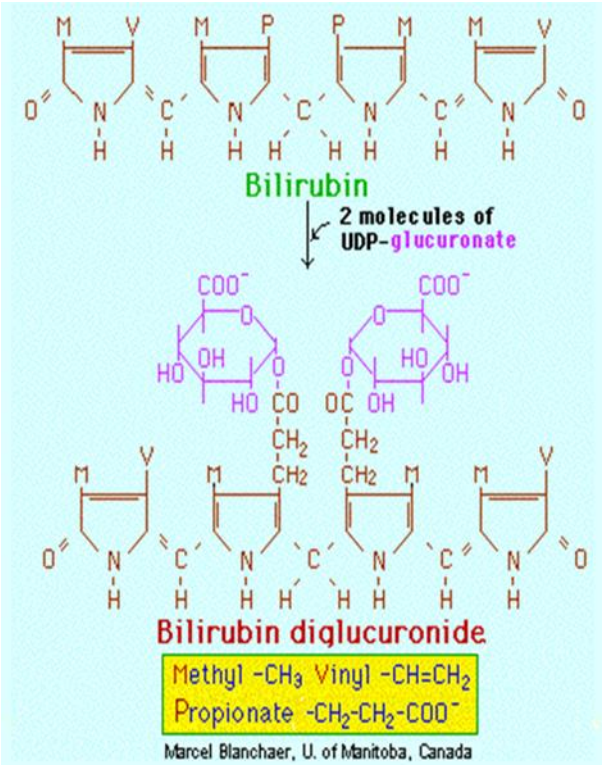
Bilirubin metabolism in the liver

- in the hepatocyte
  - proteins Y and Z
- enzyme uridin 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



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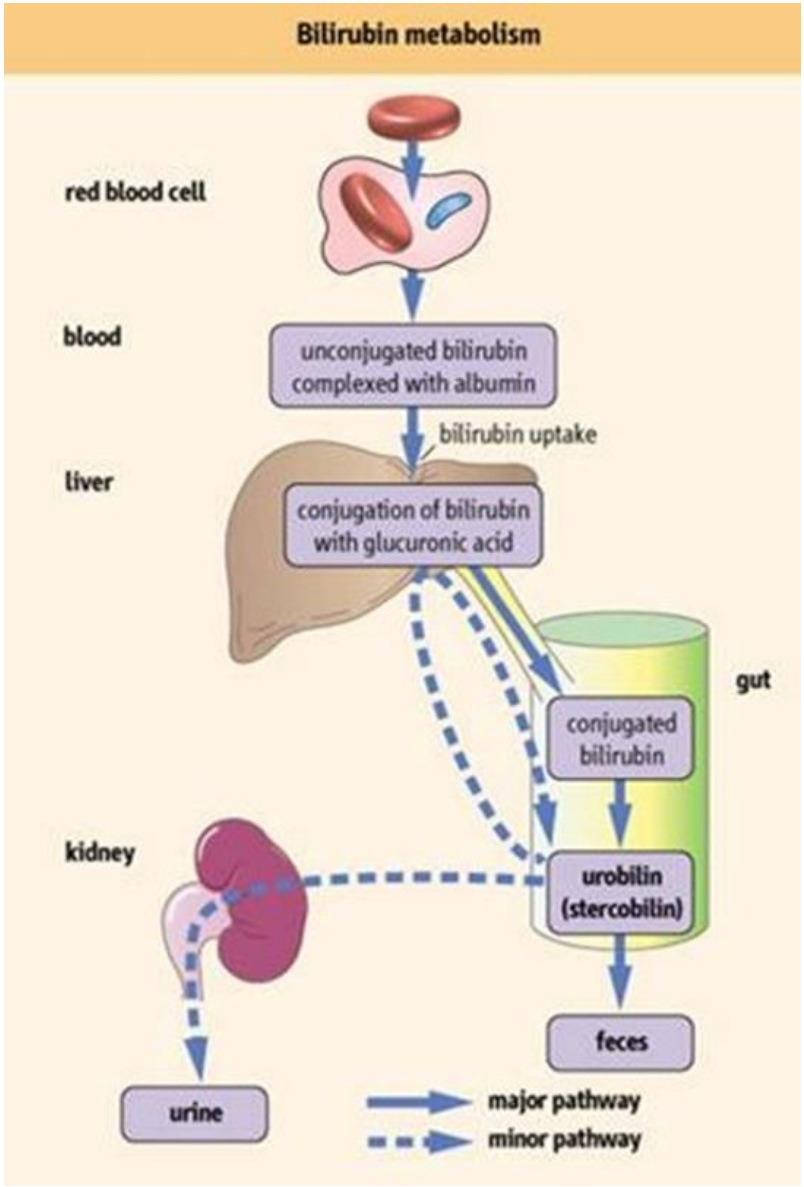
Bilirubin metabolism in the gut

