Bilirubin

- The good
- The bad, and
- The ugly

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Professor of Genetics
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Pathophysiological importance of bilirubin metabolism

- **It is the end product of heme detoxification.**
- **Serum bilirubin level is an important clinical marker of hepatobiliary excretory function.**
- **Bilirubin is an endogenous model for plasma carriage and hepatic throughput of organic anions.**
- **Hepatic uptake, storage, conjugation and excretion of bilirubin are finely balanced. Therefore, enhancement of bilirubin throughput requires coordinated induction of multiple genes, which may be mediated by nuclear receptors.**

Sources of bilirubin

Erythroid
(80%)

Normal:

- Senescent erythrocytes
- Free heme

Abnormal:

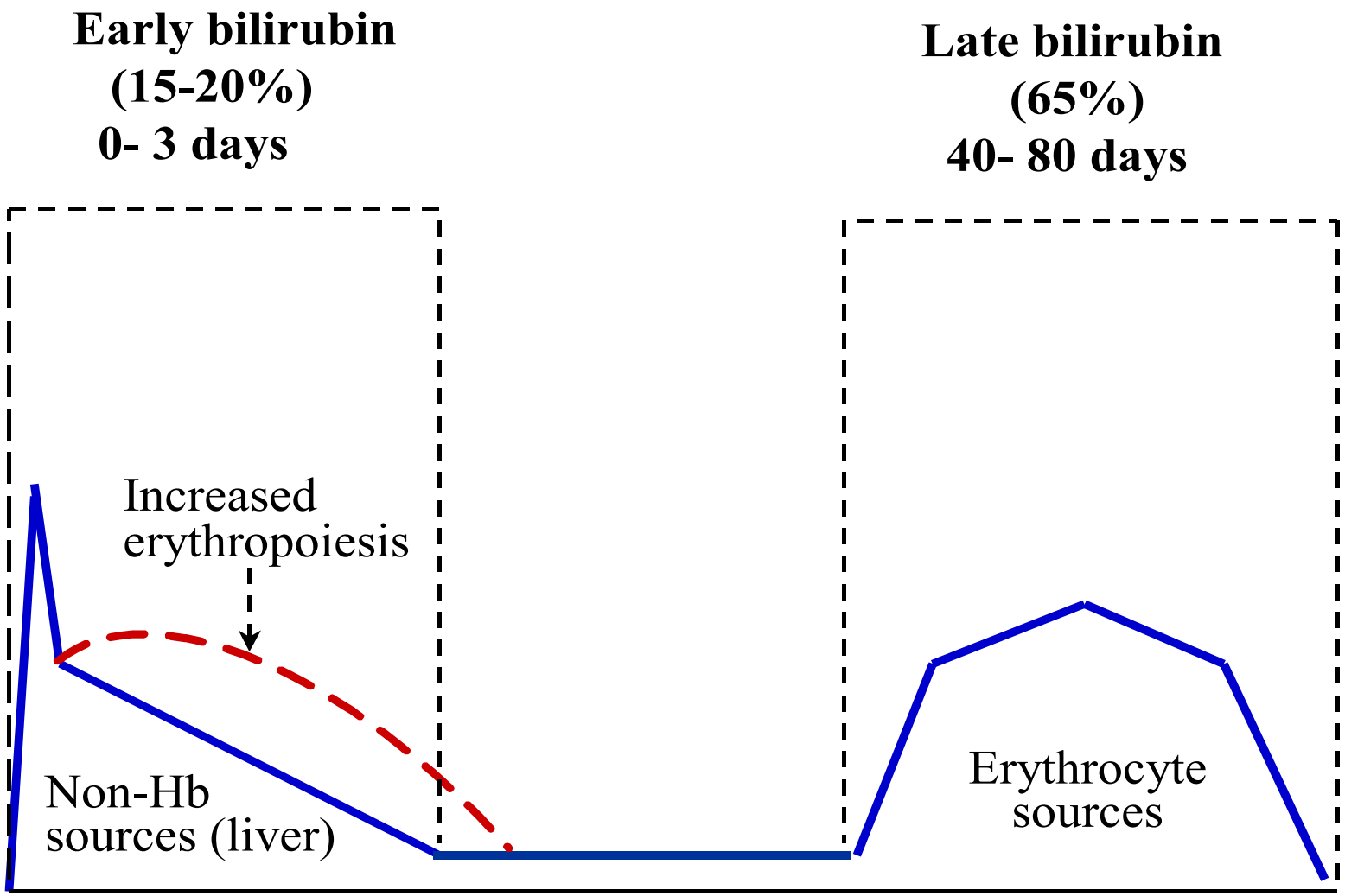
- Hemolysis:
  - Extravascular
  - Intravascular
- Ineffective erythropoiesis

Non-erythroid
(20%)

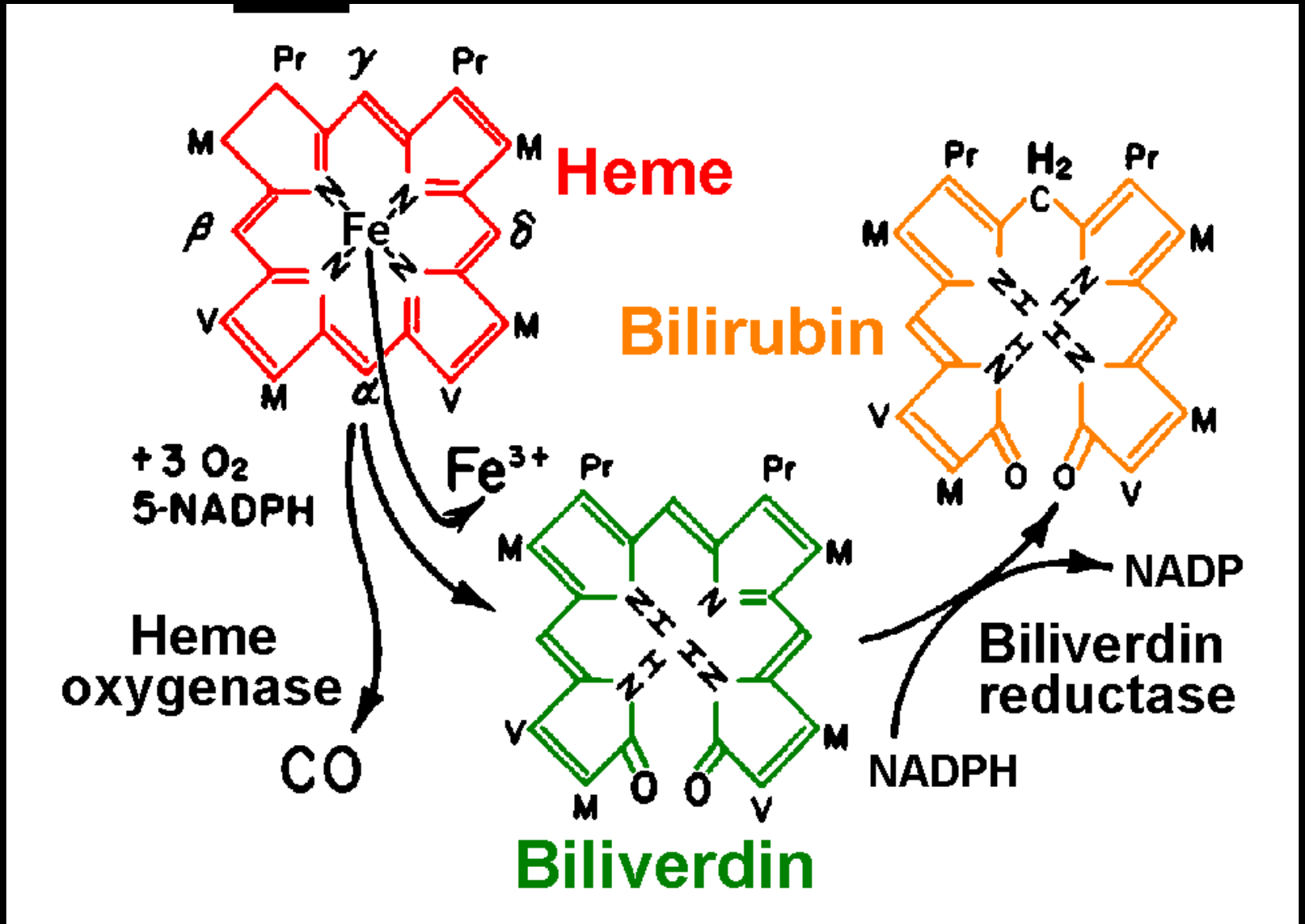
- Cytochromes
- Catalase
- Peroxidase
- Tryptophane pyrrolase

- Myoglobin

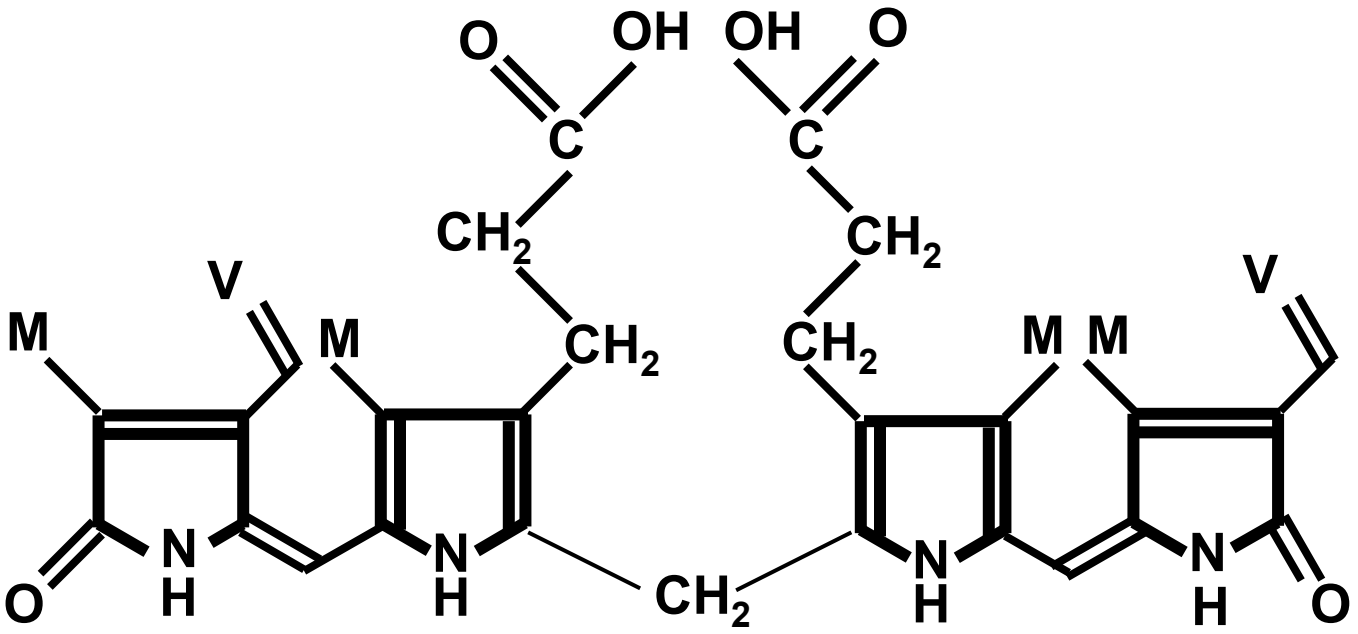
Early and late labeled peaks of radioisotope incorporation into bilirubin After injection of labeled porphyrin precursor (^{14}C-glycine)



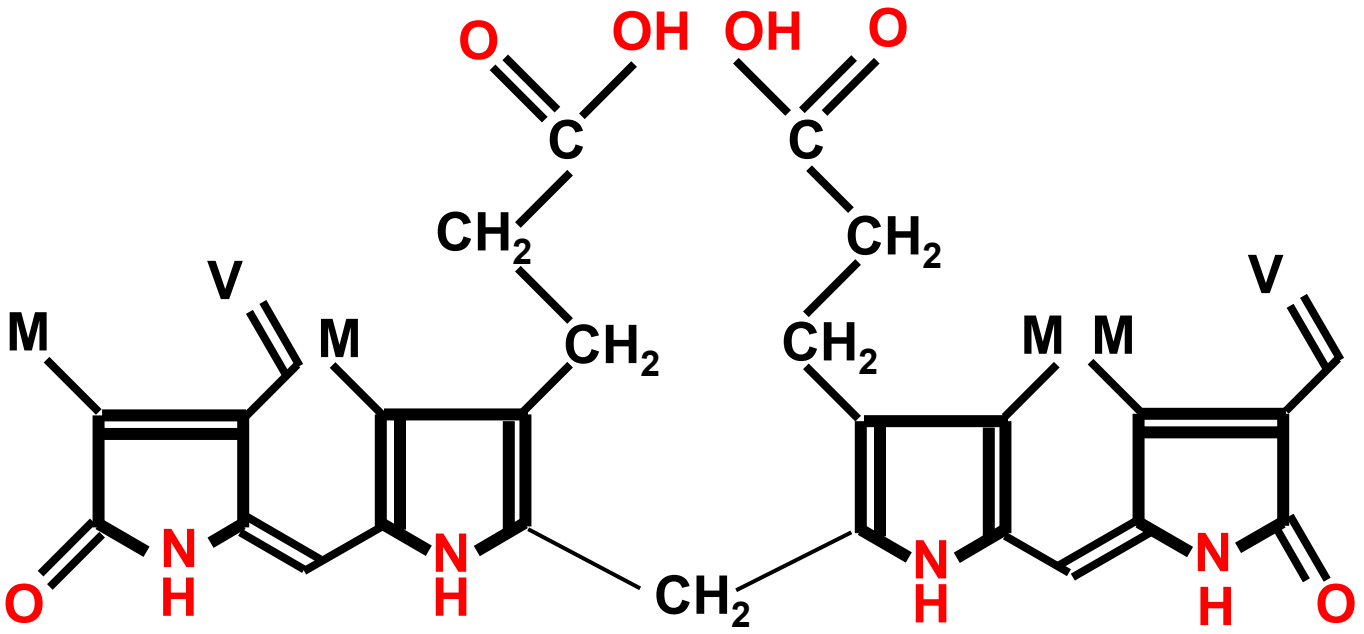
Opening of the heme ring and Enzyme-catalyzed formation of bilirubin



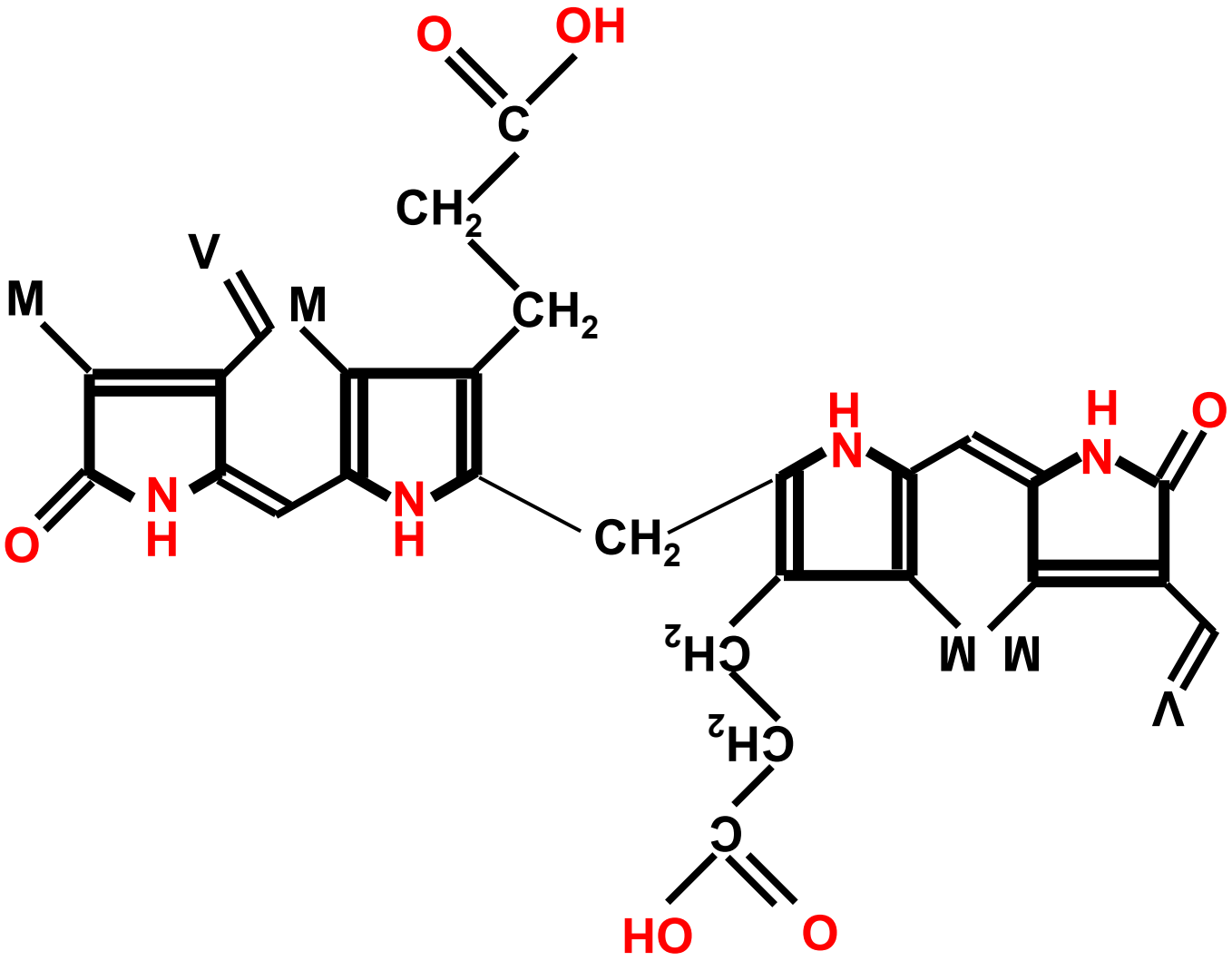
The linear structure of bilirubin:
Two dipyrroles joined by a central methene bridge



