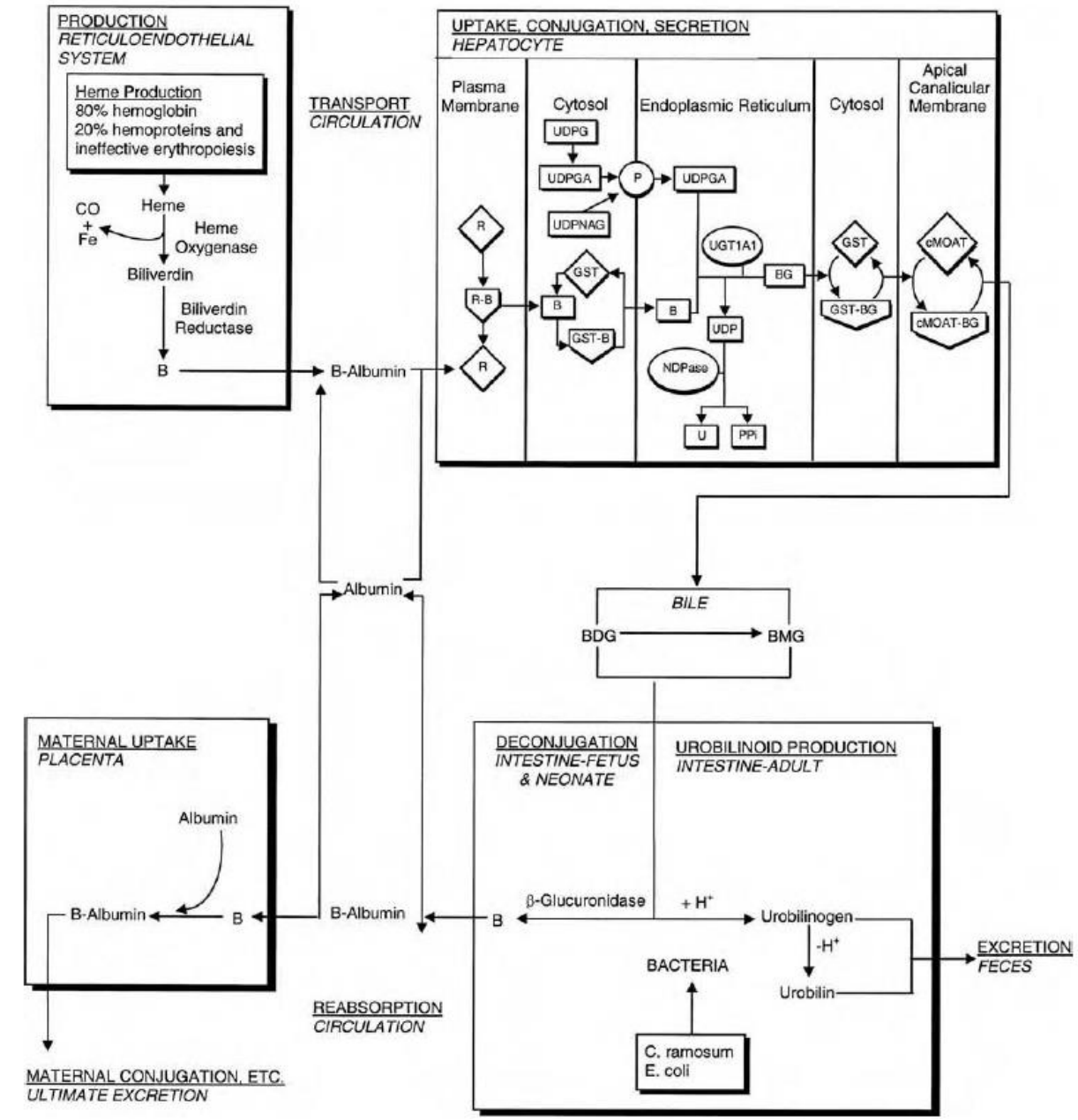
BILIRUBIN METABOLISM

DR JP,ASST PROF,ICH,MCH,KOTTAYAM



A schematic overview of bilirubin (B) metabolism in the fetus, neonate, and adult. R, membrane carrier; GST, glutathione Stransferase (ligandin); UDPG, uridine diphosphate glucose; UDPGA, uridine diphosphate glucuronic acid; UDPNAG, uridine diphosphate *N*-acetylglucosamine; P, permease; UGT1A1, bilirubin glucuronosyltransferase; NDPase, nucleoside diphosphatase; PPi, inorganic pyrophosphate; BDG/BMG, bilirubin di- or monoglucuronide; cMOAT, canalicular multispecific organic anion transporter (also known as MRP2 or ABCC2); BG, bilirubin glucuronide