- bilirubin passes through the bile ducts into the small intestine
  - deconjugation by bacterial enzyme β-glucuronidase
    - production of urobilinogen (colourless)
  - urobilinogen is
    - re-absorbed (enterohepatic circulation, approx. 20 %)
    - 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 catabolism

- 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?

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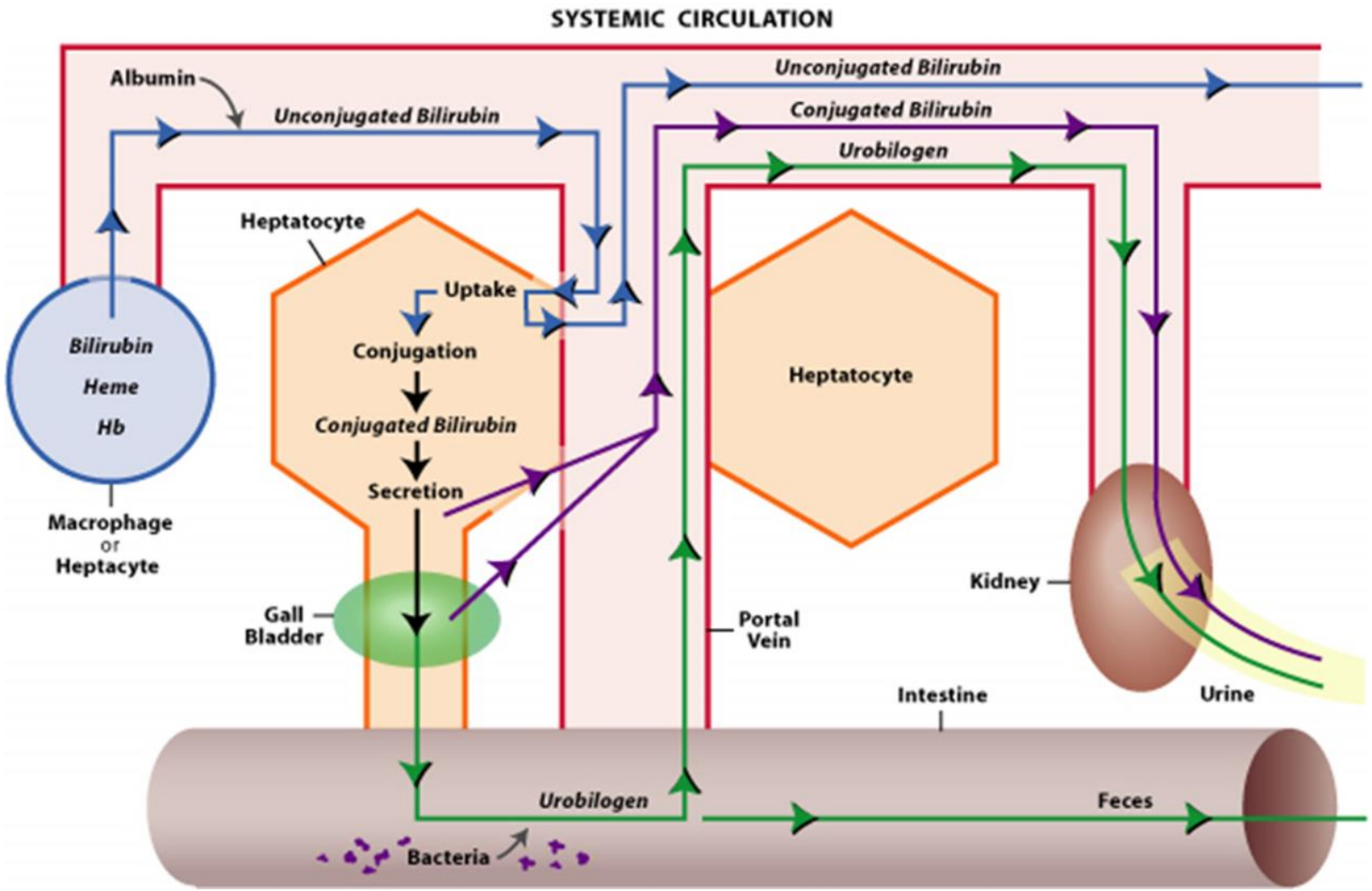
Summary