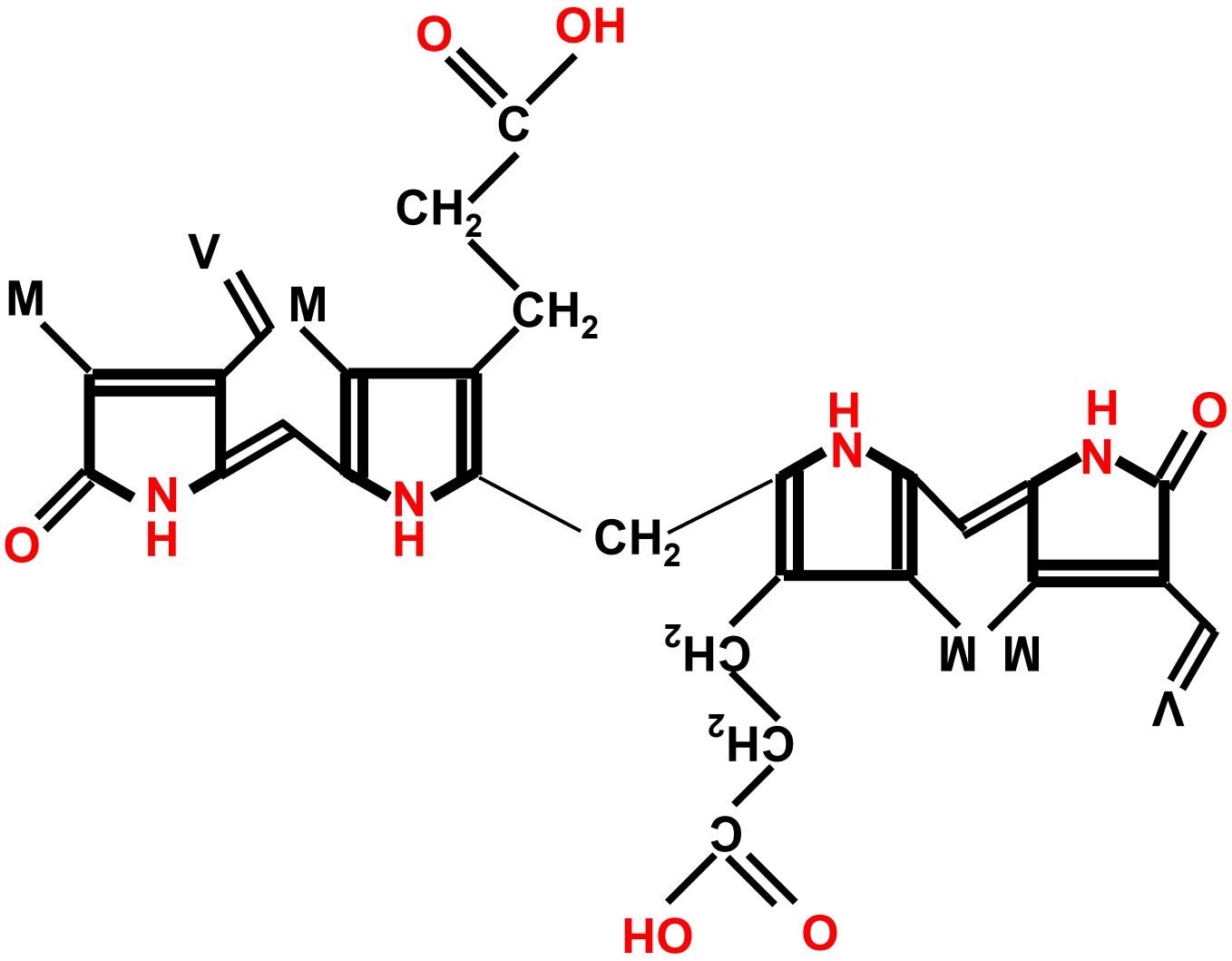
Bilirubin contains several polar groups (shown in red):
Yet, it is insoluble in water.



Water insolubility of bilirubin is explained by internal hydrogen bonding.



This is explained by internal hydrogen bonding.



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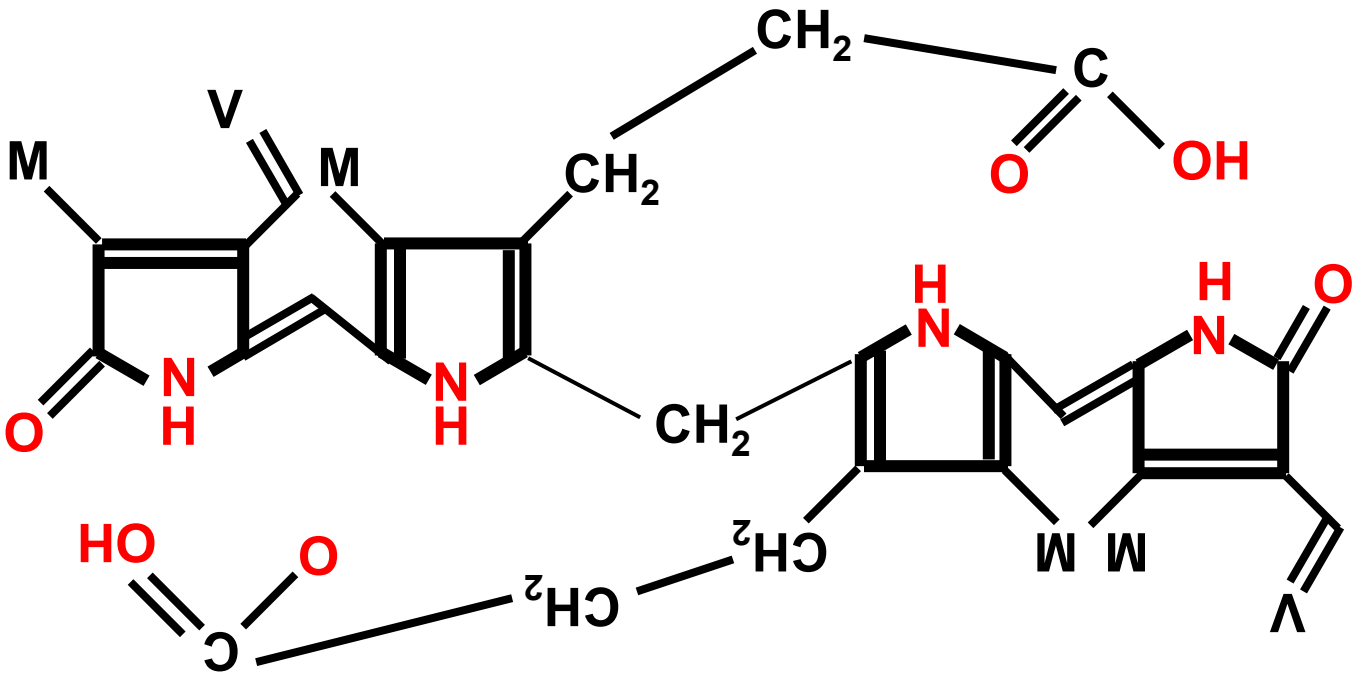

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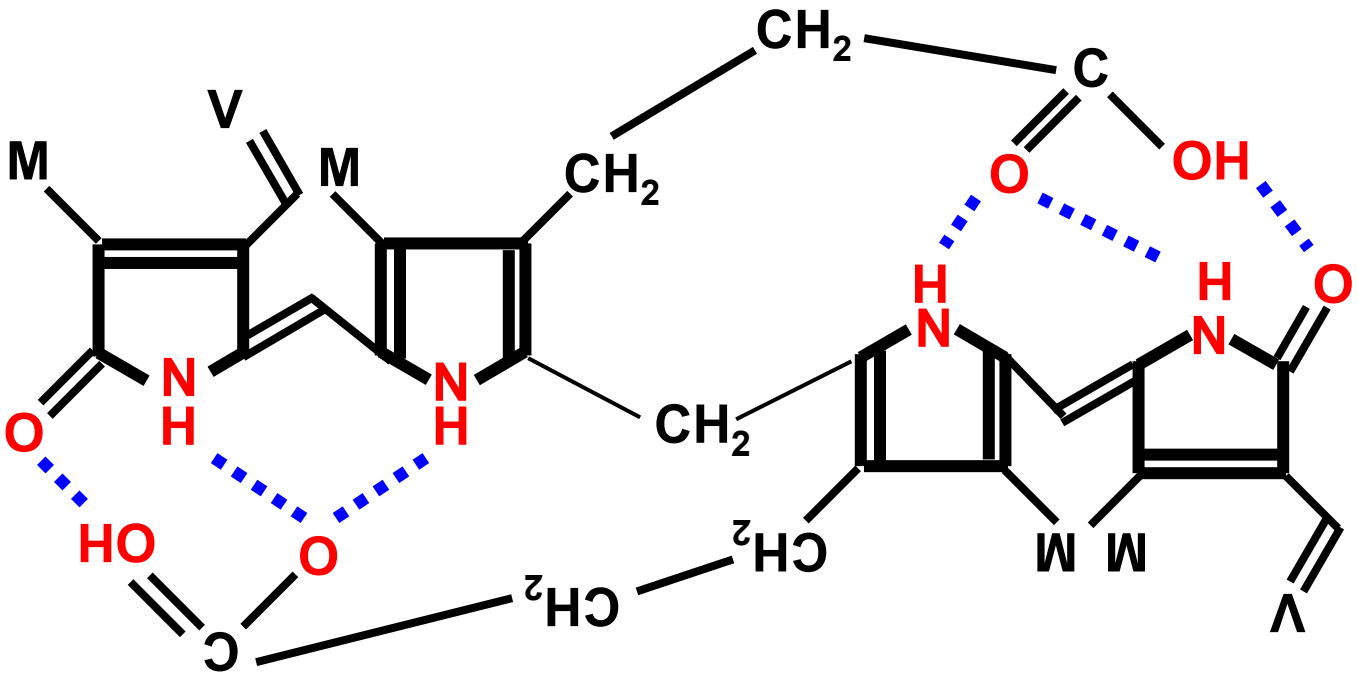
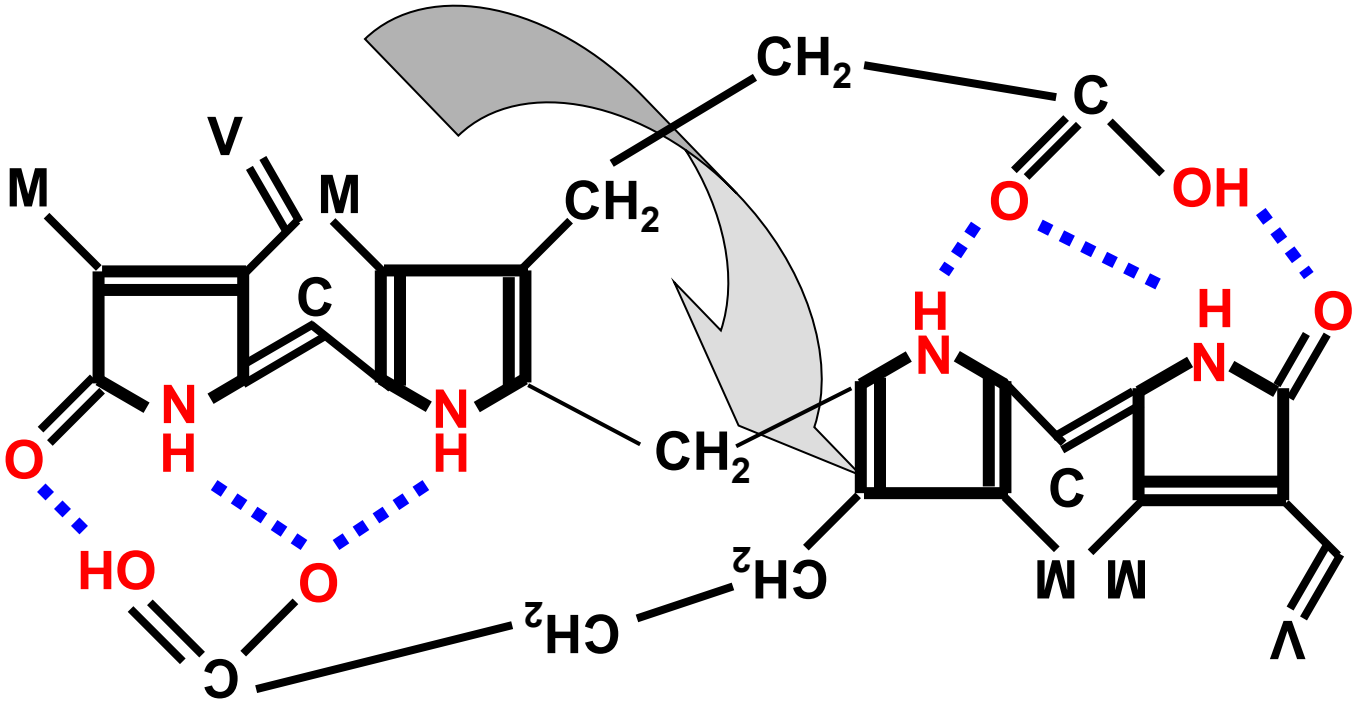
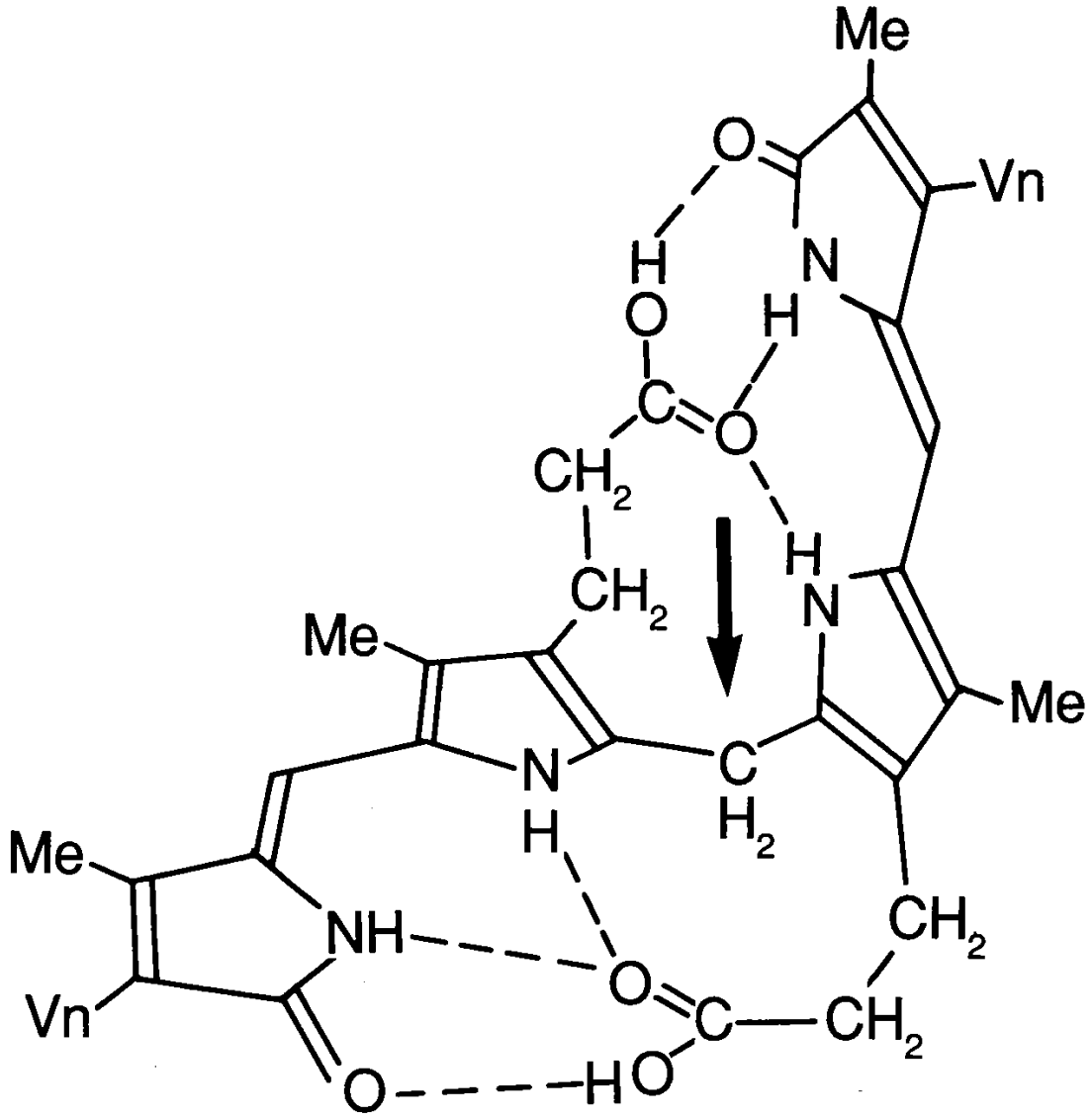


- As a consequence of hydrogen bonding, all polar groups are engaged.
- The central methene bridge becomes buried.