Production and Metabolism of Bilirubin

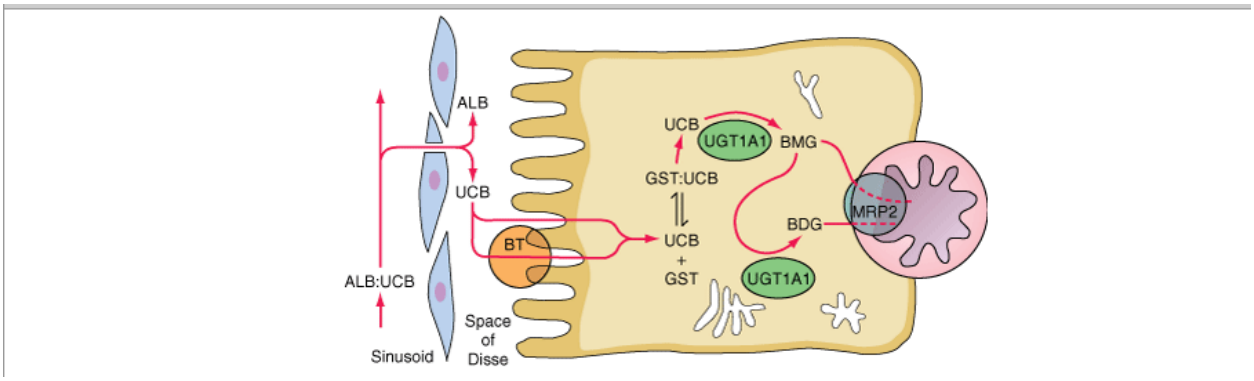
- Bilirubin, a tetrapyrrole pigment, is a breakdown product of heme (ferroprotoporphyrin IX). About 70–80% of the 250–300 mg of bilirubin produced each day is derived from the breakdown of hemoglobin in senescent red blood cells. The remainder comes from prematurely destroyed erythroid cells in bone marrow and from the turnover of hemoproteins such as myoglobin and cytochromes found in tissues throughout the body.
- The formation of bilirubin occurs in reticuloendothelial cells, primarily in the spleen and liver. The first reaction, catalyzed by the microsomal enzyme heme oxygenase,

  Oxidatively cleaves the bridge of the porphyrin group and opens the heme ring. The end products of this reaction are biliverdin, carbon monoxide, and iron. The second reaction, catalyzed by the cytosolic enzyme biliverdin reductase, reduces the central methylene bridge of biliverdin and converts it to bilirubin. Bilirubin formed in the reticuloendothelial cells is virtually insoluble in water. This is due to tight internal hydrogen bonding between the water-soluble moieties of bilirubin, proprionic acid carboxyl groups of one dipyrrolic half of the molecule with the imino and lactam groups of the opposite half. This configuration blocks solvent access to the polar residues of bilirubin and places the hydrophobic residues on the outside. To be transported in blood, bilirubin must be solubilized. This is accomplished by its reversible, noncovalent binding to albumin. Unconjugated bilirubin bound to albumin is transported to the liver, where it, but not the albumin, is taken up by hepatocytes via a process that at least partly involves carrier-mediated membrane transport. No specific bilirubin transporter has yet been identified
- After entering the hepatocyte, unconjugated bilirubin is bound to the cytosolic protein ligandin, or glutathione S-transferase B. Whereas ligandin was initially thought to be a transport protein, responsible for delivering unconjugated bilirubin from the plasma membrane to the endoplasmic reticulum, it now appears that its role may in fact be to reduce bilirubin efflux back into the plasma. Studies suggest that unconjugated bilirubin may well rapidly diffuse unaided through the aqueous cytosol between membranes.
- In the endoplasmic reticulum, bilirubin is solubilized by conjugation to glucuronic acid, a process that disrupts the internal hydrogen bonds and yields bilirubin monoglucuronide and diglucuronide. The conjugation of glucuronic acid to bilirubin is catalyzed by bilirubin uridine diphosphate-glucuronosyl transferase (UDPGT). The now hydrophilic bilirubin conjugates diffuse from the endoplasmic reticulum to the canalicular membrane, where bilirubin monoglucuronide and diglucuronide are actively transported into canalicular bile by an energy-dependent mechanism involving the multiple drug resistance protein 2.
- The conjugated bilirubin excreted into bile drains into the duodenum and passes unchanged through the proximal small bowel. Conjugated bilirubin is not taken up by the intestinal mucosa. When the conjugated bilirubin reaches the distal ileum and colon, it is