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Icterus/jaundice

- yellow discoloration of skin, mucose membranes and sclera
  - occurs when bilirubin concentration > 30 – 50 umol/l
  - deposition of bilirubin in tissues rich in elastin
- normal plasma level < 17 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

- shades of jaundice
  - rubin
    - redish (hepatitis)
  - flavin
    - yellow (hemolysis)
  - verdin
    - greenish (obstruction)
  - melas
    - greyish (prolonged obstruction)

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Jaundice

- jaundice can result from
  - increased production of bilirubin
  - decreased clearance of bilirubin
- conditions that produce jaundice
  - disorders of bilirubin metabolism
    - increased bilirubin production
    - decreased hepatocellular uptake of unconjugated bilirubin
    - decreased bilirubin conjugation
  - liver disease
  - obstruction of the bile ducts

- increased bilirubin production
  - hemolysis
  - ineffective erythropoiesis
  - resorption of a hematoma
  - massive blood transfusions
    - shortened lifespan of transfused RBC
- decreased bilirubin uptake
  - drugs
    - cyclosporine A
  - Gilbert‘s syndrome
- decreased bilirubin conjugation

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Premicrosomal hyperbilirubinemias

Bilirubin overproduction	Disorder of liver bilirubin uptake	Disorder of bilirubin conjugation
Hemolytic anemia	Immature transport systems	Immature conjugation system
Ineffective erythropoiesis	Acquired defect of bilirubin transport at the sinusoidal pole of hepatocyte	Inborn defect of bilirubin conjugation
Extravascular hemolysis	Inborn defect of bilirubin transport at the sinusoidal pole of hepatocyte	Acquired defect of bilirubin conjugation
Overproduction from non-hemoglobin sources		

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Bilirubin overproduction

- hemolytic anemias
  - the most common cause of bilirubin overproduction
  - stimulation of bilirubin metabolism
    - serum bilirubin < 70 µmol/l
- ineffective erythropoiesis
  - dysfunctional RBC maturation in bone marrow
    - thalassemia, megaloblastic anemia
  - defective RBC undergo premature hemolysis in the spleen
    - physiologic (10 – 20 %)
  - usually only mild bilirubin elavation (<100 µmol/l
- extravascular hemolysis
  - hematoma, surgery
- hemolysis of RBC from blood transfusions
  - 10 % of RBC from blood transfusion undergo hemolysis within 24 hours
    - Normally processed by the liver
      - Possible jaundice in paitnets with liver disease

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Disorders of liver bilirubin uptake

- neonatal hyperbilirubinemia
  - multifactorial etiopathogenesis
  - immature transport systems of the hepatocyte
- acquired
  - interference of bilirubin transport with some drugs sharing same transporter
    - statins, fibrates, cyclosporine A
      - insufficient clinical data

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Disorders of bilirubin conjugation

- inborn defects of conjugation
  - Gilbert‘s syndrome
  - Crigler-Najjar syndrome
- acquired deficit of UGT1A1
  - potentially in any diffuse hepatocellular damage
    - steatosis, steatohepatitis, fibrosis, cirhosis
  - xenobiotics
    - mild inhibition might be beneficial
      - under investigation

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Postmicrosomal hyperbilirubinemia