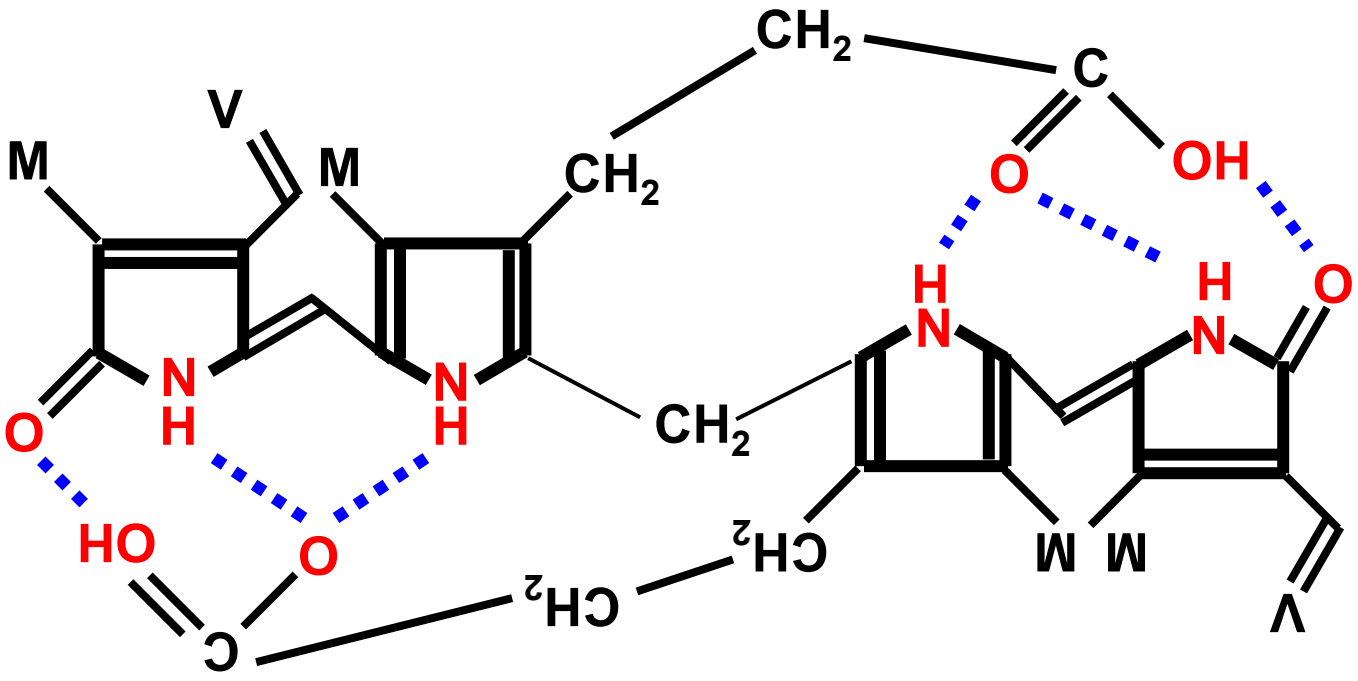


Ridge-tile structure of bilirubin



Conjugation with glucuronic acid makes bilirubin water soluble

The internal hydrogen bonds of bilirubin are disrupted by conjugation of the propionic acid carboxyl group with glucuronic acid



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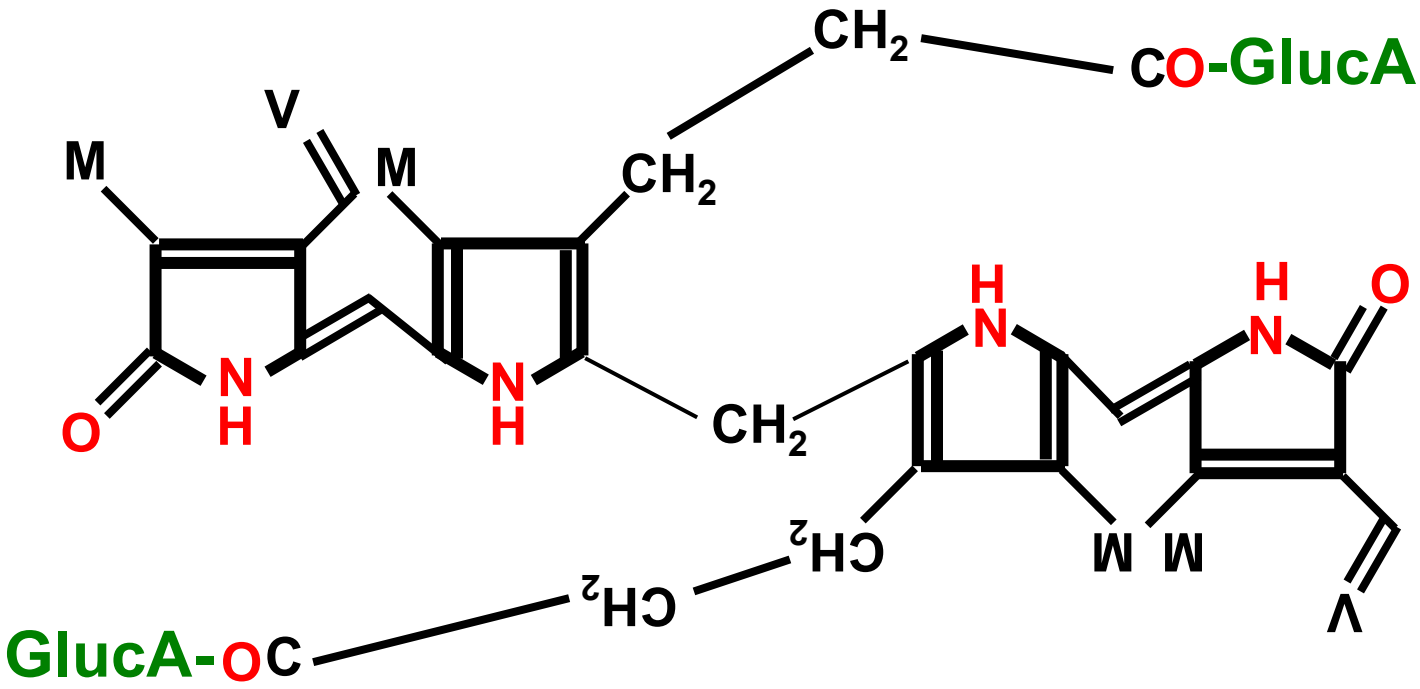
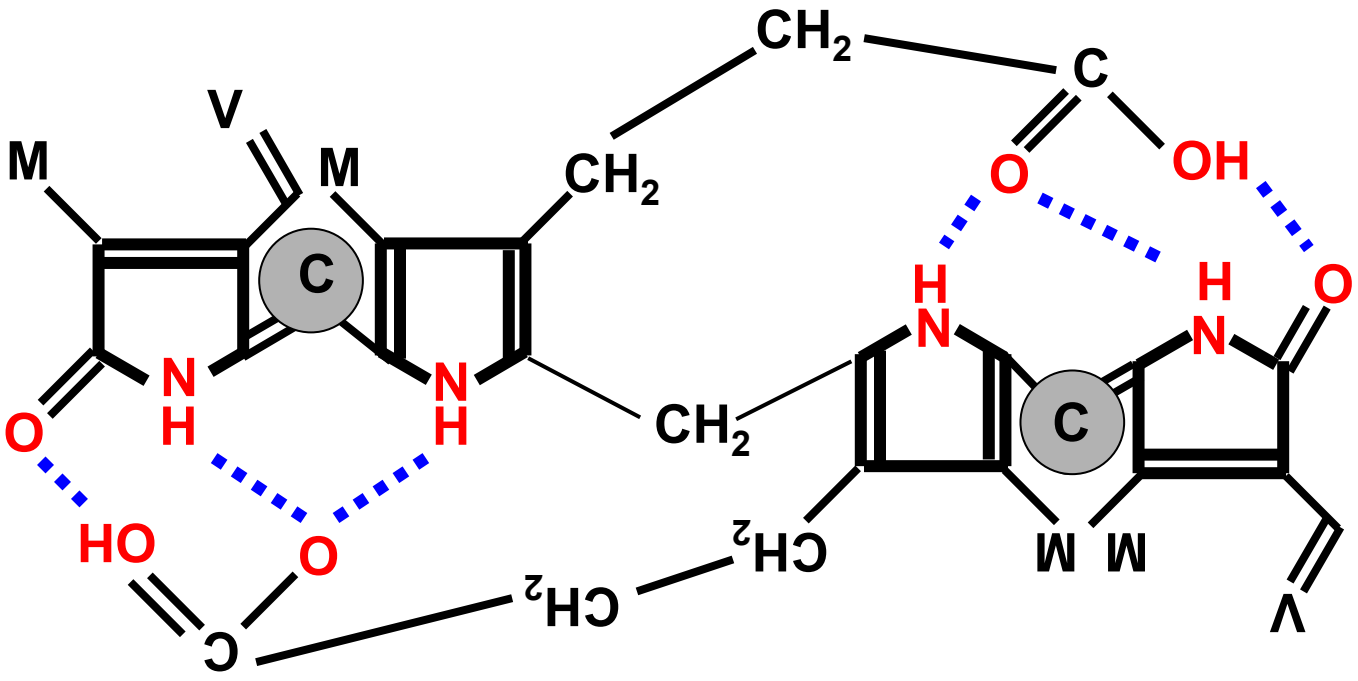


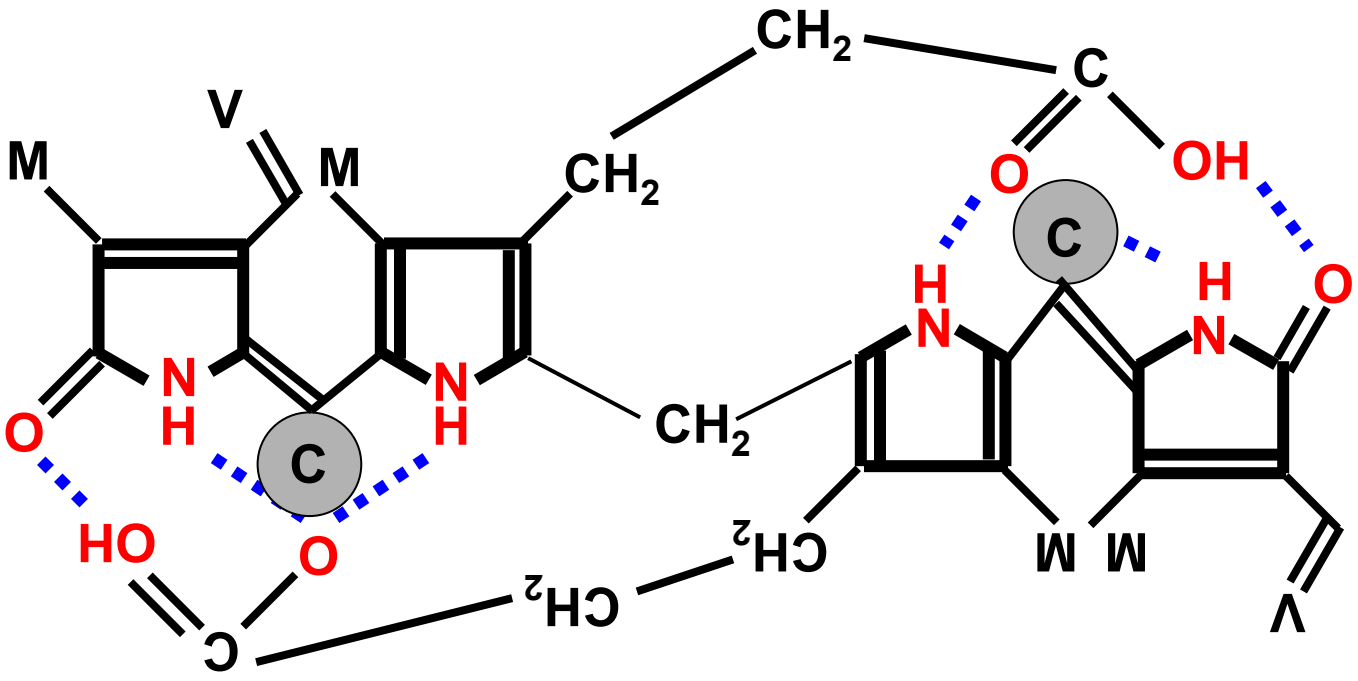
Phototherapy changes the configuration of bilirubin making it transiently water soluble

- **Internal hydrogen bonds are disrupted transiently upon exposure of bilirubin to light.**

- **The dipyrrole carbon bridges switch direction.**



- **The bulky carbon atom disrupts the hydrogen bonds by steric hindrence.**



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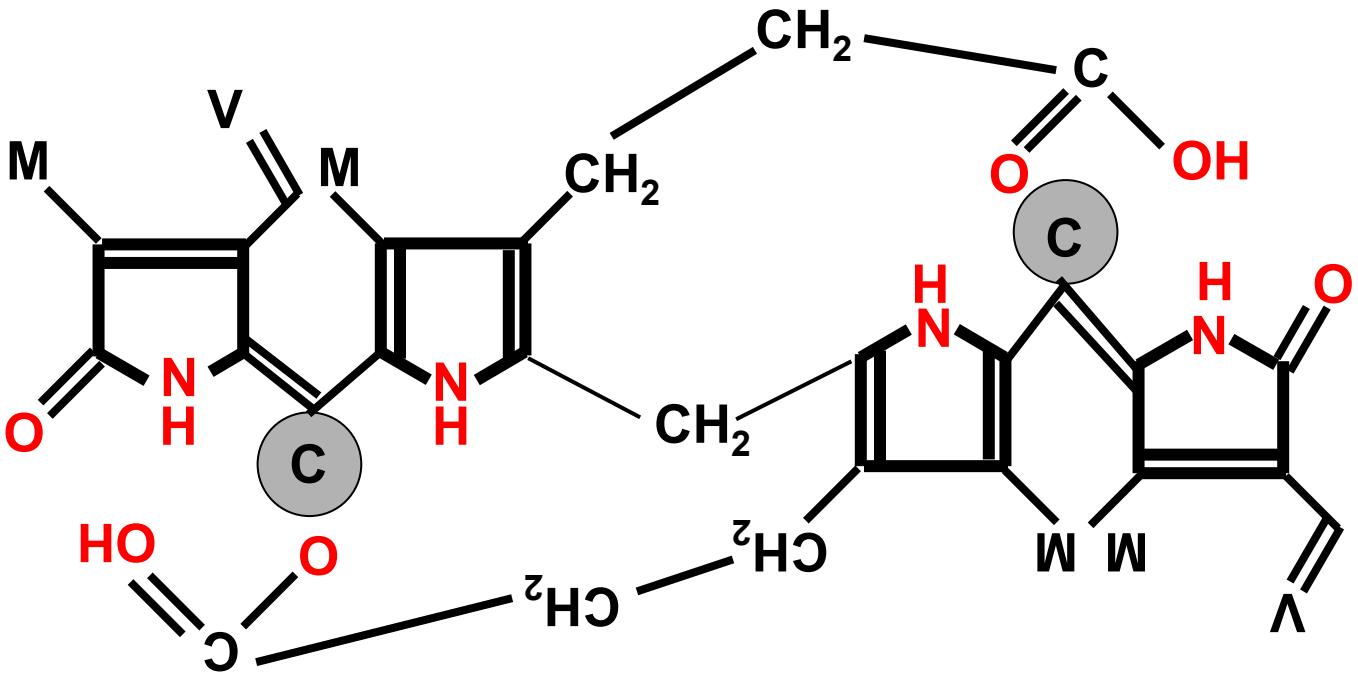
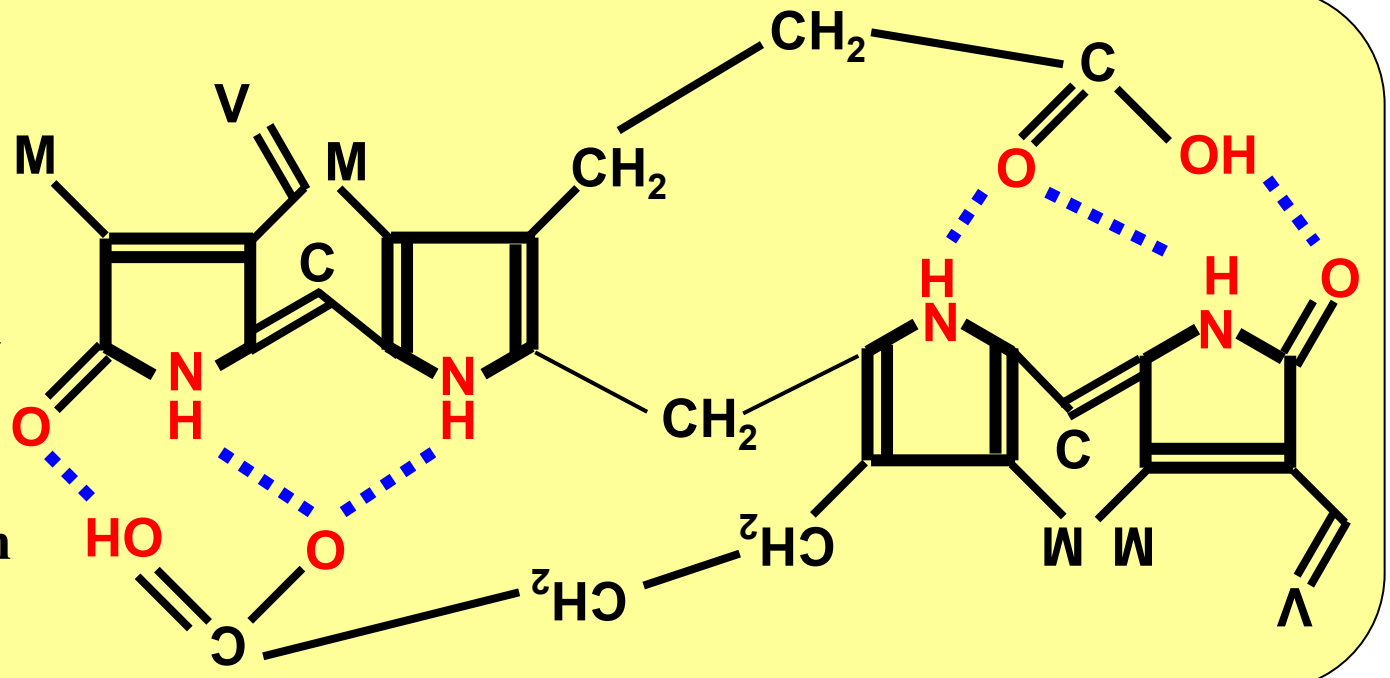
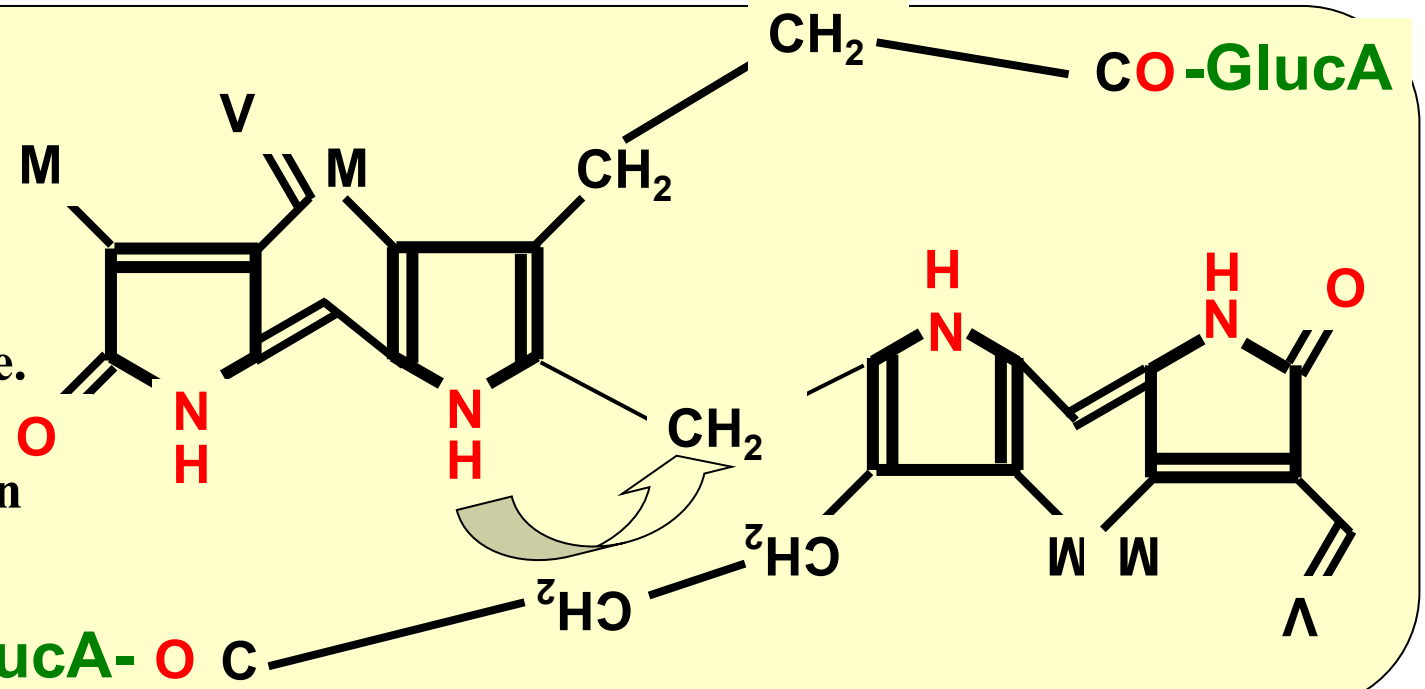


Exposure to diazo reagents result in “direct” and “indirect” van den Burgh reaction, roughly corresponding to conjugated and unconjugated fractions of bilirubin.

