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TABLE 348-1 Liver Test Patterns in Hepatobiliary Disorders TYPE OF DISORDER BILIRUBIN AMINOTRANSFERASES ALKALINE PHOSPHATASE ALBUMIN PROTHROMBIN TIME Hemolysis/Gilbert’s syndrome Normal to 86 μmol/L (5 mg/dL) 85% due to indirect fractions No bilirubinuria Normal Normal Normal Normal Acute hepatocellular necrosis (viral, ischemic, and drug- or toxin-induced hepatitis) Both fractions may be elevated Peak usually follows aminotransferases Bilirubinuria Elevated, often >500 IU, ALT > AST Chronic hepatocellular disorders Both fractions may be elevated Bilirubinuria Elevated, but usually <300 IU Alcoholic hepatitis, cirrhosis Both fractions may be elevated Bilirubinuria AST:ALT >2 suggests alcoholic hepatitis or cirrhosis Intra- and extrahepatic cholestasis (obstructive jaundice) Both fractions may be elevated Bilirubinuria Normal to moderate elevation Rarely >500 IU Infiltrative diseases (tumor, granulomata) Usually normal Normal to slight elevation Elevated, often >4× normal elevation Fractionate, or confirm liver origin with 5′-nucleotidase or γ-glutamyl transpeptidase Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase. judicious use of the other tests described in this chapter. Table 348-1 shows how patterns of liver tests can lead the clinician to a category of disease that will direct further evaluation. However, it is important to remember that no single set of liver tests will necessarily provide a diagnosis. It is often necessary to repeat these tests on several occasions over days to weeks for a diagnostic pattern to emerge. Figure 348-1 is an algorithm for the evaluation of chronically abnormal liver tests. ■ ■GLOBAL CONSIDERATIONS The tests and principles presented in this chapter are applicable world wide. The causes of liver test abnormalities vary according to region. In developing nations, infectious diseases are more commonly the etiol ogy of abnormal serum liver tests than in developed nations. ■ ■FURTHER READING Berzigotti A et al: EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis: 2021 update. J Hepatology 75:659, 2021. Kaplan M: Alkaline phosphatase. Gastroenterology 62:452, 1972. Kim WR et al: MELD 3.0: The model for end-stage liver disease updated for the modern era. Gastroenterology 161:1887, 2021. Prati D et al: Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 137:1, 2002. Allan W. Wolkoff

The Hyperbilirubinemias ■ ■BILIRUBIN METABOLISM The details of bilirubin metabolism are presented in Chap. 52. However, the hyperbilirubinemias are best understood in terms of perturbations of specific aspects of bilirubin metabolism and transport, and these will be briefly reviewed here as depicted in Fig. 349-1. Bilirubin is the end product of heme degradation. Some 70–90% of bilirubin is derived from degradation of the hemoglobin of senescent

Normal to <3× normal elevation Normal Usually normal. If >5× above control and not corrected by parenteral vitamin K, suggests poor prognosis Normal to <3× normal elevation Often decreased Often prolonged Fails to correct with parenteral vitamin K Normal to <3× normal elevation Often decreased Often prolonged Fails to correct with parenteral vitamin K Elevated, often >4× normal elevation Normal, unless chronic Normal If prolonged, will correct with parenteral vitamin K Normal Normal CHAPTER 349 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 dis tinct but interrelated steps (Fig. 349-1). The Hyperbilirubinemias

1. Hepatocellular uptake: Uptake of bilirubin by the hepatocyte has carrier-mediated kinetics. Although a number of candidate bilirubin transporters have been proposed, the identity of the actual trans porter remains elusive.
2. Intracellular binding: Within the hepatocyte, bilirubin is kept in solution by binding as a nonsubstrate ligand to several of the gluta thione-S-transferases, formerly called ligandins.
3. Conjugation: Bilirubin is conjugated with one or two glucuronic acid moieties by a specific UDP-glucuronosyltransferase to form OATP1B1 OATP1B3 ALB UCB BMG BDG UGT1A1 BMG UGT1A1 MRP3 GST:UCB UCB BMG BDG MRP2 MRP2 UCB

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GST BDG BT ALB:UCB Space of Disse Sinusoid FIGURE 349-1 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. In addition to this direct excretion of bilirubin glucuronides, a portion are transported into the portal circulation by MRP3 and subjected to reuptake into the hepatocyte by OATP1B1 and OATP1B3. ALB, albumin; BDG, bilirubin diglucuronide; BMG, bilirubin monoglucuronide; BT, proposed bilirubin transporter; GST, glutathioneS-transferase; MRP2 and MRP3, multidrug resistance–associated proteins 2 and 3; OATP1B1 and OATP1B3, organic anion transport proteins 1B1 and 1B3; UCB, unconjugated bilirubin; UGT1A1, bilirubin-UDP-glucuronosyltransferase.