- disorder of bilirubin metabolism after conjugation in hepatocytes
- usually conjugated hyperbilirubinemia
  - familial
    - Dubin-Johnson, Rotor
  - acquired
    - intrahepatal cholestasis
    - extrahepatal cholestasis

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Mixed hyperbilirubinemia

- diffuse damage of liver parenchyma
  - hepatitis, steatohepatitis
  - excessive fibrous tissue deposition
  - tumour
  - metabolic disease
  - drugs, toxins

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Unconjugated hyperbilirubinemia

- increased bilirubin production
  - hemolysis
  - ineffective erythropoiesis
  - resorption of a hematoma
  - massive blood transfusions
    - shortened lifespan of transfused RBC
- decreased bilirubin uptake
  - drugs
    - cyclosporine A
  - Gilbert‘s syndrome
- decreased bilirubin conjugation
  - Gilbert‘s syndrome
  - Crigler-Najjar syndrome
  - physiologic jaundice of the newborn

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Liver disease

- jaundice is a common feature of generalized hepatic dysfunction
  - abnormalities in biochemical liver tests are commonly present
- acute hepatocellular injury
  - viral hepatitis
  - toxins
    - amanitin
  - hepatic ischemia
  - metabolic derangements
    - Wilson disease
- chronic hepatocellular injury
  - jaundice does not typically develop in chronic hepatocellular injury unless cirrhosis is present
  - chronic viral hepatitis
  - nonalcoholic fatty liver disease
  - alcoholic liver disease
  - hereditary hemochromatosis
- intrahepatic cholestatic disorders
  - impaired bile formation in the absence of widespread hepatocellular injury

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Obstruction of the bile ducts

- choledocholithiasis
  - the most common cause of biliary obstruction
  - gallstones typically originate in the gallbladder, migrate into the common bile duct and occlude the ampulla of Vater
- disease of the bile ducts
  - intrinsic narrowing of the bile ducts due to
    - inflammation
    - infection
    - neoplastic biliary disease
- extrinsic compression
  - neoplasm, inflammation
    - jaundice is a classic feature of carcinoma of the head of the pancreas

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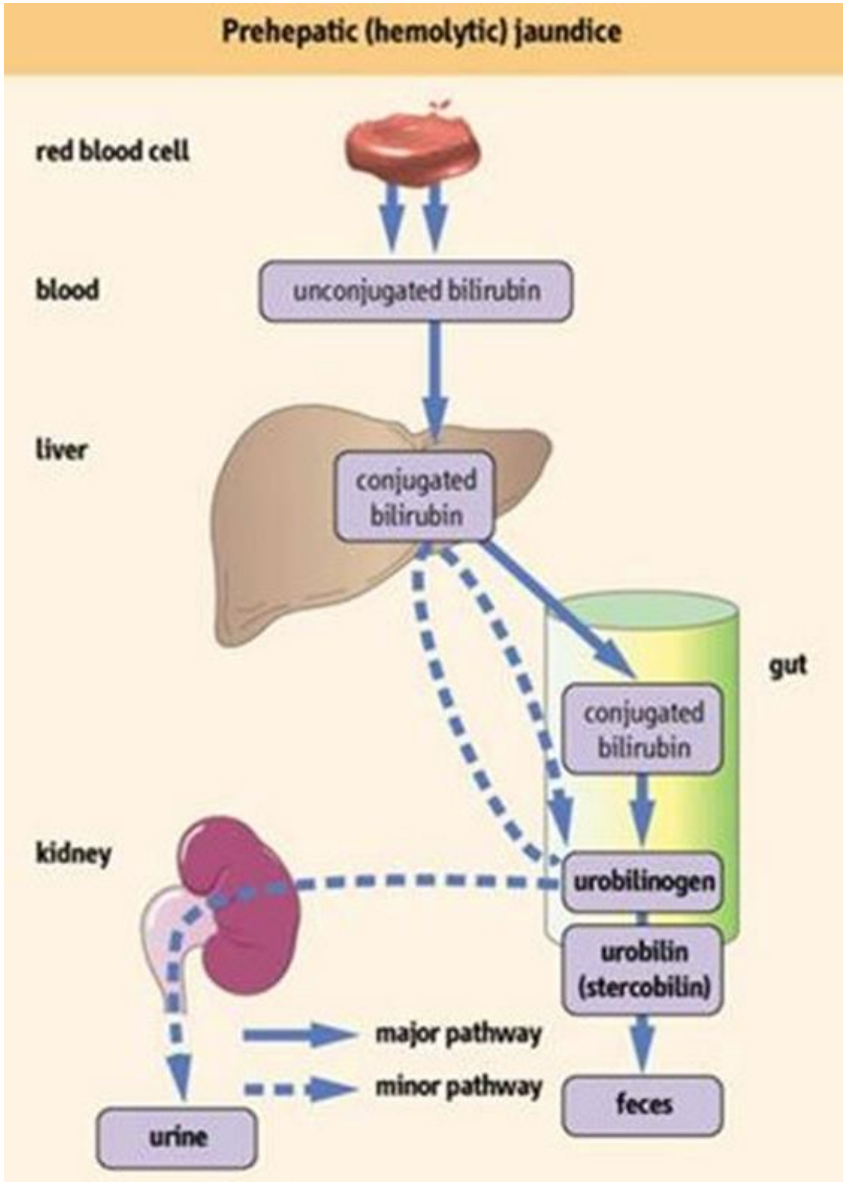
The causes of jaundice