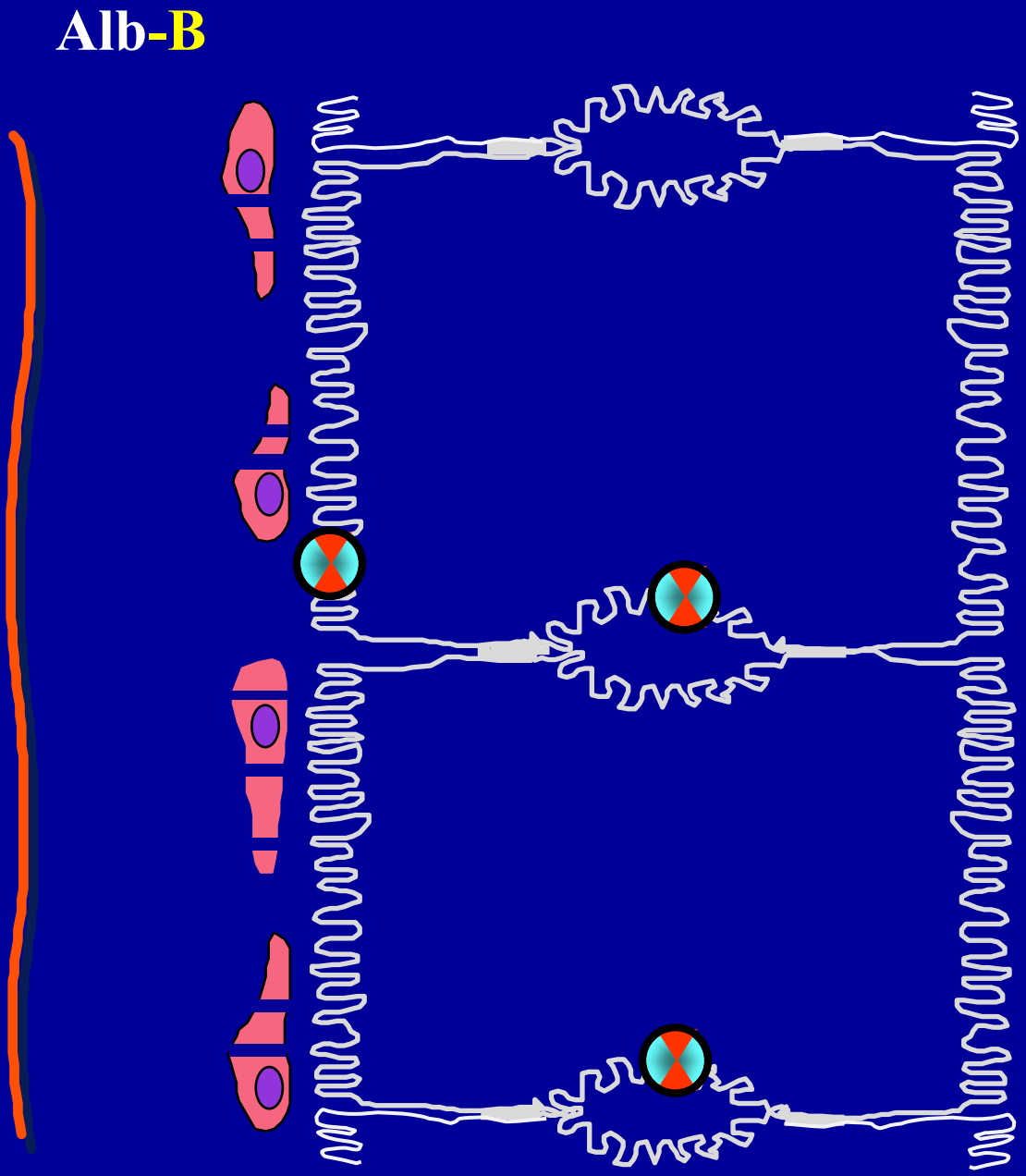
In unconjugated bilirubin, the central carbon bridge is buried by hydrogen bonds. Therefore, the van den Burgh reaction is “indirect”.



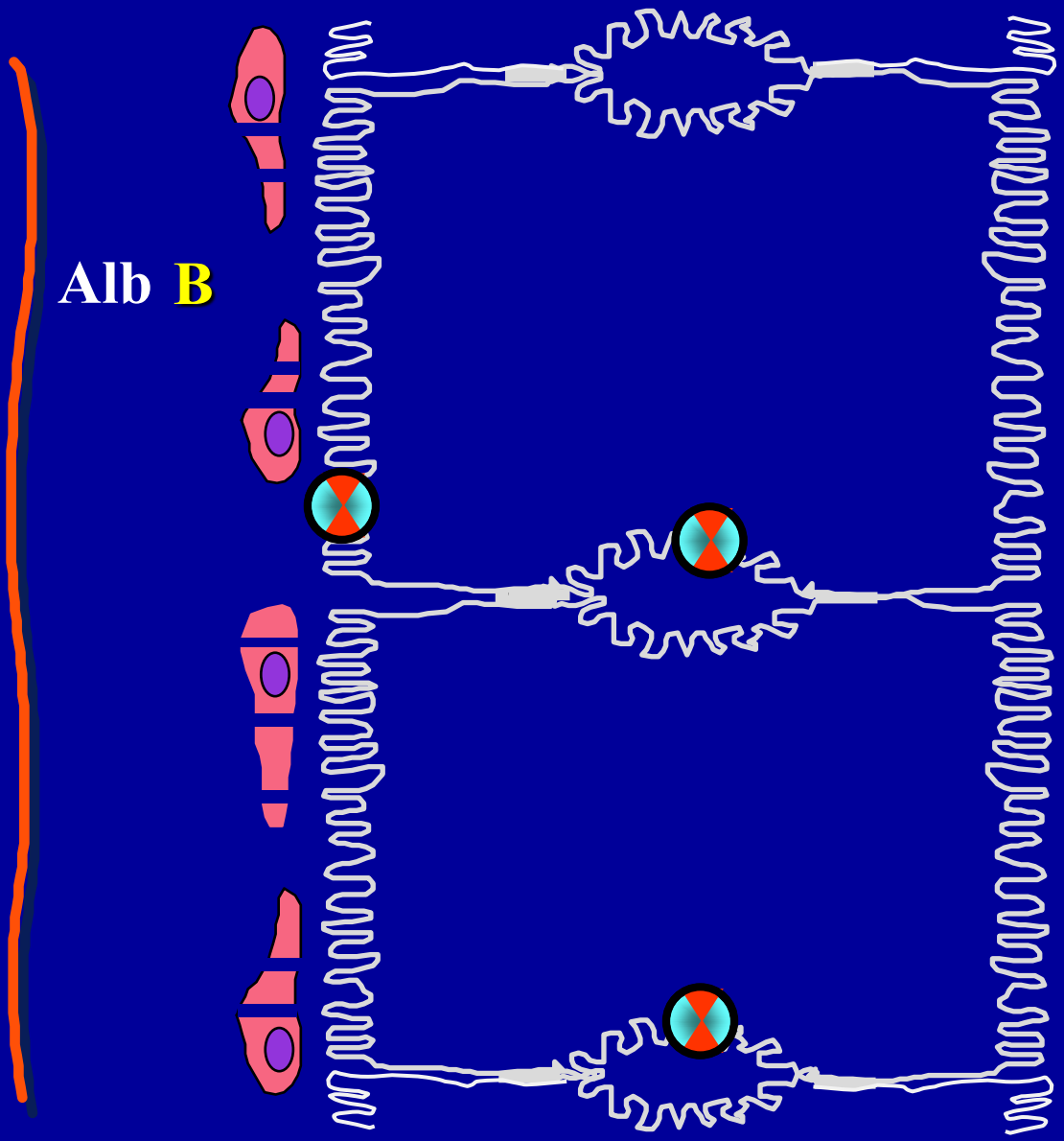
In conjugated bilirubin, the central carbon bridge is accessible. Therefore, the van den Burgh reaction is “direct”.



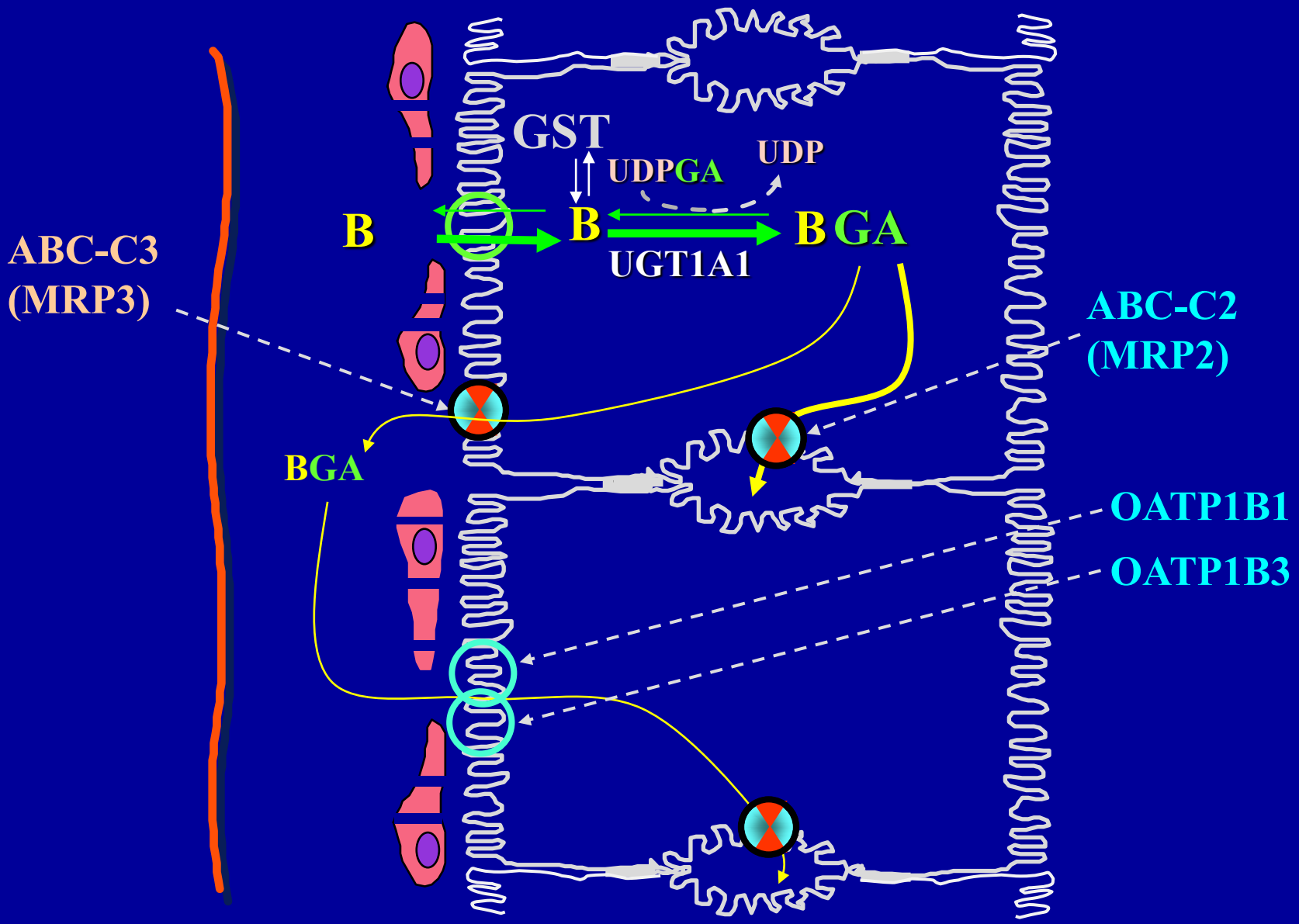
Bilirubin throughput: schema of hepatocytes

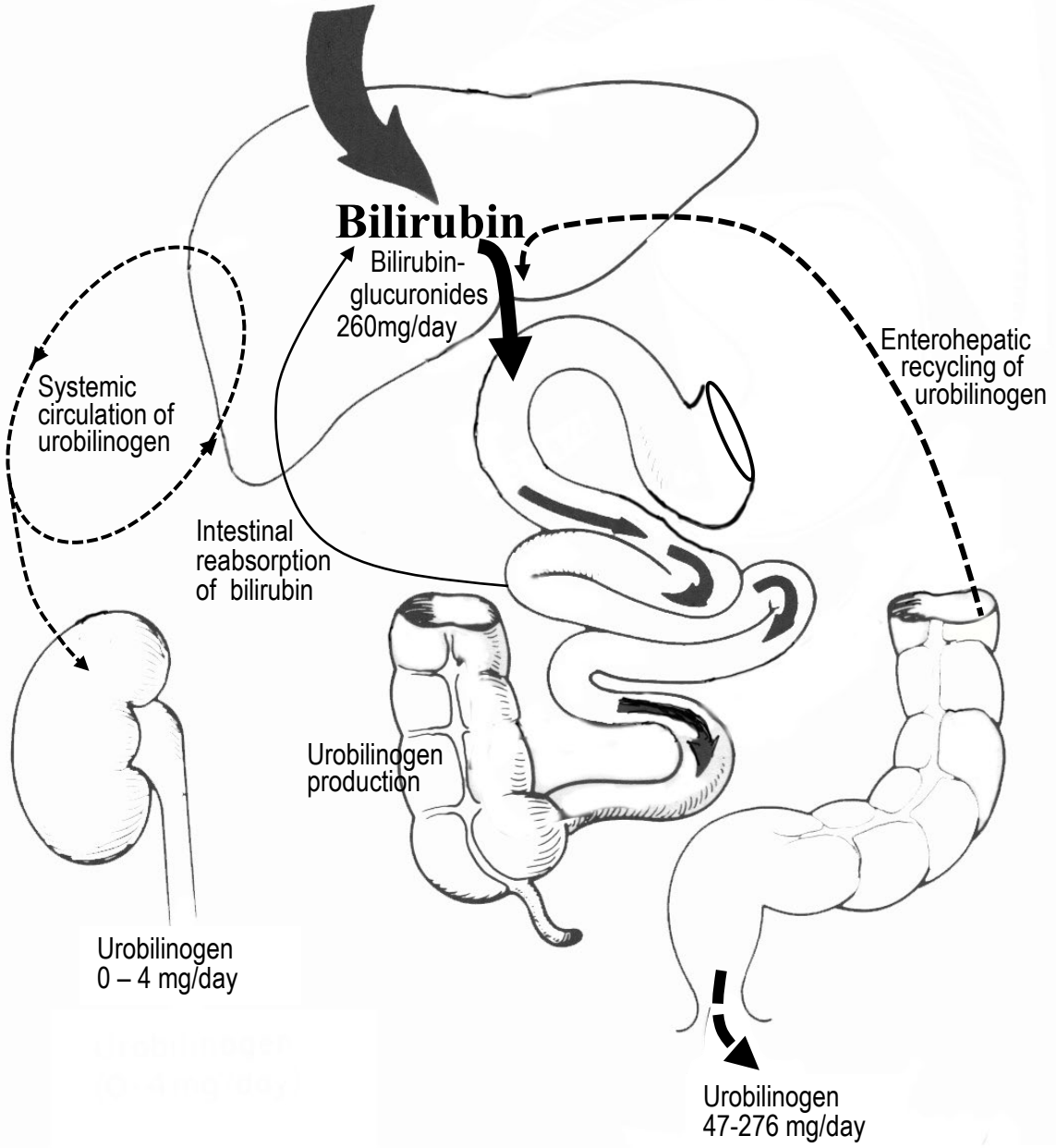


Bilirubin throughput: schema of hepatocytes



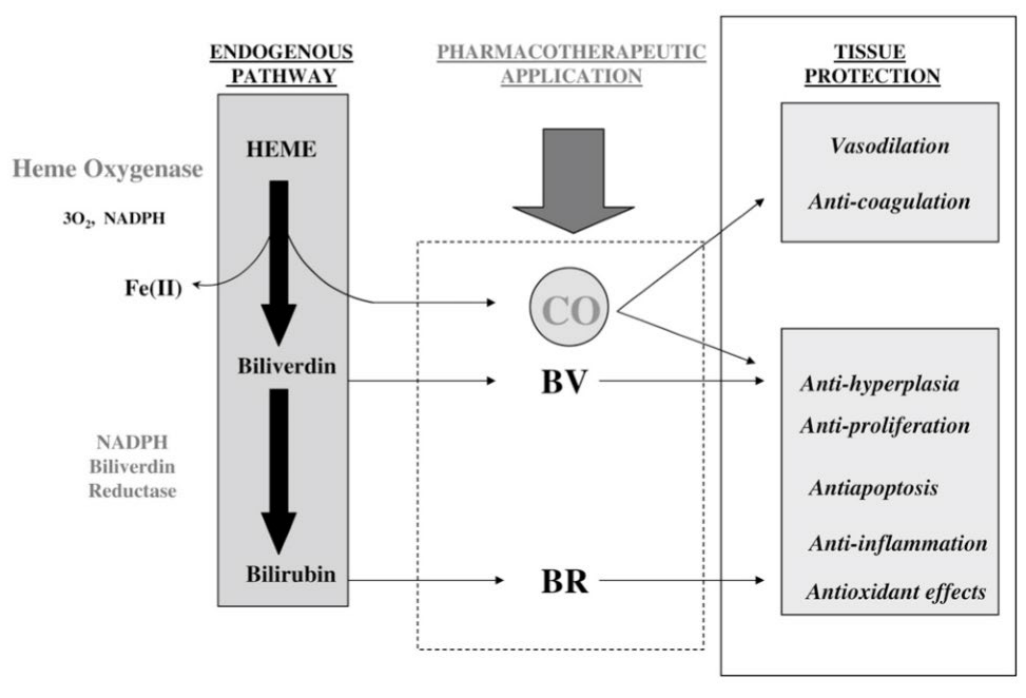
Bilirubin throughput: schema of hepatocytes




Bilirubin
Bilirubin-
glucuronides
260mg/day
Enterohepatic
recycling of
urobilinogen
Systemic
circulation of
urobilinogen
Intestinal
reabsorption
of bilirubin
Urobilinogen
production
Urobilinogen
0 – 4 mg/day
Urobilinogen
47-276 mg/day

THE GOOD

Physiological benefits of HO-1 products



Ryter SW, et al.Am J Respir Cell Mol Biol Vol 36. pp 175–182, 2007

Bilirubin is antioxidant and antiinflammatory

Superoxide and H_2O_2, generated by intermediary metabolism give rise to reactive oxygen species (ROS)

ROS-mediated lipid peroxidation injures cell membranes.