5′ 3′ 500 kb Variable (Substrate Specific) First Exons A13 A12 A11 A10 A9 A8 A7 A6 A5 A4 A3 A2 A1 A(TA)6TAA TATA Box FIGURE 349-2 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 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. bilirubin mono- and diglucuronide,

respectively. Conjugation dis rupts 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-glucuro nosyltransferases have been classified into gene families based on the degree of homology among the mRNAs for the various iso forms. 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, desig nated A1, A2, etc. (Fig. 349-2), 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 vari ous 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 desig nated as the UGT1A1 gene (Fig. 349-2), 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. 4. Biliary excretion: It has been thought until recently that biliru PART 10 Disorders of the Gastrointestinal System bin mono- and diglucuronides are excreted directly 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, ABCC2). Mutations of MRP2 result in the Dubin-Johnson syndrome (see below). However, studies in patients with Rotor syndrome (see below) indicate that after formation, a portion of the glucuronides is transported into the portal circulation by a sinusoidal membrane protein called multidrug resistance–associated protein 3 (MRP3, ABCC3) and is subjected to reuptake into the hepatocyte by the sinusoidal membrane uptake transporters organic anion transport protein 1B1 (OATP1B1, SLCO1B1) and OATP1B3 (SLCO1B3). ■ ■EXTRAHEPATIC ASPECTS OF BILIRUBIN DISPOSITION Bilirubin in the Gut Following secretion into bile, conjugated bili rubin 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 col orless compound urobilinogen. Urobilinogen undergoes enterohepatic

cycling. Urobilinogen not taken up by the liver reaches the systemic circula tion, from which some is cleared by the kidneys. Unconjugated bilirubin ordi narily does not reach the gut except in neonates or, by ill-defined alterna tive 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. Common Exons

~245 AA ~286 AA Renal Excretion of Bilirubin Con jugates Unconjugated bilirubin is not excreted in urine, as it is too tightly bound to albumin for effective glomeru lar 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. It should be kept in mind that the kidney can serve as an “overflow valve” for conjugated bilirubin. Consequently, the level of jaundice in individuals with conjugated hyperbilirubinemia can be amplified in the presence of renal failure. DISORDERS OF BILIRUBIN METABOLISM LEADING TO UNCONJUGATED HYPERBILIRUBINEMIA ■ ■INCREASED BILIRUBIN

PRODUCTION Hemolysis Increased destruction of erythrocytes leads to increased bilirubin turnover and unconjugated hyperbilirubinemia; the hyperbil irubinemia is usually modest in the presence of normal liver function. In particular, the bone marrow is only capable of a sustained eightfold increase in erythrocyte production in response to a hemolytic stress. Therefore, hemolysis alone cannot result in a sustained hyperbilirubi nemia of more than ~68 µmol/L (4 mg/dL). Higher values imply con comitant hepatic dysfunction. When hemolysis is the only abnormality in an otherwise healthy individual, the result is a purely unconjugated hyperbilirubinemia, with the direct-reacting fraction as measured in a typical clinical laboratory being ≤15% of the total serum bilirubin. In the presence of systemic disease, which may include a degree of hepatic dysfunction, hemolysis may produce a component of conjugated hyperbilirubinemia in addition to an elevated unconjugated bilirubin concentration. Prolonged hemolysis may lead to the precipitation of bilirubin salts within the gallbladder or biliary tree, resulting in the for mation of gallstones in which bilirubin, rather than cholesterol, is the major component. Such pigment stones may lead to acute or chronic cholecystitis, biliary obstruction, or any other biliary tract consequence of calculous disease. Ineffective Erythropoiesis During erythroid maturation, small amounts of hemoglobin may be lost at the time of nuclear extrusion, and a fraction of developing erythroid cells is destroyed within the marrow. These processes normally account for a small proportion of bilirubin that is produced. In various disorders, including thalassemia major, megaloblastic anemias due to folate or vitamin B12 deficiency, congenital erythropoietic porphyria, lead poisoning, and various congenital and acquired dyserythropoietic anemias, the fraction of total bilirubin production derived from ineffective erythropoiesis is increased, reaching as much as 70% of the total. This may be sufficient to produce modest degrees of unconjugated hyperbilirubinemia. Miscellaneous Degradation of the hemoglobin of extravascu lar collections of erythrocytes, such as those seen in massive tissue infarctions or large hematomas, may lead transiently to unconjugated hyperbilirubinemia.