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

Prehepatic icterus

- 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 icterus

- 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
- hypocholic stool
  - lower production of bilirubin

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Gilbert‘s syndrome

- genetically determined disorder
  - autosomal recessive
  - mutations in the promoter of UDPGT gene
    - decreease of enzyme activity by 70 %
      - responds to phenobarbital
- common
  - 5 – 10 % prevalence
  - more common in males
- typically presents during or after adolescence
- benign
  - lifelong hyperbilirubinemia
    - Up to 100 µmol/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

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Hereditary disorders of bilirubin metabolism

	Syndrome				
PARAMETER	**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

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Posthepatic (cholestatic) icterus

- 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

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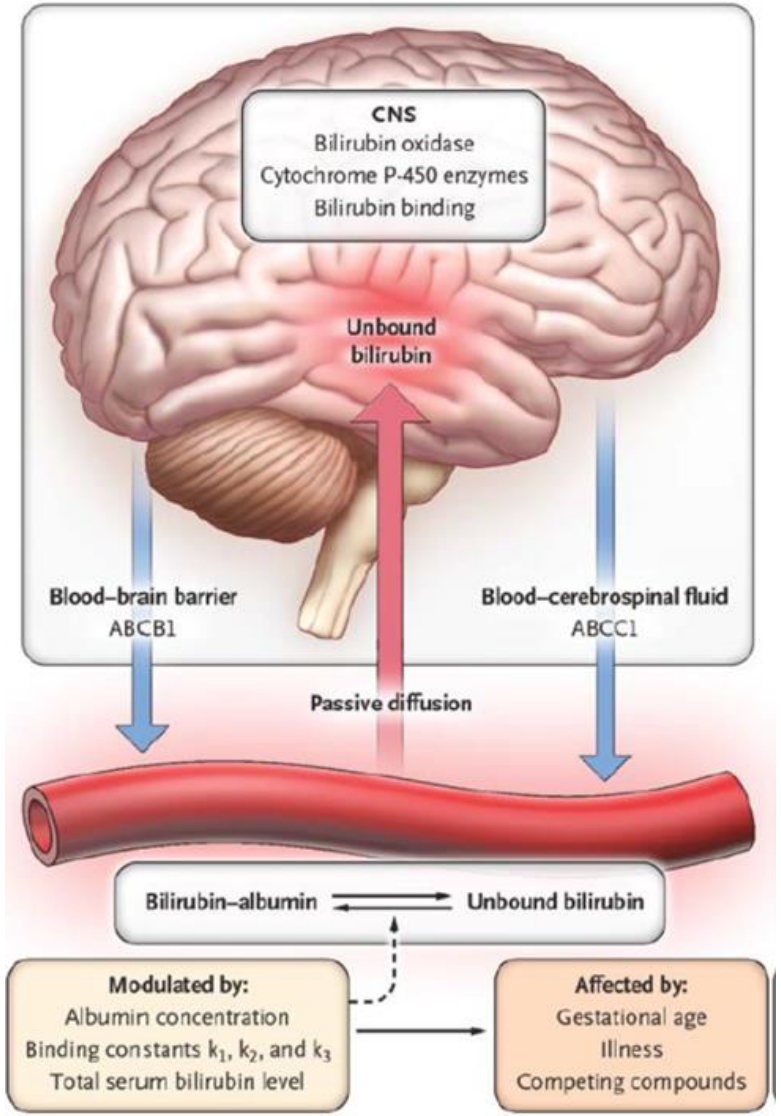
Neonatal hyperbilirubinemia