Oxidative stress-associated diseases in adults:

- Cardiovascular disease
- Cancer
- Metabolic syndrome, NAFLD
- SLE

- As a powerful lipipophilic antioxidant, bilirubin complements the ROS neutralizing function of hydrophilic antioxidants (Vit C, Vit E and glutathione).
- Bilirubin also has an anti-inflammatory effect.

Within normal range, serum bilirubin is inversely related to the risk of CAD

CAD and CVD risk	Reference
Ischemic heart disease in middle age British men: Serum bilirubin **<0.41 mg/dl** vs. **0.47 – 0.53 mg/dl**, showed a **30% reduction in relative risk** ERR = 0.68 (95% CI 0.51-0.89)]	Breimer, L.H., et al *Clin Chem*, 41, 1504 (1995)
A **50% decrease** in serum bilirubin is associated with a **47% increase** in CAD risk	Schwertner HA, et al. *Clin Chem* 40:18-23 (1994).
In the Famingham Heart Study individuals with **higher serum bilirubin** (1.14±0.44 mg/dL) had about **one third the CVD risk** of those with lower levels (0.69±0.27 mg/dL) HR ratio 0.36 (95% CI 0.18 to 0.74).	Lin JP, et al *Circulation*; 114:1476-1481 (2006)
In overweight individuals, log-total bilirubin was positively correlated with %flow-mediated coronary dilatation and HDL; and inversely correlated with log-hs-CRP suggesting that bilirubin increases coronary endothelial function via anti-inflammatory effects.	Yoshino S, et al. J Atheroscler Thromb 2011;18:403-412.

Within normal range, serum bilirubin is inversely related to cancer risk

Condition	Relative risk	Reference
>0.6 mg/dl vs <0.2 mgdl serum bilirubin: • Death from all causes in Belgian men: • Death from cancers in Belgian men:	0.73 (95% CI 0.57 - 0.94) 0.42 (95% CI0.26 - 0.68)	Temme EHL, et al. *Cancer Causes and Control* 12: 887–894 (2001)
In NHANES-III (n=176000) marked decrease in colorectal cancer prevalence per 1-mg/dl increment in serum bilirubin	0.257 (95% CI: 0.254-0.260)	Zucker SD, et al. Hepatology 40:827-835, 2004
Metabolomic study in a large case control and prospective study showed higher lung cancer incidence and mortality in male smokers with low (<0.75 mg/dl) vs high (>1.0 mg/dl) bilirubin. ($P < 0.001$)	Incidence and mortality increased 5% and 6%, respectively per 0.1 mg/dl decrease of bilirubin	Chi-Pang Wen, et al. *Clin Cancer Res*

Within normal range, bilirubin is inversely related to non-alcoholic fatty liver disease

Condition	Relative risk	Reference
Serum bilirubin levels are inversely correlated with incidence of NAFLD in 9096 men and 8222 women.	OR=0.83 and 0.80, in the 3rd and 4th quartile (vs. lowest quartile), P for trend <0.001)	Kwak et al. *Clin Mol Hepatol* 18:383-390 (2012)
NAFLD patients with serum UCB >1.2 had less frequent high NASH score (>4) and advanced fibrosis (p = 0.005). Moreover, significantly higher number of patients with UCHB had no liver fibrosis (53.3% vs. 11%, p = 0.008).	***NAS >4***: UCB >1.2: 40% UCB <1.2:60% (p=0.05) ***Fibrosis:*** UCB >1.2: 48% UCB <1.2: 89% (p=0.008)	Kumar R, et al, *Clin Biochem* 45:272-274 (2012)
In 640 patients, serum bilirubin was inversely related with NASH incidence.	***Total bilirubin >1.0 vs >1.0 mg/dl:*** OR 16.1, 95% CI 3.7–70.8 P < 0.001).	Hjelkrem M, et al. Aliment Pharmacol Ther 35:1416-1423 (2012).

THE BAD

- **Mixed hyperbilirubinemia is a marker of liver disease**
- **Conjugated hyperbilirubinemia (as in Dubin-Johnson syndrome and Rotor syndrome) can cause confusion with liver disease.**

THE UGLY

Neonatal jaundice:

Exaggerated neonatal jaundice can cause bilirubin-induced neural damage (BIND).

Inherited jaundice:

Particularly Crigler-Najjar syndrome type-1, is associated with a life-long risk of BIND.

Neonatal hyperbilirubinemia

- Human neonates have higher serum bilirubin levels than adults ("physiological jaundice").
- In most cases, bilirubin levels peak at 96 hr and resolve by 1 week.
- Neonatal hyperbilirubinemia is exacerbated by feeding human milk.
- Greatly exacerbated neonatal hyperbilirubinemia can cause BIND.
  Kernicterus refers to yellow discoloration of the brain.
- As albumin binding prevents bilirubin toxicity, the treatment target is to keep bilirubin/albumin molar ratio under 0.7.
- Determination of free (unbound) bilirubin fraction can be helpful, but is used only in Japan.

Neonatal hyperbilirubinemia

Neonatal hyperbilirubinemia is caused by:

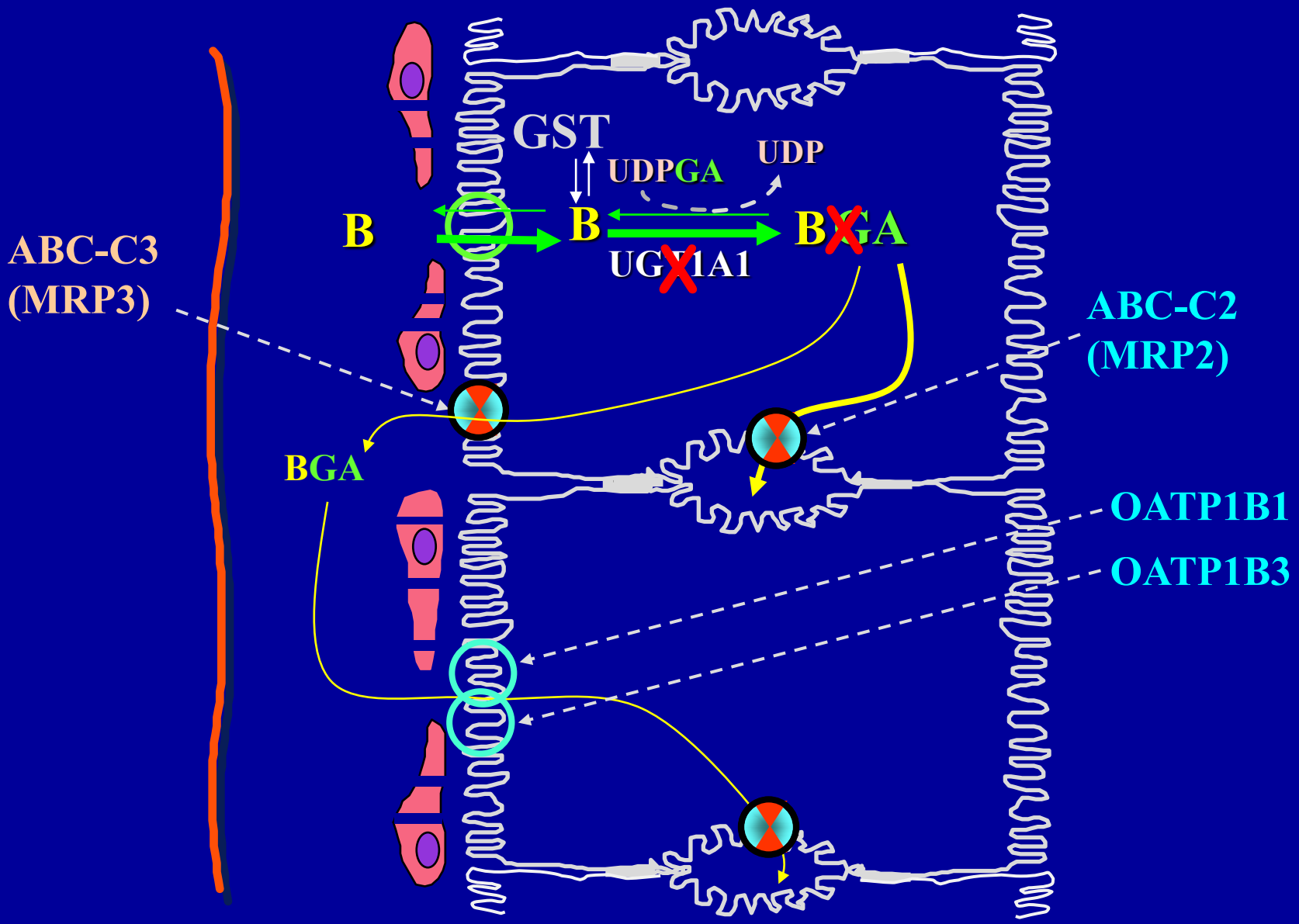
- Increased bilirubin production due to enhanced hemolysis.
- Delayed expression of hepatic UGT1A1
  (Note: in human neonates intestinal UGT1A1 is the major mechanism of bilirubin glucuronidation).
- At later time points, bile canalicular excretion may become rate limiting, whereby conjugated bilirubin also accumulates in plasma.

Breast feeding enhances neonatal hyperbilirubinemia by:

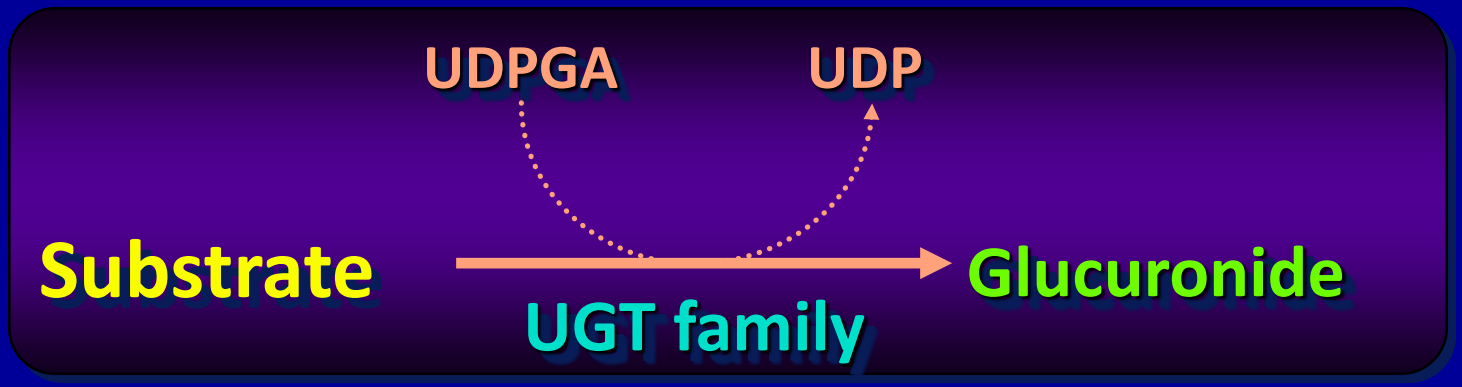
- Reducing caloric intake
- Inhibiting intestinal UGT1A1
- Increasing the reabsorption of bilirubin secreted in bile

Inherited disorders causing unconjugated hyperbilirubinemia

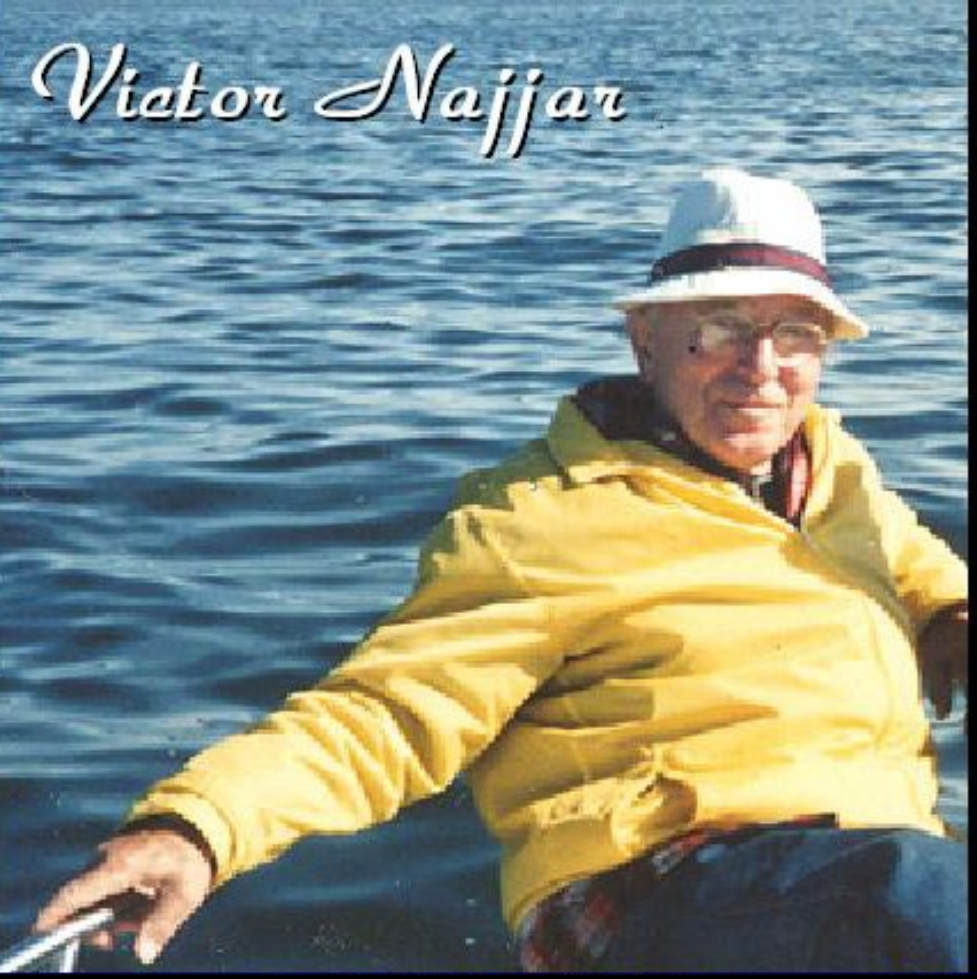
Bilirubin throughput: schema of hepatocytes

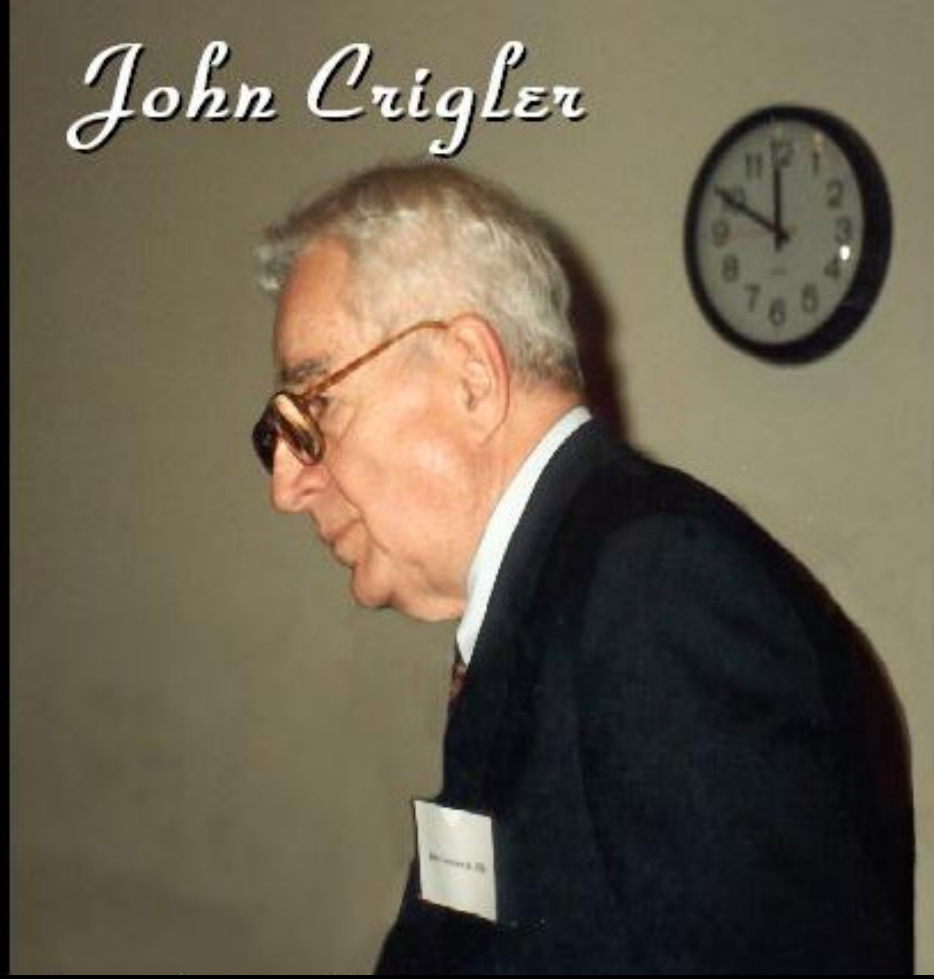


UDP-glucuronosyltransferases (UGTs)