■ ■DECREASED HEPATIC BILIRUBIN CLEARANCE Decreased Hepatic Uptake Decreased hepatic bilirubin uptake is believed to contribute to the unconjugated hyperbilirubinemia of Gilbert syndrome (GS), although the molecular basis for this find ing remains unclear (see below). Several drugs, including flavaspidic acid, novobiocin, and rifampin, as well as various cholecystographic contrast agents, have been reported to inhibit bilirubin uptake. The resulting unconjugated hyperbilirubinemia resolves with cessation of the medication. Impaired Conjugation • PHYSIOLOGIC NEONATAL JAUNDICE

Bilirubin produced by the fetus is cleared by the placenta and elimi nated by the maternal liver. Immediately after birth, the neonatal liver must assume responsibility for bilirubin clearance and excretion. However, many hepatic physiologic processes are incompletely devel oped at birth. Levels of UGT1A1 are low, and alternative excretory pathways allow passage of unconjugated bilirubin into the gut. Since the intestinal flora that convert bilirubin to urobilinogen are also undeveloped, an enterohepatic circulation of unconjugated bilirubin ensues. As a consequence, most neonates develop mild unconjugated hyperbilirubinemia between days 2 and 5 after birth. Peak levels are typically <85–170 µmol/L (5–10 mg/dL) and decline to normal adult concentrations within 2 weeks, as mechanisms required for bilirubin disposition mature. Prematurity, often associated with more pro found immaturity of hepatic function and hemolysis, can result in higher levels of unconjugated hyperbilirubinemia. A rapidly rising unconjugated bilirubin concentration, or absolute levels >340 µmol/L (20 mg/dL), puts the infant at risk for bilirubin encephalopathy, or kernicterus. Under these circumstances, bilirubin crosses an imma ture blood-brain barrier and

precipitates in the basal ganglia and other areas of the brain. The consequences range from appreciable neurologic deficits to death. Treatment options include phototherapy, which converts bilirubin into water-soluble photoisomers that are excreted directly into bile, and exchange transfusion. The canalicular mechanisms responsible for bilirubin excretion are also immature at birth, and their maturation may lag behind that of UGT1A1; this can lead to transient conjugated neonatal hyperbilirubinemia, especially in infants with hemolysis. ACQUIRED CONJUGATION DEFECTS A modest reduction in bilirubin conjugating capacity may be observed in advanced hepatitis or cirrho sis. However, in this setting, conjugation is better preserved than other aspects of bilirubin disposition, such as canalicular excretion. Various drugs, including pregnanediol, novobiocin, chloramphenicol, genta micin, and atazanavir, may produce unconjugated hyperbilirubinemia by inhibiting UGT1A1 activity. Bilirubin conjugation may be inhibited by certain fatty acids that are present in breast milk, but not serum, TABLE 349-1 Principal Differential Characteristics of Gilbert and Crigler-Najjar Syndromes CRIGLER-NAJJAR SYNDROME FEATURE GILBERT SYNDROME TYPE I TYPE II Total serum bilirubin, μmol/L (mg/dL) 310–755 (usually >345) (18–45

[usually >20]) Routine liver tests Response to phenobarbital Kernicterus Hepatic histology Normal None Usual Normal Bile characteristics Color Bilirubin fractions Pale or colorless

> 90% unconjugated Bilirubin UDP-glucuronosyltransferase activity Inheritance (all autosomal) Typically absent; traces in some patients Recessive

of mothers whose infants have excessive neonatal hyperbilirubinemia (breast milk jaundice). Alternatively, there may be increased entero hepatic circulation of bilirubin in these infants. The pathogenesis of breast milk jaundice appears to differ from that of transient familial neonatal hyperbilirubinemia (Lucey-Driscoll syndrome), in which there may be a UGT1A1 inhibitor in maternal serum.