  hydrolyzed to unconjugated bilirubin by bacterial -glucuronidases. The unconjugated bilirubin is reduced by normal gut bacteria to form a group of colorless tetrapyrroles called urobilinogens. About 80–90% of these products are excreted in feces, either unchanged or oxidized to orange derivatives called urobilins. The remaining 10–

20% of the urobilinogens are passively absorbed, enter the portal venous blood, and are reexcreted by the liver. A small fraction (usually <3 mg/dL) escapes hepatic uptake, filters across the renal glomerulus, and is excreted in urine

- Bilirubin is the end product of heme degradation. From 70–90% of bilirubin is derived from degradation of the hemoglobin of senescent red blood cells. Bilirubin produced in the periphery is transported to the liver within the plasma, where, due to its insolubility in aqueous solutions, it is tightly bound to albumin. Under normal circumstances, bilirubin is removed from the circulation rapidly and efficiently by hepatocytes. Transfer of bilirubin from blood to bile involves four distinct but interrelated steps



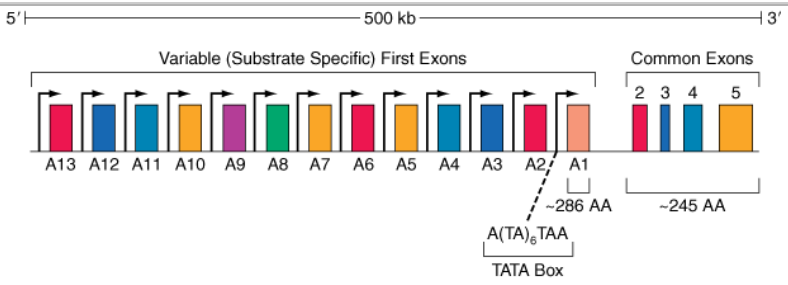
Hepatocellular bilirubin transport.

Albumin-bound bilirubin in sinusoidal blood passes through endothelial cell fenestrae to reach the hepatocyte surface, entering the cell by both facilitated and simple diffusional processes. Within the cell it is bound to glutathione-S-transferases and conjugated by bilirubin-UDP-glucuronosyltransferase (UGT1A1) to mono- and diglucuronides, which are actively transported across the canalicular membrane into the bile. ALB, albumin; UCB, unconjugated bilirubin, UGT1A1, bilirubin-UDP-glucuronosyltransferase; BMG, bilirubin monoglucuronide; GST, glutathione-S-transferase; MRP2, multidrug resistance–associated protein 2; BDG, bilirubin diglucuronide; BT, proposed bilirubin transporter

- 1. *Hepatocellular uptake:* Uptake of bilirubin by the hepatocyte has carrier-mediated kinetics. Although a number of candidate bilirubin transporters have been proposed, the actual transporter remains elusive.
- 2. *Intracellular binding:* Within the hepatocyte, bilirubin is kept in solution by binding as a nonsubstrate ligand to several of the glutathione-S-transferases, formerly called ligandins.
- 3. *Conjugation:* Bilirubin is conjugated with one or two glucuronic acid moieties by a specific UDP-glucuronosyltransferase to form bilirubin mono- and diglucuronide, respectively. Conjugation disrupts the internal hydrogen bonding that limits aqueous solubility of bilirubin, and the resulting glucuronide conjugates are highly soluble in water. Conjugation is obligatory for excretion of bilirubin across the bile canalicular membrane into bile. The UDP-glucuronosyltransferases have been classified into gene families based on the degree of homology among the mRNAs for the various isoforms. Those that conjugate bilirubin and certain other substrates have been designated the *UGT1* family. These are expressed from a single gene complex by alternative promoter