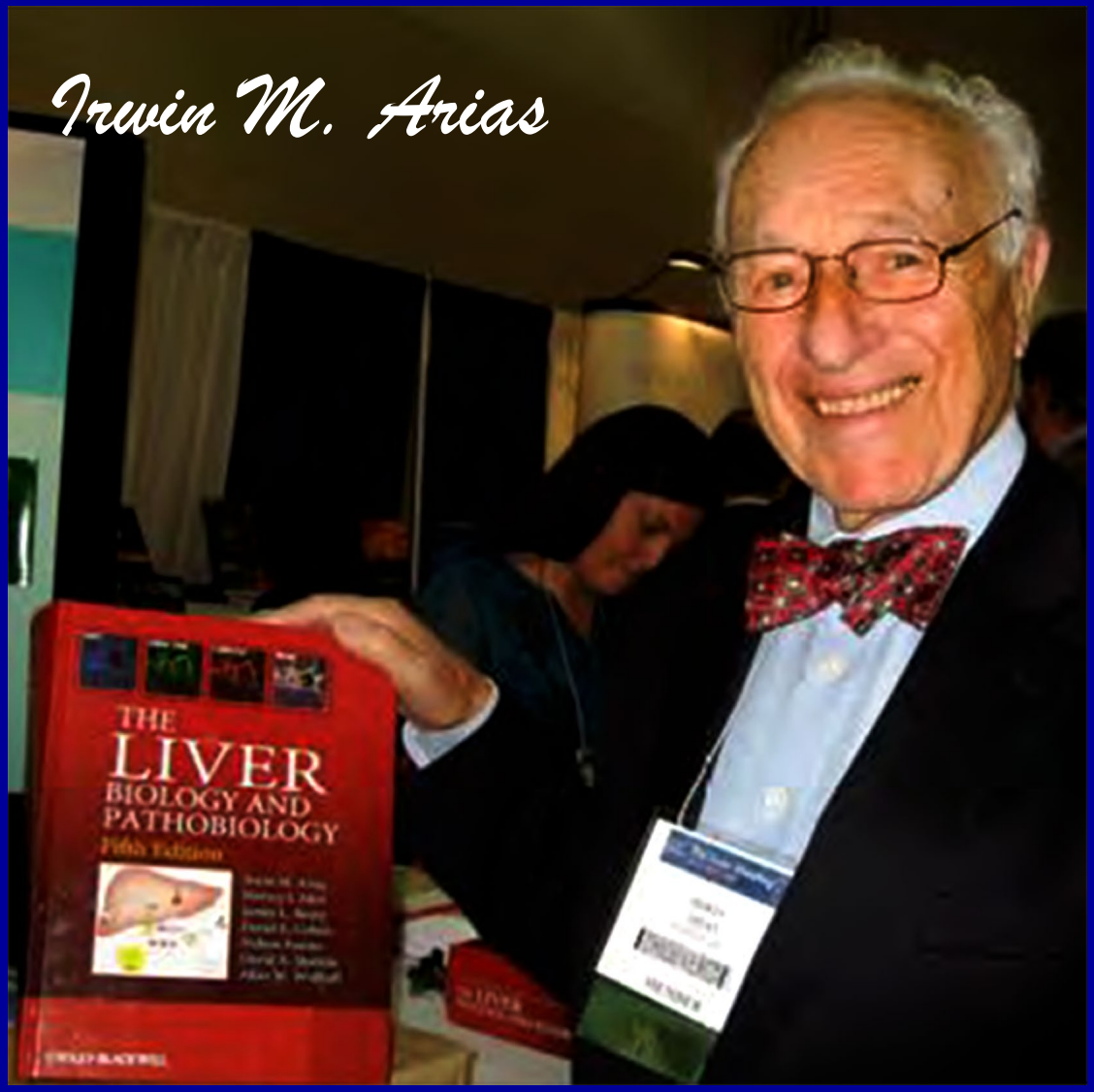
- UGTs are ER proteins that convert many internal and exogenous toxins to non-toxic metabolites.
- UGTs are a **family of enzymes** concentrated in the liver.
- One UGT isoform, **UGT1A1, conjugates bilirubin** and is essential for its excretion.
- Inherited UGT1A1 deficiency causes jaundice.





In 1953, Crigler and Najjar described “a mysterious illness that caused jaundice and severe neurological damage”

Crigler JF & Najjar VA *Pediatrics* 10:169 (1952)



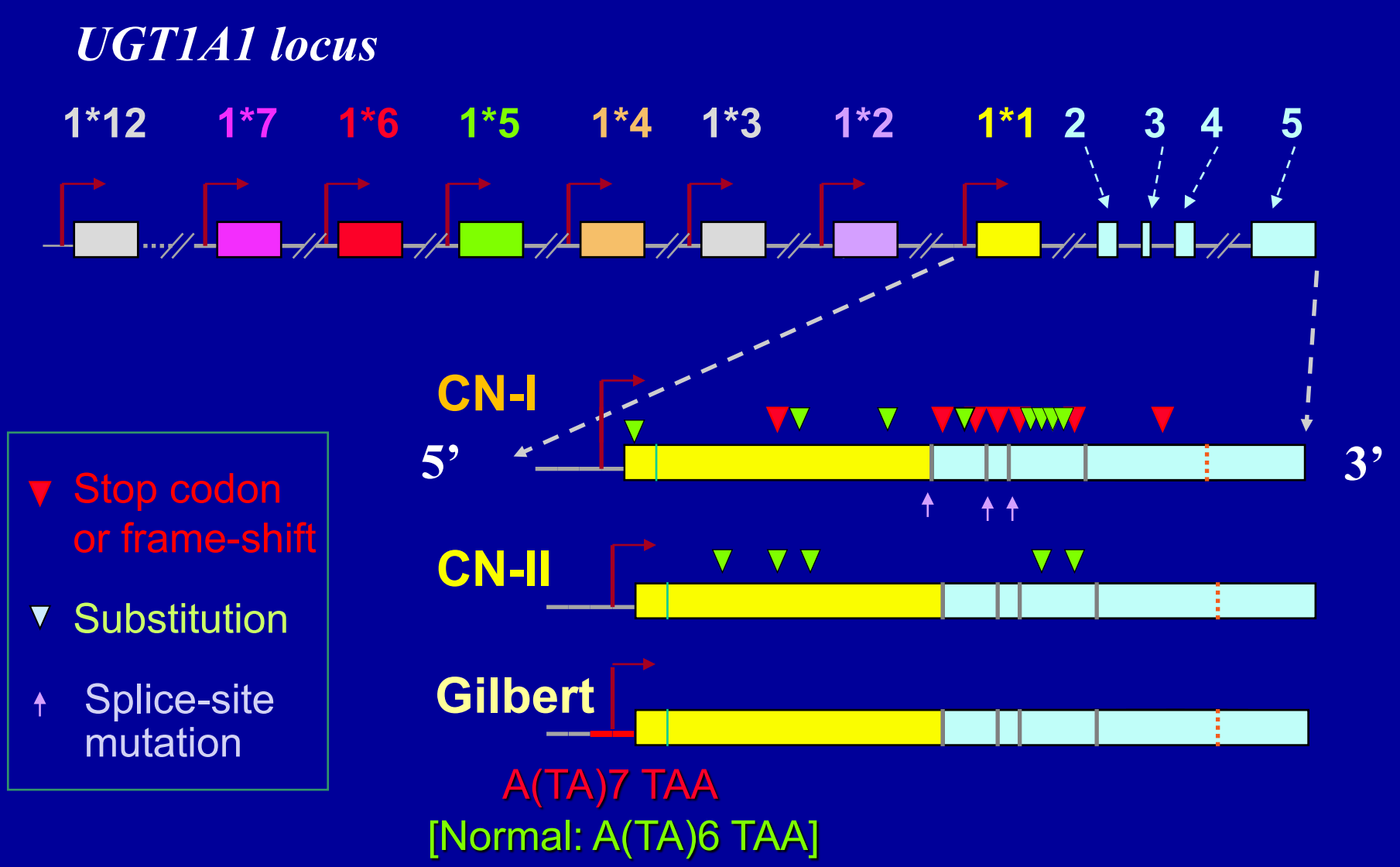
In 1962, Irwin Arias described a milder variant of Crigler-Najjar syndrome, now known as CN-2 or Arias syndrome

Arias IM *J Clin Investig* 41:2233 (1962)

In 1901, A. Gilbert and P. Lereboullet described a condition characterized by mild, intermittent, non-hemolytic jaundice, now termed Gilbert syndrome.

Gilbert A & Lereboullet *Semin Medicine* 21:241 (1901)

Genetic lesions causing UGT1A1 deficiency

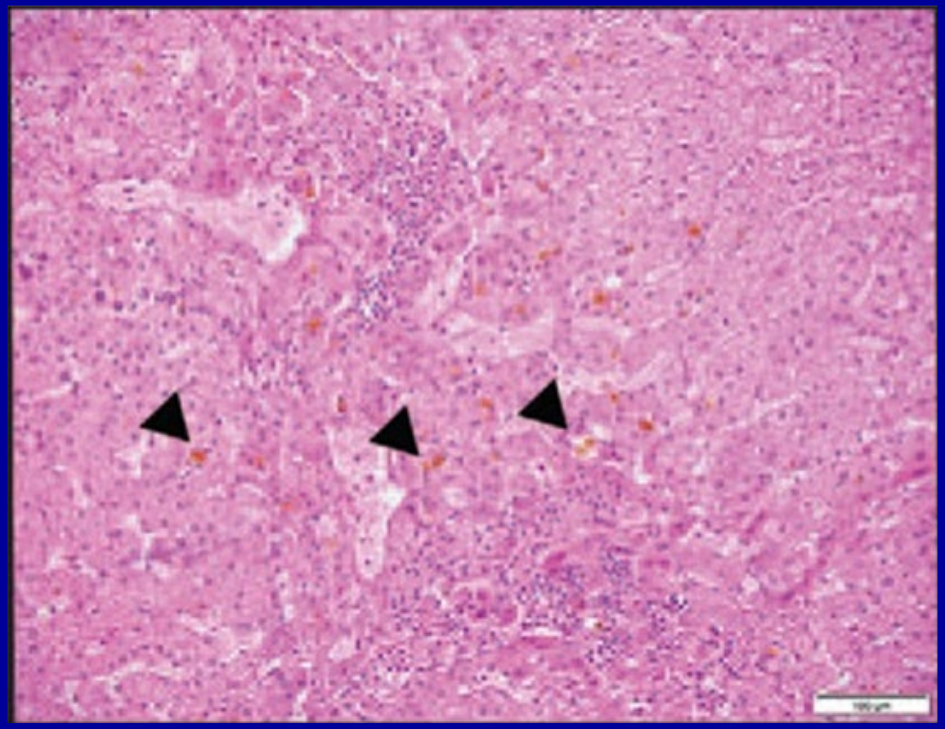


Bosma PJ, Roy-Chowdhury N, et al. *Hepatology* 15:941 (1992)
Bosma PJ, Roy-Chowdhury J, et al. *New Eng J Med* 333:1171 (1995)

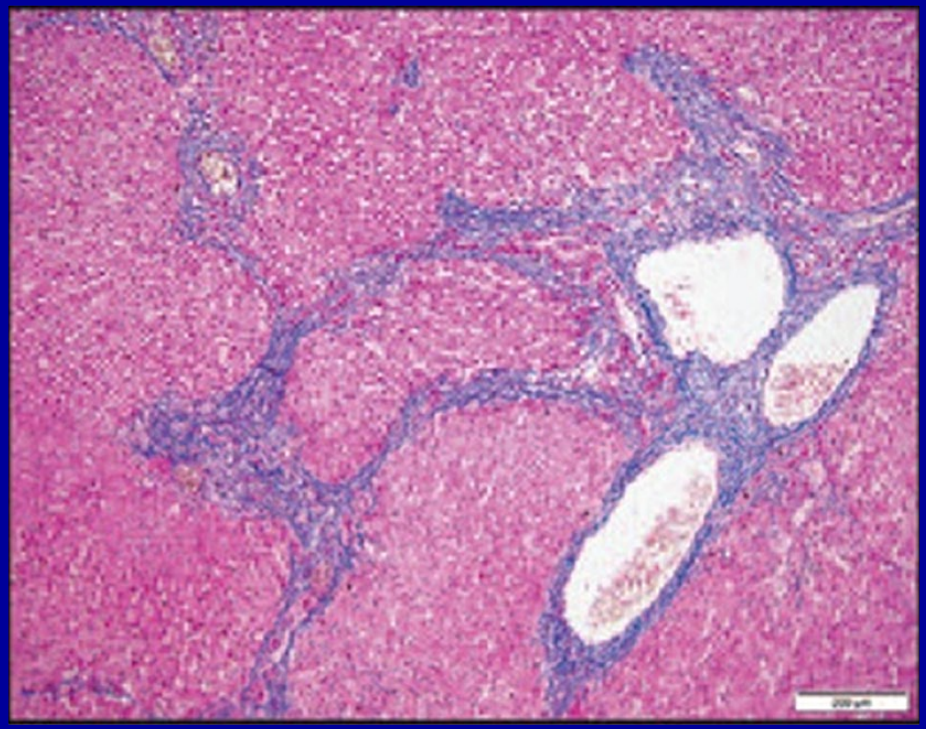
Characteristics of UGT1A1 deficiency syndromes

Parameters	Crigler-Najjar Syndrome type 1	Crigler-Najjar Syndrome type 2	Gilbert Syndrome
Serum Bilirubin	20 - 50 mg/dl (340-510 μmol/L)	3-30 mg/dl (fluctuates) (51-510 μmol/L)	1-5 mg/dl (fluctuates) (17-85 μmol/L)
Routine LFT's	Normal	Normal	Normal
Histology	Normal	Normal	Normal
BUGT Activity	Absent	Markedly reduced	Reduced to ~30%
Bile Pigments	Only traces of BMG and BDG	Increased BMG : BDG ratio	Increased BMG : BDG ratio
Phenobarbital	No effect	Reduces bilirubin by >25%	Jaundice disappears
Prevalence	Rare	Rare	Common (<5% of the population)
Age of Diagnosis	1 - 3 d postnatal	Usually during first year	Usually early adulthood
Prognosis	Kernicterus (unless OLT)	Kernicterus is unusual	No neuropathy (benign)

Liver histology in Crigler-Najjar syndrome-1



Historically, liver was considered to be structurally and histologically normal in CN-1, except bilirubin "plugs" in bile canaliculi caused by precipitation of a small amount of secreted unconjugated bilirubin.



Recent systematic analysis of explanted livers during transplantation has shown significant fibrosis, especially in some older patients. But cirrhotic nodules were not seen and portal hypertension was absent.

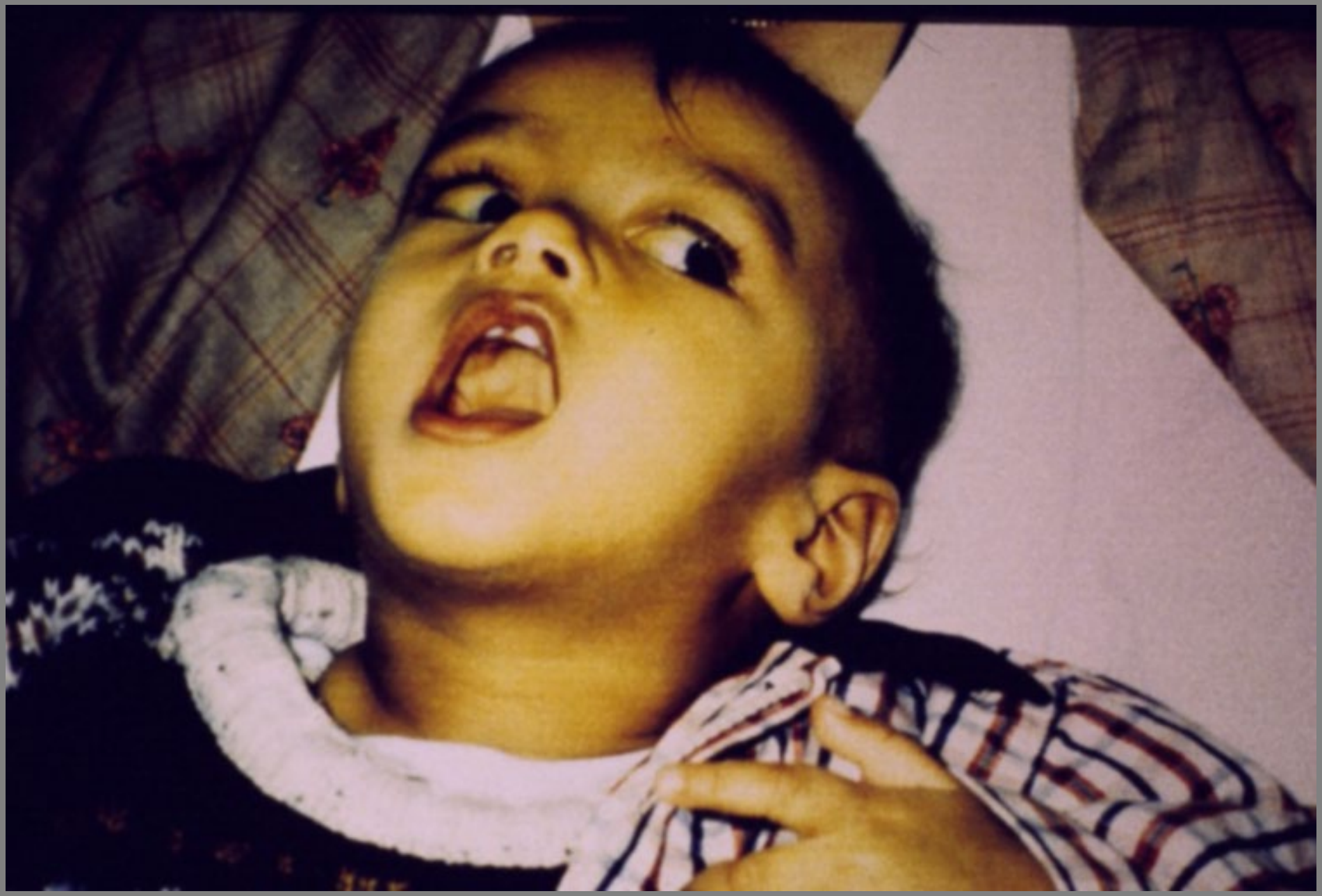
Michell et al. J Pediatr Gastroenterol Nutr 66:588 (2018)