■ ■HEREDITARY DEFECTS IN BILIRUBIN CONJUGATION Three familial disorders characterized by differing degrees of uncon jugated hyperbilirubinemia have long been recognized. The defining clinical features of each are described below (Table 349-1). While these disorders have been recognized for decades to reflect differing degrees of deficiency in the ability to conjugate bilirubin, recent advances in the molecular biology of the UGT1 gene complex have elucidated their interrelationships and clarified previously puzzling features. Crigler-Najjar Syndrome, Type I CN-I is characterized by striking unconjugated hyperbilirubinemia of ~340–765 μmol/L (20– 45 mg/dL) that appears in the neonatal period and persists for life. Other conventional hepatic biochemical tests such as serum amino transferases and alkaline phosphatase are normal, and there is no evidence of hemolysis. Hepatic histology is also essentially normal except for the occasional presence of bile plugs within canaliculi. Bilirubin glucuronides are virtually absent from the bile, and there is no detectable constitutive expression of UGT1A1 activity in hepatic tissue. Neither UGT1A1 activity nor the serum bilirubin concentra tion responds to administration of phenobarbital or other enzyme inducers. Unconjugated bilirubin accumulates in plasma, from which it is eliminated very slowly by alternative pathways that include direct passage into the bile and small intestine, possibly via bilirubin pho toisomers. This accounts for the small amount of urobilinogen found in feces. No

bilirubin is found in the urine. First described in 1952, the disorder is rare (estimated prevalence, 0.6–1.0 per million). Many patients are from geographically or socially isolated communities in which consanguinity is common, and pedigree analyses show an auto somal recessive pattern of inheritance. The majority of patients (type IA) exhibit defects in the glucuronide conjugation of a spectrum of substrates in addition to bilirubin, including various drugs and other xenobiotics. These individuals have mutations in one of the com mon exons (2–5) of the UGT1 gene (Fig. 349-2). In a smaller subset (type IB), the defect is limited largely to bilirubin conjugation, and the causative mutation is in the bilirubin-specific exon A1. Estrogen glucuronidation is mediated by UGT1A1 and is defective in all CN-I patients. More than 30 different genetic lesions of UGT1A1 respon sible for CN-I have been identified, including deletions, insertions, alterations in intron splice donor and acceptor sites, exon skipping, CHAPTER 349 The Hyperbilirubinemias 100–430 (usually ≤345) (6–25

[usually ≤20]) Typically ≤70 μmol/L (≤4 mg/dL) in absence of fasting or hemolysis Normal Decreases bilirubin by >25% Rare Normal Normal Decreases bilirubin to normal No Usually normal; increased lipofuscin pigment in some Pigmented Largest fraction (mean: 57%) monoconjugates Normal dark color Mainly diconjugates but monoconjugates increased (mean: 23%) Markedly reduced: 0–10% of normal Predominantly recessive Reduced: typically 10–33% of normal Promoter mutation: recessive Missense mutations: 7 of 8 dominant;

1 reportedly recessive

and point mutations that introduce premature stop codons or alter critical amino acids. Their common feature is that they all encode proteins with absent or, at most, traces of bilirubin-UDP-glucuronos yltransferase enzymatic activity.

Prior to the use of phototherapy, most patients with CN-I died of bilirubin encephalopathy (kernicterus) in infancy or early childhood. A few lived as long as early adult life without overt neurologic damage, although more subtle testing usually indicated mild but progressive brain damage, now termed bilirubin-induced neurologic dysfunction (BIND). In the absence of liver transplantation, death eventually super vened from late-onset bilirubin encephalopathy, which often followed a nonspecific febrile illness. Although isolated hepatocyte transplanta tion has been used in a small number of cases of CN-I, early liver trans plantation (Chap. 356) remains the best hope to prevent brain injury and death at present. It is anticipated that gene replacement therapy may be an option in the future. Crigler-Najjar Syndrome, Type II (CN-II) This condition was recognized as a distinct entity in 1962 and is characterized by marked unconjugated hyperbilirubinemia in the absence of abnormalities of other conventional hepatic biochemical tests, hepatic histology, or hemolysis. It differs from CN-I in several specific ways (Table 349-1): (1) although there is considerable overlap, average bilirubin concentra tions are lower in CN-II; (2) accordingly, CN-II is only infrequently associated with kernicterus; (3) bile is deeply colored, and bilirubin glucuronides are present, with a striking, characteristic increase in the proportion of monoglucuronides; (4) UGT1A1 in liver is usually pres ent at reduced levels (typically ≤10% of normal); and (5) while typically detected in infancy, hyperbilirubinemia was not recognized in some cases until later in life and, in one instance, at age 34. As with CN-I, most CN-II cases exhibit abnormalities in the conjugation of other compounds, such as salicylamide and menthol, but in some instances, the defect appears limited to bilirubin. Reduction of serum bilirubin concentrations by >25% in response to enzyme inducers such as phenobarbital distinguishes CN-II from CN-I,