usage. This gene complex contains multiple substrate-specific first exons, designated A1, A2, etc.J, each with its own promoter and each encoding the amino-terminal half of a specific isoform. In addition, there are four common exons (exons 2–5) that encode the shared carboxyl-terminal half of all of the *UGT1* isoforms. The various first exons encode the specific aglycone substrate–binding sites for each isoform, while the shared exons encode the binding site for the sugar donor, UDP-glucuronic acid, and the transmembrane domain. Exon A1 and the four common exons, collectively designated the *UGT1A1* gene (Fig. 297-2), encode the physiologically critical enzyme bilirubin-UDP-glucuronosyltransferase (UGT1A1). A functional corollary of the organization of the *UGT1* gene is that a mutation in one of the first exons will affect only a single enzyme isoform. By contrast, a mutation in exons 2–5 will alter all isoforms encoded by the *UGT1* gene complex.

- *Biliary excretion:* Bilirubin mono- and diglucuronides are excreted across the canalicular plasma membrane into the bile canaliculus by an ATP-dependent transport process mediated by a canalicular membrane protein called *multidrug resistance–associated protein 2* (MRP2). Mutations of MRP2 result in the Dubin-Johnson syndrome (see below



Structural organization of the human *UGT1* gene complex. This large complex on chromosome 2 contains at least 13 substrate-specific first exons (A1, A2, etc.). Since four of these are pseudogenes, nine UGT1 isoforms with differing substrate specificities are expressed. Each exon 1 has its own promoter and encodes the amino-terminal substrate-specific ~286 amino acids of the various *UGT1*-encoded isoforms, and common exons 2–5 that encode the 245 carboxyl-terminal amino acids common to all of the isoforms. mRNAs for specific isoforms are assembled by splicing a particular first exon such as the bilirubin-specific exon A1 to exons 2 to 5. The resulting message encodes a complete enzyme, in this particular case bilirubin-UDP-glucuronosyltransferase (UGT1A1). Mutations in a first exon affect only a single isoform. Those in exons 2–5 affect all enzymes encoded by the UGT1 complex.