Treatment of Crigler-Najjar syndrome type 1

Conventional

- Phototherapy: Daily
- Plasmapheresis: In emergency
- Liver transplantation: OLT or auxiliary: definitive treatment, but prolonged immunosuppression needed

Phototherapy bed

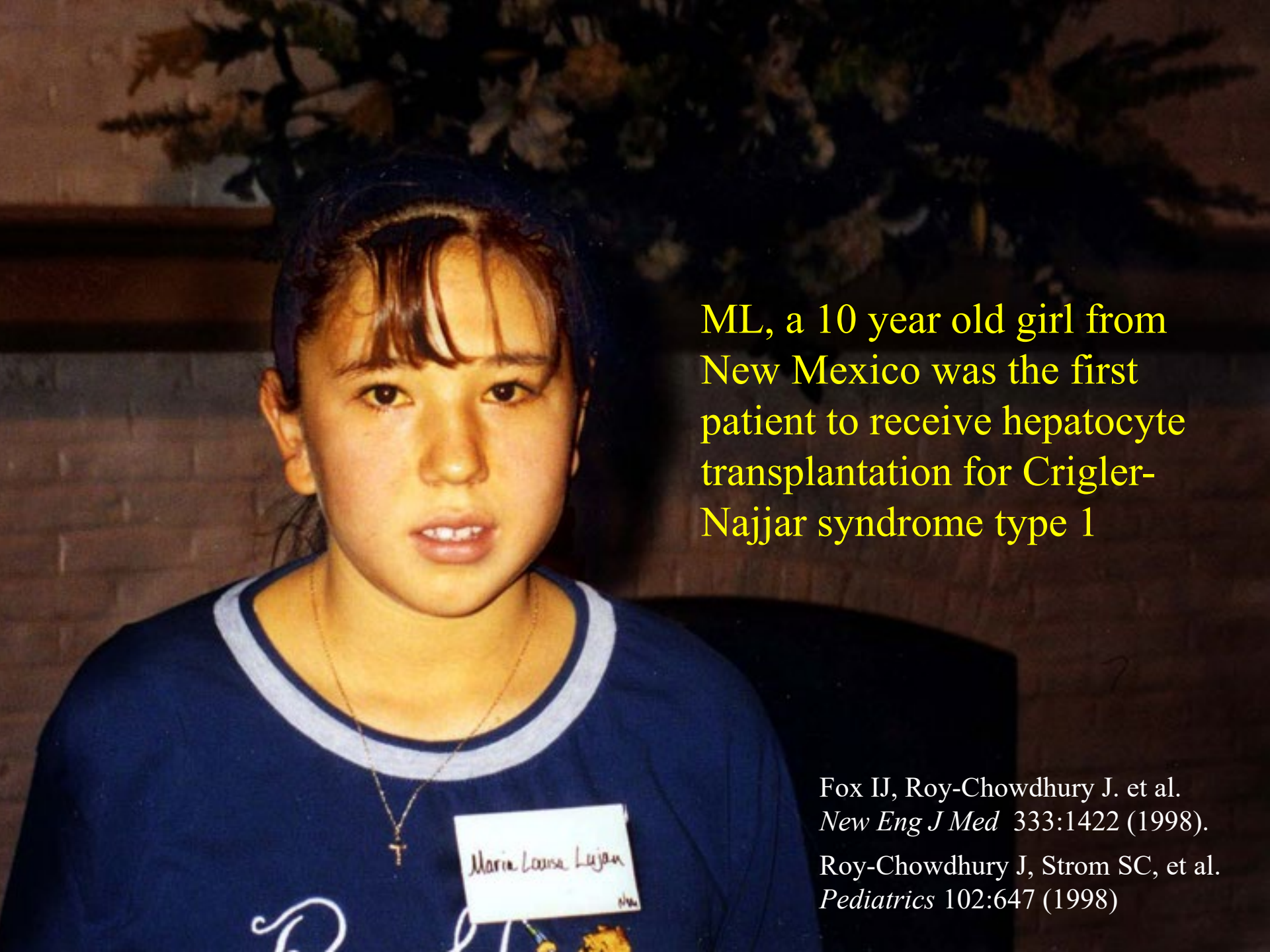


CN-1 syndrome-1: permanent brain damage

Treatment of Crigler-Najjar syndrome type 1

Emerging therapies

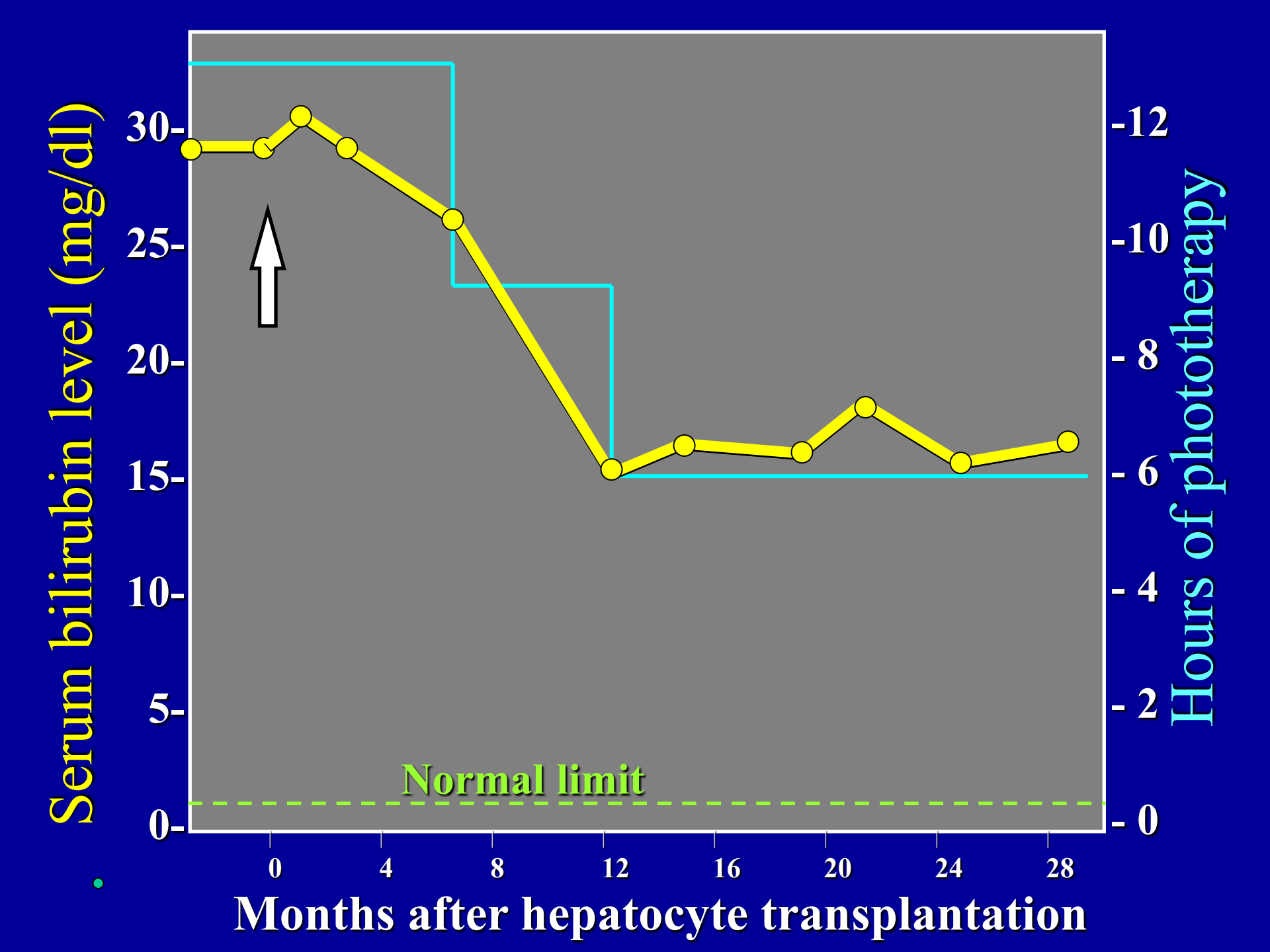
- **Hepatocyte transplantation**:
  Requires immunosuppression to prevent allograft rejection.
- **Gene therapy**:
  Recombinant adeno-associated virus.
- **Gene editing:**
  On the horizon.



ML, a 10 year old girl from New Mexico was the first patient to receive hepatocyte transplantation for Crigler-Najjar syndrome type 1

Fox IJ, Roy-Chowdhury J. et al. *New Eng J Med* 333:1422 (1998).

Roy-Chowdhury J, Strom SC, et al. *Pediatrics* 102:647 (1998)


Serum bilirubin level (mg/dl)
30
25
20
15
10
5
0
Hours of phototherapy
12
10
8
6
4
2
0
Normal limit
0
4
8
12
16
20
24
28
Months after hepatocyte transplantation

Follow-up of the first hepatocyte transplantation for Crigler-Najjar syndrome-1

- Two and a half year later, the serum bilirubin started to increase. [The patient may have stopped taking tacrolimus].
- HPLC of bile showed continued presence of bilirubin glucuronides.
- The patient was transplanted with an auxiliary liver lobe and serum bilirubin became normal in 3 days.

- This case showed that hepatocyte transplantation is feasible, safe and can be effective in inherited metabolic diseases.
- However, the number of hepatocytes that can be engrafted in the liver is not sufficient to obviate the need for phototherapy.
- Continuous immunosuppression is needed to prevent allograft rejection.

Inherited disorders causing conjugated hyperbilirubinemia

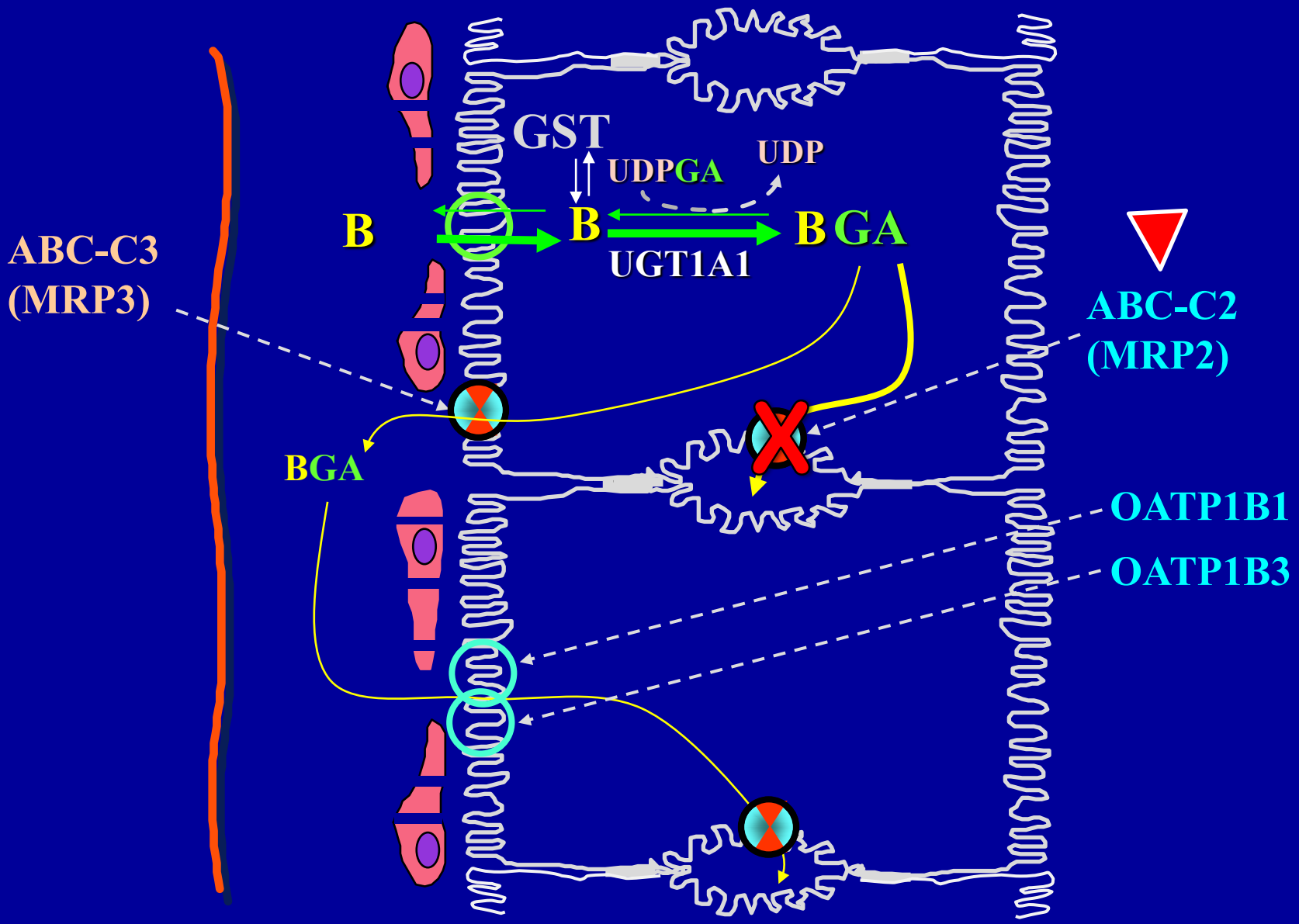
- Dubin-Johnson syndrome
- Rotor syndrome

Dubin-Johnson syndrome

In 1954, I.N. **Dubin** and FB **Johnson**, and H. **Sprinz** and RS **Nelson** reported simultaneously a syndrome characterized by:

- Conjugated hyperbilirubinemia
- **Black liver**
- Dark brown pigments in hepatocytes
- Otherwise normal liver histology and normal routine liver chemistries.

In Dubin-Johnson syndrome, ABCC2 is mutated



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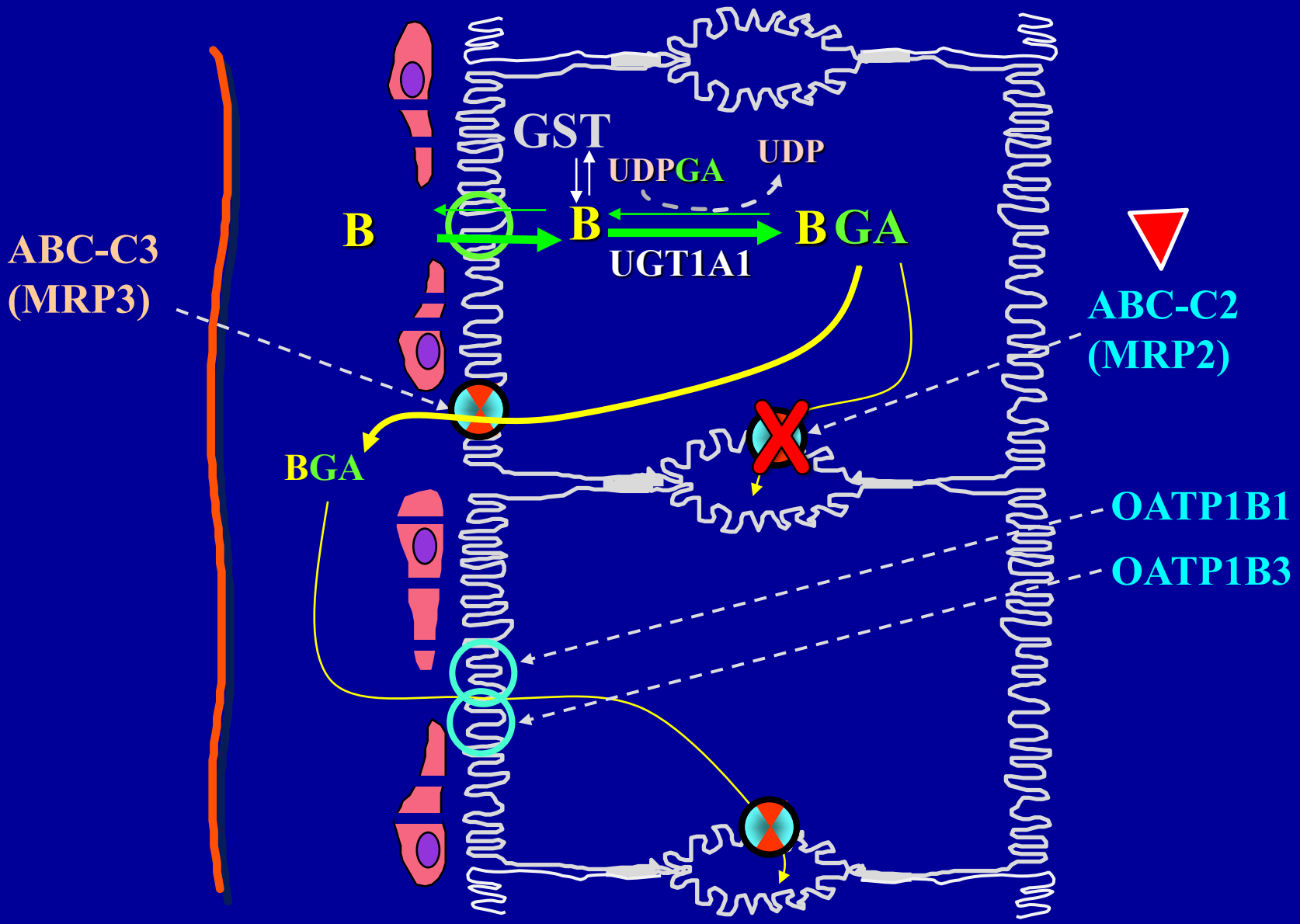
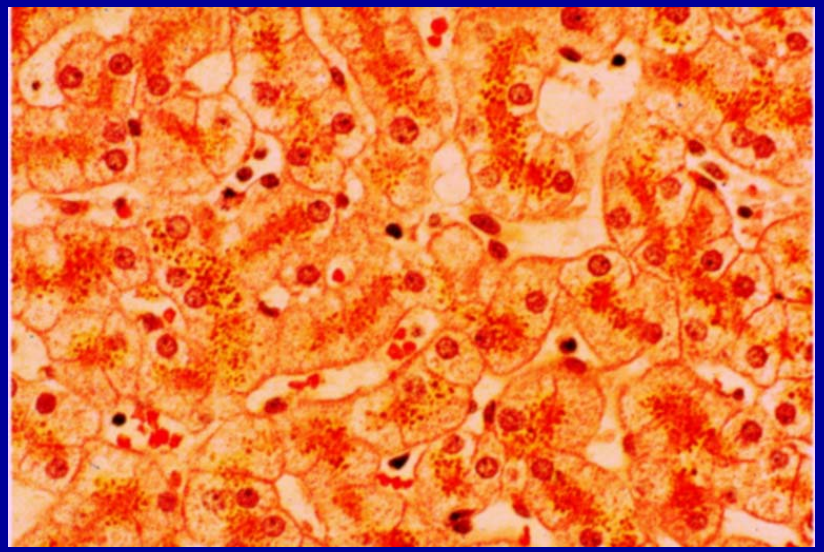