although this response may not be elicited in early infancy and often is not accompanied by measurable UGT1A1 induction. Bilirubin concentrations during phe nobarbital administration do not return to normal but are typically in the range of 51–86 μmol/L (3–5 mg/dL). Although the incidence of kernicterus in CN-II is low, instances have occurred, not only in infants but also in adolescents and adults, often in the setting of an intercur rent illness, fasting, or another factor that temporarily raises the serum bilirubin concentration above baseline and reduces serum albumin levels. For this reason, phenobarbital therapy is widely recommended, a single bedtime dose often sufficing to maintain clinically safe serum bilirubin concentrations. PART 10 Disorders of the Gastrointestinal System Over 100 different mutations in the UGT1 gene have been identi fied as causing CN-I or CN-II. It was found that missense mutations are more common in CN-II patients, as would be expected in this less severe phenotype. Their common feature is that they encode for a bilirubin-UDP-glucuronosyltransferase with markedly reduced, but detectable, enzymatic activity. The spectrum of residual enzyme activ ity explains the spectrum of phenotypic severity of the resulting hyper bilirubinemia. Molecular analysis has established that a large majority of CN-II patients are either homozygotes or compound heterozygotes for CN-II mutations and that individuals carrying one mutated and one entirely normal allele have normal bilirubin concentrations. Gilbert Syndrome This syndrome is characterized by mild uncon jugated hyperbilirubinemia, normal values for standard hepatic bio chemical tests, and normal hepatic histology other than a modest increase of lipofuscin pigment in some patients. Serum bilirubin concentrations are most often <51 μmol/L (<3 mg/dL), although both higher and lower values are frequent. The clinical spectrum of hyper bilirubinemia fades into that of CN-II at serum bilirubin concentra tions of 86–136 μmol/L (5–8 mg/dL). At the other end of the scale, the distinction between mild cases of GS and a normal state is often blurred. Bilirubin concentrations may fluctuate substantially in any given individual, and at least 25% of patients will exhibit temporarily

normal values during prolonged follow-up. More elevated values are associated with stress, fatigue, alcohol use, reduced caloric intake, and intercurrent illness, while increased caloric intake or administra tion of enzyme-inducing agents produces lower bilirubin levels. GS is most often diagnosed at or shortly after puberty or in adult life during routine examinations that include multichannel biochemical analyses. UGT1A1 activity is typically reduced to 10–35% of normal, and bile pigments exhibit a characteristic increase in bilirubin mono glucuronides. Studies of radiobilirubin kinetics indicate that hepatic bilirubin clearance is reduced to an average of one-third of normal. Administration of phenobarbital normalizes both the serum biliru bin concentration and hepatic bilirubin clearance; however, failure of UGT1A1 activity to improve in many such instances suggests the possible coexistence of an additional defect. Compartmental analysis of bilirubin kinetic data suggests that GS patients may have a defect in bilirubin uptake as well as in conjugation, although this has not been shown directly. Defects in the hepatic uptake of other organic anions that at least partially share an uptake mechanism with bilirubin, such as sulfobromophthalein and indocyanine green (ICG), are observed in a minority of patients. The metabolism and transport of bile acids that do not utilize the bilirubin uptake mechanism are normal. The magnitude of changes in the serum bilirubin concentration induced by provocation tests such as 48 h of fasting or the IV administration of nicotinic acid has been reported to be of help in separating GS patients from normal individuals. Other studies dispute this assertion. Moreover, on theoretical grounds, the results of such studies should provide no more information than simple measurements of the base line serum bilirubin concentration. Family studies indicate that GS and hereditary hemolytic anemias such as hereditary spherocytosis, glucose-6-phosphate