Extrahepatic Aspects of Bilirubin Disposition

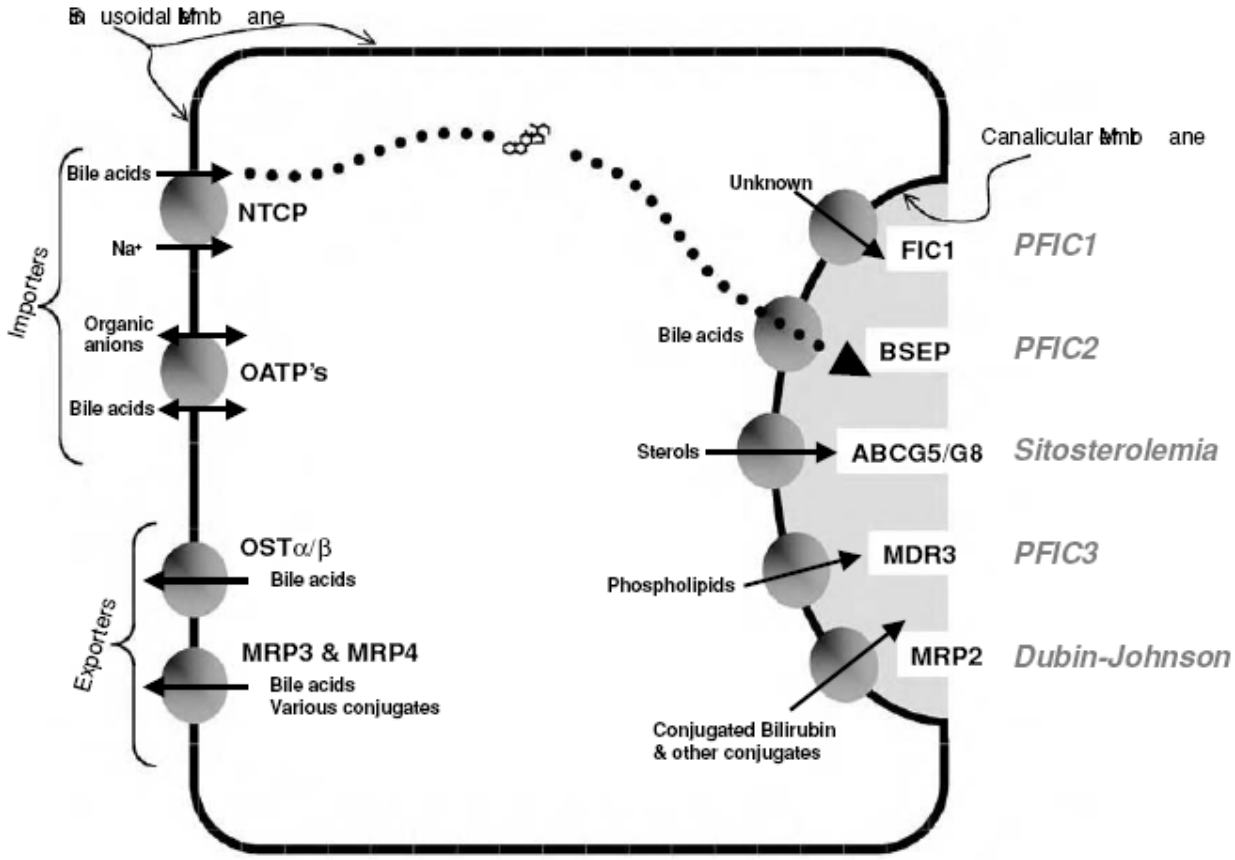
Bilirubin in the Gut

- Following secretion into bile, conjugated bilirubin reaches the duodenum and passes down the gastrointestinal tract without reabsorption by the intestinal mucosa. An appreciable fraction is converted by bacterial metabolism in the gut to the water-soluble colorless compound, urobilinogen. Urobilinogen undergoes enterohepatic cycling. Urobilinogen not taken up by the liver reaches the systemic circulation, from which some is cleared by the kidneys. Unconjugated bilirubin ordinarily does not reach the gut except in neonates or, by ill-defined alternative pathways, in the presence of severe unconjugated hyperbilirubinemia [e.g., Crigler-Najjar syndrome, type I (CN-I)]. Unconjugated bilirubin that reaches the gut is partly reabsorbed, amplifying any underlying hyperbilirubinemia. Recent reports suggest that oral administration of calcium phosphate with or without the lipase inhibitor orlistat may be an efficient means to

interrupt bilirubin enterohepatic cycling to reduce serum bilirubin levels in this situation; however, this remains to be validated in larger clinical trials.

Renal Excretion of Bilirubin Conjugates

- Unconjugated bilirubin is not excreted in urine as it is too tightly bound to albumin for effective glomerular filtration and there is no tubular mechanism for its renal secretion. In contrast, the bilirubin conjugates are readily filtered at the glomerulus and can appear in urine in disorders characterized by increased bilirubin conjugates in the circulation



Roles for critical hepatic transporters in the formation of bile and adaptation to cholestasis.
On the left is a representation of the sinusoidal surface and on the right, a canalicular surface. Diseases associated with defects in specific canalicular transporter genes are noted in *italics*. Note that bile acids have several means of transport across the sinusoidal membrane, both import and export, whereas there is one canalicular bile acid transporter, BSEP. These transporters allow for fine-tuning of intracellular bile acid concentrations as a means to adapt to a variety of cholestatic conditions. The principal means for bile acid flux across the hepatocyte is noted with the dotted line. NTCP, Na+/taurocholate cotransporting polypeptide;OATP,organic acid transporting polypeptide;OST,organic solute transporter;MRP,multidrug resistance–related protein; FIC1, familial intrahepatic cholestasis 1; BSEP, bile salt export pump; MDR, multidrug resistance protein. Official gene designations: *FIC1* (*ATP8B1*), *BSEP* (*ABCB11*),*MDR3*(*ABCB4*), and *MRP2* (*ABCC2*).