Hepatic pigmentation in Dubin-Johnson syndrome

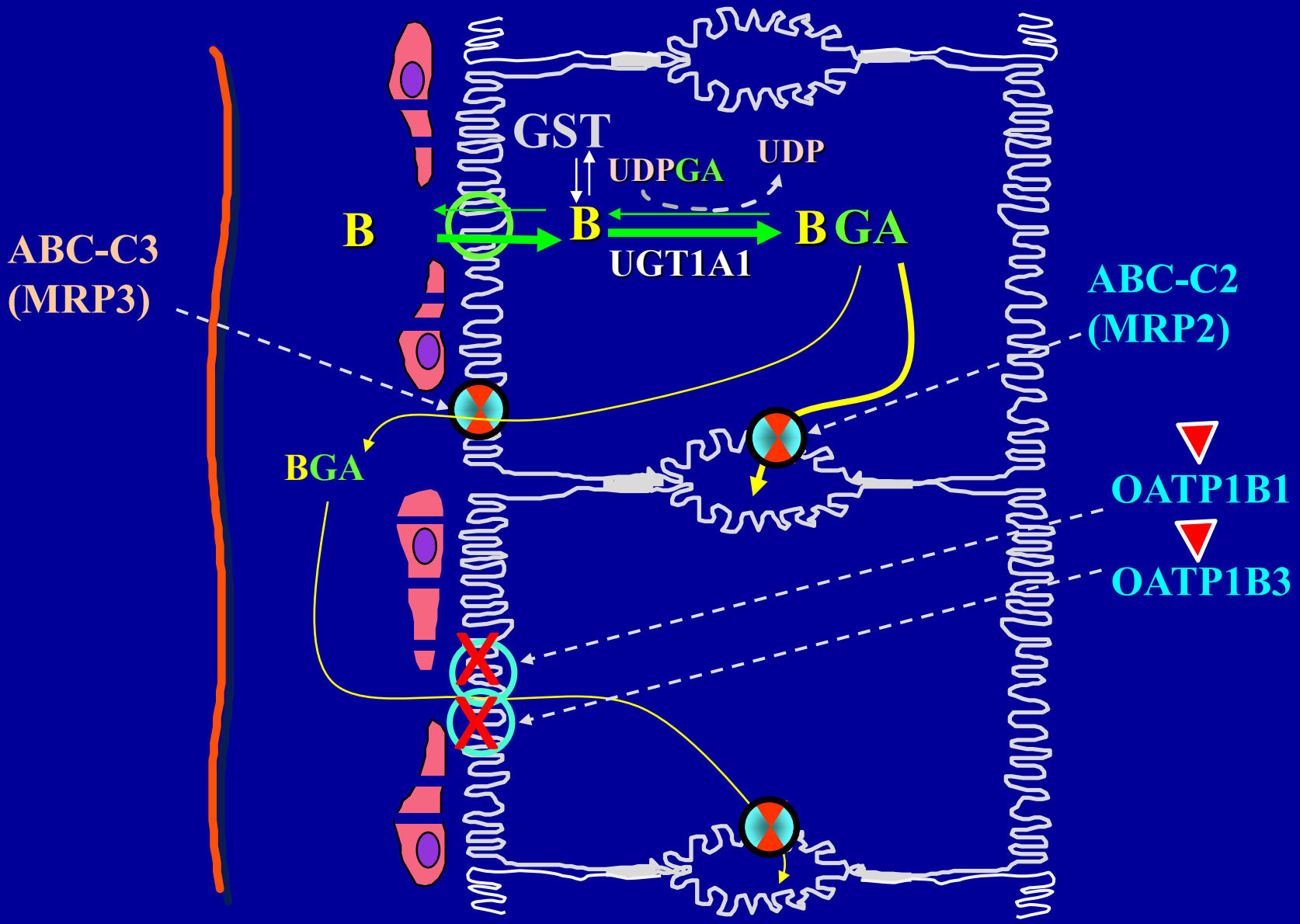
- Biliary excretion of many organic anions, but not most bile acids, is deficient in Dubin-Johnson syndrome.
- Abnormality of biliary excretion causes accumulation of a pigment in the liver.
- However, serum bilirubin is mildly elevated (3-5 mg/dl), suggesting the existence of additional pathways for bilirubin glucuronide excretion into bile



In 1948, A.B. **Rotor,** L. Manahan and A. Florentine reported a syndrome characterized by:

- Conjugated hyperbilirubinemia
- **Normal colored liver**, without pigmentation
- Otherwise normal liver histology and normal routine liver chemistries.

In Rotor syndrome, OATP1B1 & OATP1B2 are both mutated



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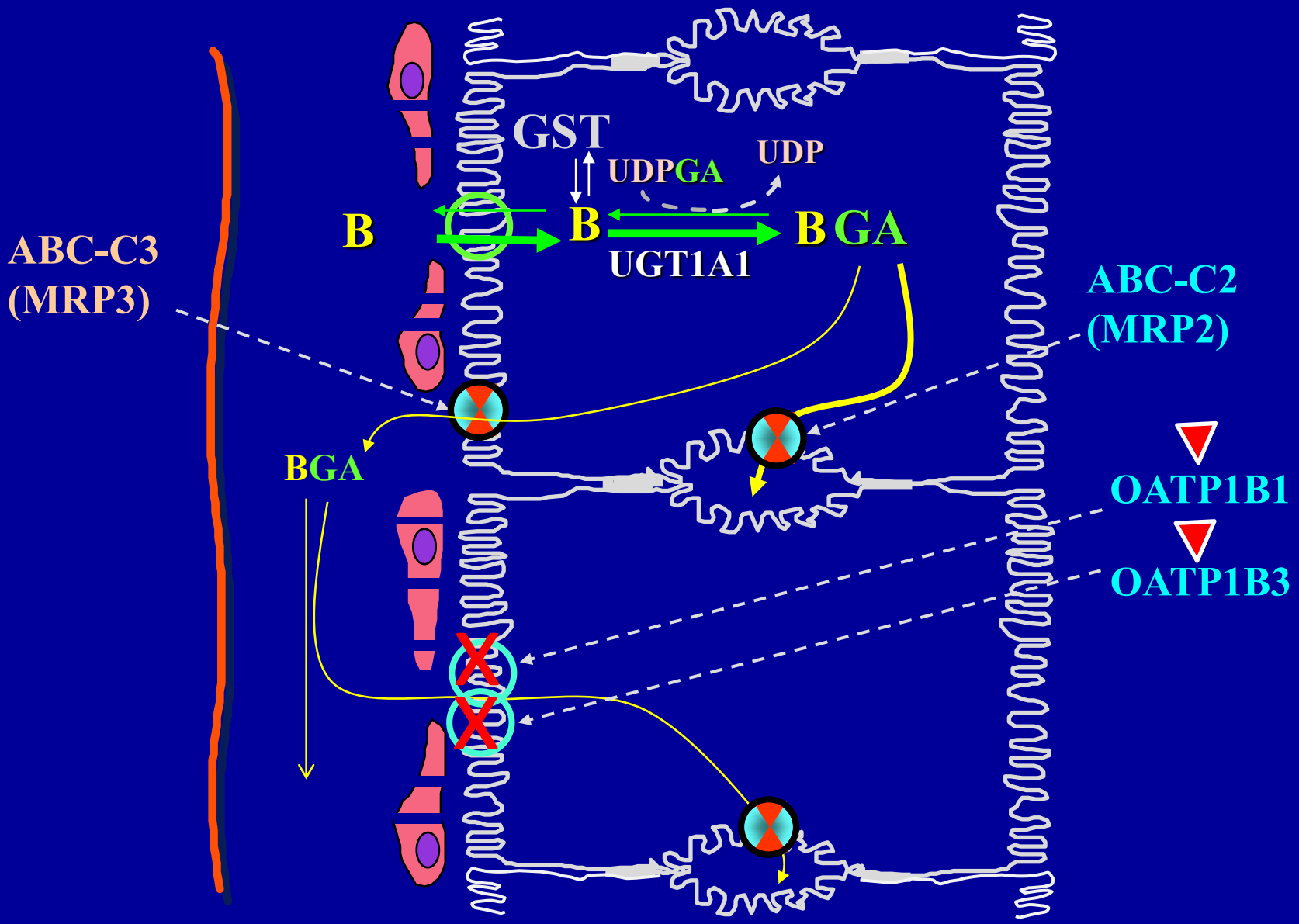
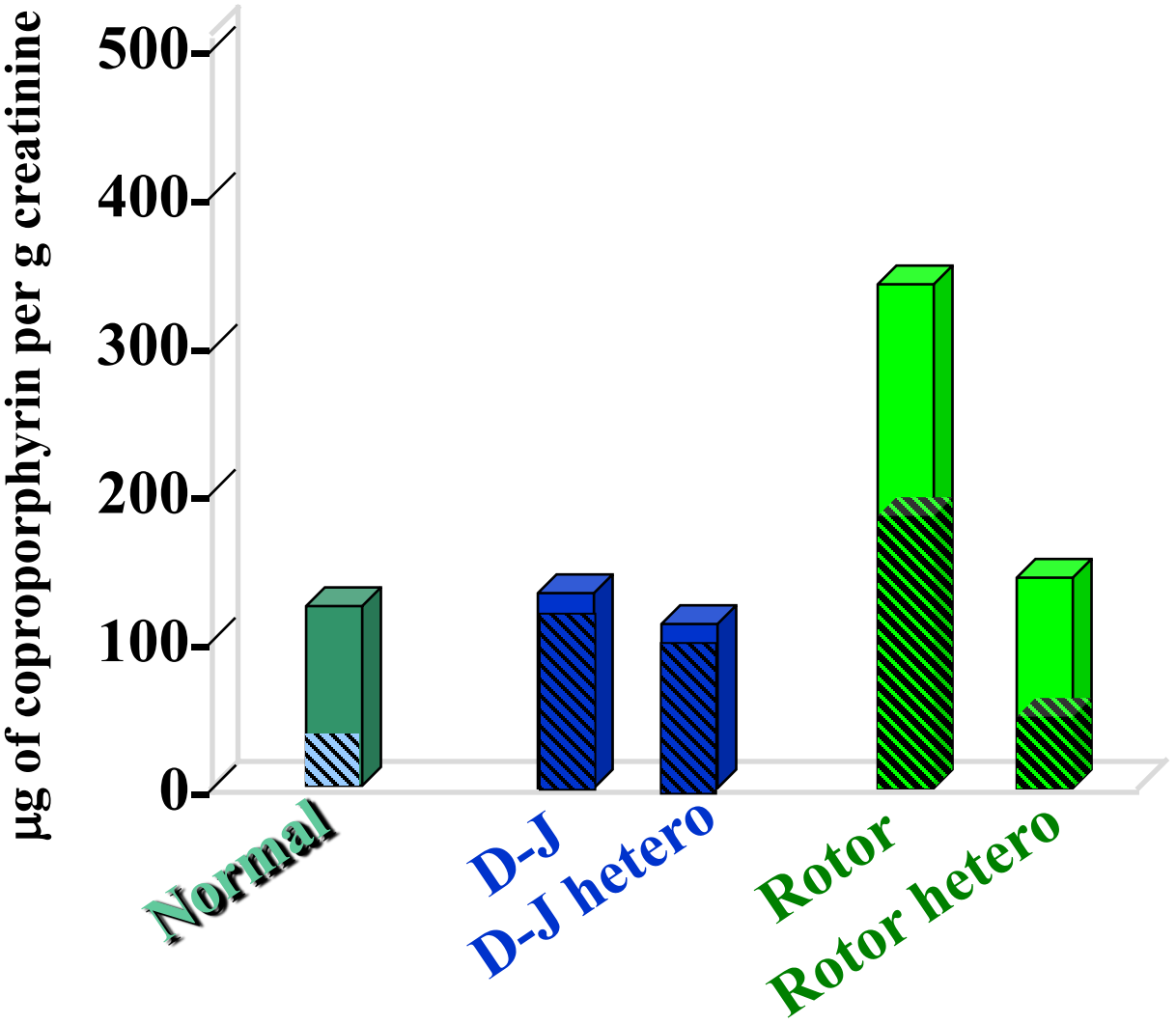


Parameters	Dubin-Johnson Syndrome	Rotor Syndrome
Serum Bilirubin	2 - 5 (-20) mg/dl (~60% conjugated)	2 - 5 (-20) mg/dl (~60% conjugated)
Routine LFT's	Normal	Normal
Histology	Dark pigment, centrilobular	Normal
Plasma BSP Retention (45 minutes)	Normal or slightly elevated Secondary rise at 90 min	Elevated No secondary rise at 90 min
BSP Infusion Studies	T_{max} reduced to 10% S normal	T_{max} reduced to 50% S reduced to 10 - 25%
Oral Cholecystogram	Gallbladder usually not visualized	Gallbladder usually visualized
Urinary Coproporphyrin	Normal total > 80% as coproporphyrin I	Elevated total Elevated coproporphyrin I, but < 80%
Mode of Inheritance	Autosomal recessive	Autosomal recessive
Prevalence	Uncommon (1:1300 in Persian Jews)	Rare
Prognosis	Benign (occasionally hepatosplenomegaly)	Benign

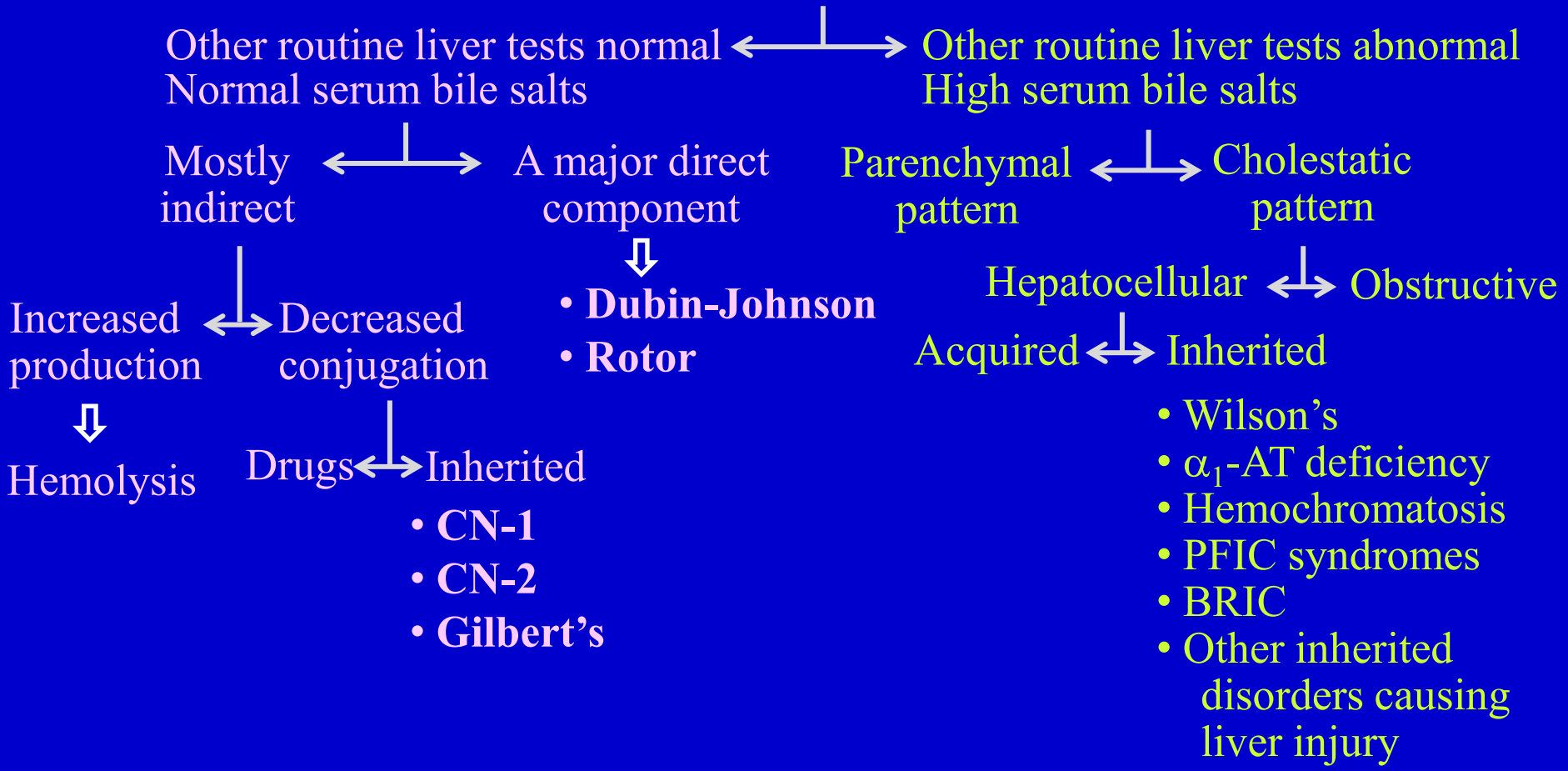
LFT: Liver Function Test; BSP: Bromsulphalein; Tmzx: Transport maximum; S: Hepatic storage capacity

Urinary coproporphyrin excretion pattern in Dubin-Johnson and Rotor syndromes



Wolkoff AW et al. Am J Med 60:173 (1976)

Hyperbilirubinemia




Thank you for
your attention!