- 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



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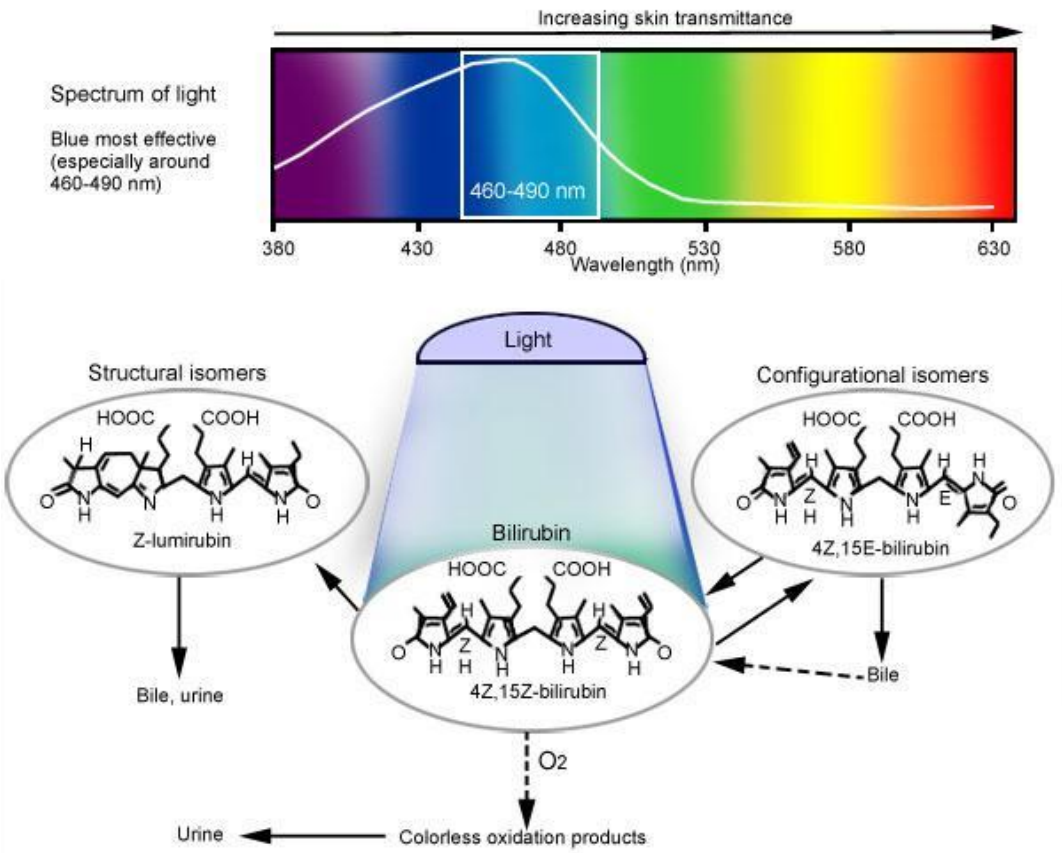
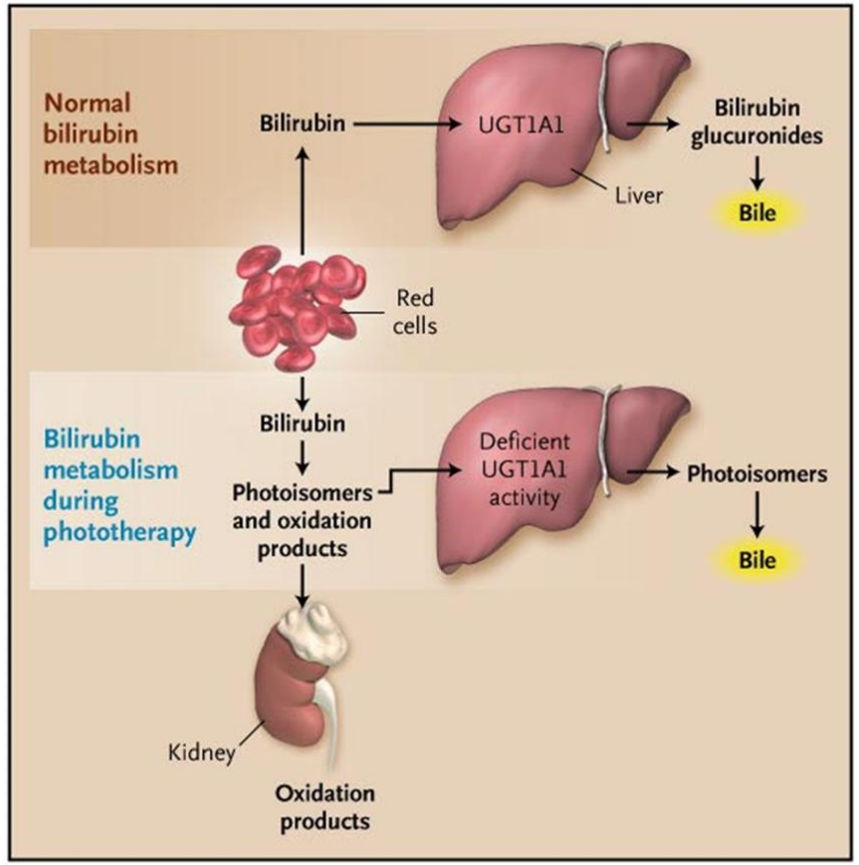
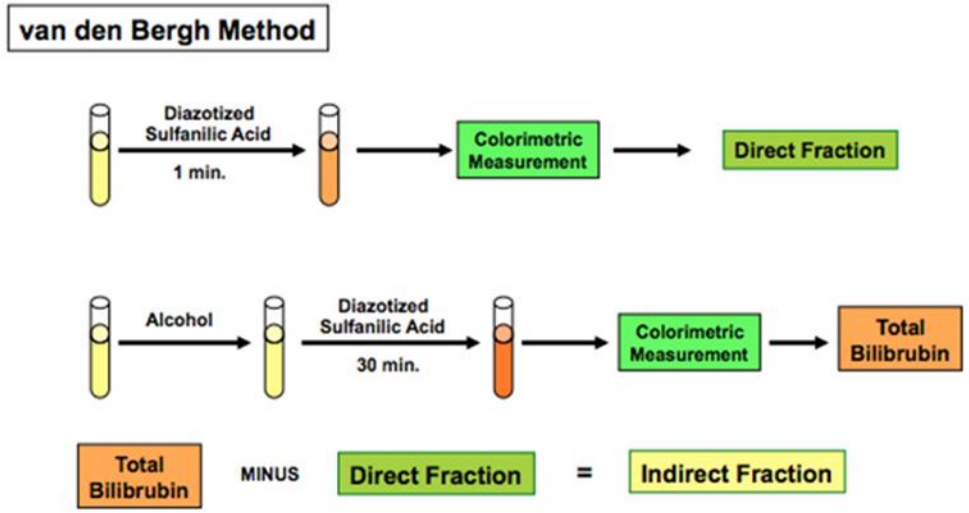
Phototherapy





Bilirubin measurement

- van den Bergh reaction
  - conventional colorimetric method
  - bilirubin reacts with sulphanilic acid to produce purple coloured azo bilirubin
- conjugated bilirubin is cleaved rapidly
- unconjugated bilirubin reacts slowly
  - requires addition of an accelerator
    - ethanol, urea
    - releases bilirubin from albumin
- (pre)analytic phase
  - prevent hemolysis
  - protect from sunlight



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