dehydrogenase deficiency, and β-thalassemia trait sort independently. Reports of hemolysis in up to 50% of GS patients are believed to reflect better case finding, since patients with both GS and hemolysis have higher bilirubin concentrations and are more likely to be jaundiced than patients with either defect alone. GS is common, with many series placing its prevalence as high as 8%. Males predominate over females by reported ratios ranging from 1.5:1 to >7:1. However, these ratios may have a large artifactual component since normal males have higher mean bilirubin levels than normal females, but the diagnosis of GS is often based on comparison to normal ranges established in men. The high prevalence of GS in the general population may explain the reported frequency of mild uncon jugated hyperbilirubinemia in liver transplant recipients. The disposi tion of most xenobiotics metabolized by glucuronidation appears to be normal in GS, as is oxidative drug metabolism in the majority of reported studies. The principal exception is the metabolism of the anti tumor agent irinotecan (CPT-11), whose active metabolite (SN-38) is glucuronidated specifically by bilirubin-UDP-glucuronosyltransferase. Administration of CPT-11 to patients with GS has resulted in several toxicities, including intractable diarrhea and myelosuppression. Some reports also suggest abnormal disposition of menthol, estradiol benzo ate, acetaminophen, tolbutamide, and rifamycin SV. Although some of these studies have been disputed, and there have been no reports of clinical complications from use of these agents in GS, prudence should be exercised in prescribing them or any agents metabolized primarily by glucuronidation in this condition. It should also be noted that the HIV protease inhibitors indinavir and atazanavir (Chap. 208) can inhibit UGT1A1, resulting in hyperbilirubinemia that is most pro nounced in patients with preexisting GS. Most older pedigree studies of GS were consistent with autosomal dominant inheritance with variable expressivity. However, studies of the UGT1 gene in GS have indicated a variety of molecular genetic bases for the phenotypic picture and several different patterns of inheritance. Studies in Europe and the United States found that nearly all patients had normal coding regions for UGT1A1 but were homo zygous for the insertion of an extra TA (i.e., A[TA]7TAA rather than A[TA]6TAA) in the promoter region of the first exon. This appeared to be necessary, but not sufficient, for clinically expressed GS, since 15% of normal controls were also homozygous for this variant. While normal by standard criteria, these individuals had somewhat higher

bilirubin concentrations than the rest of the controls studied. Hetero zygotes for this abnormality had bilirubin concentrations identical to those homozygous for the normal A[TA]6TAA allele. The prevalence of the A[TA]7TAA allele in a general Western population is 30%, in which case 9% would be homozygotes. This is slightly higher than the prevalence of GS based on purely phenotypic parameters. It was sug gested that additional variables, such as mild hemolysis or a defect in bilirubin uptake, might be among the factors enhancing phenotypic expression of the defect. Phenotypic expression of GS due solely to the A[TA]7TAA promoter abnormality is inherited as an autosomal recessive trait. A number of CN-II kindreds have been identified in whom there is also an allele containing a normal coding region but the A[TA]7TAA promoter abnormality. CN-II heterozygotes, who have the A[TA]6TAA promoter, are phenotypically normal, whereas those with the A[TA]7TAA pro moter express the phenotypic picture of GS. GS in such kindreds may also result from homozygosity for the A[TA]7TAA promoter abnormal ity. Seven different missense mutations in the UGT1 gene that report edly cause GS with dominant inheritance have been found in Japanese individuals. Another Japanese patient with mild unconjugated hyper bilirubinemia was homozygous for a missense mutation in exon 5. GS in her family appeared to be recessive. DISORDERS OF BILIRUBIN METABOLISM LEADING TO MIXED OR PREDOMINANTLY CONJUGATED HYPERBILIRUBINEMIA In hyperbilirubinemia due to acquired liver disease (e.g., acute hepa titis,

common bile duct stone), there are usually elevations in the serum concentrations of both conjugated and unconjugated bilirubin. Although biliary tract obstruction or hepatocellular cholestatic injury may present on occasion with a predominantly conjugated hyper bilirubinemia, it is generally not possible to differentiate intrahepatic from extrahepatic causes of jaundice based on the serum levels or relative proportions of unconjugated and conjugated bilirubin. The major reason for determining the amounts of conjugated and uncon jugated bilirubin in the serum is for the initial differentiation of hepatic parenchymal and obstructive disorders (mixed conjugated and unconjugated hyperbilirubinemia) from the inheritable and hemo lytic disorders discussed above that are associated with unconjugated hyperbilirubinemia. ■ ■FAMILIAL DEFECTS IN HEPATIC

EXCRETORY FUNCTION Dubin-Johnson Syndrome (DJS) This benign, relatively rare disorder is characterized by low-grade, predominantly conjugated hyperbilirubinemia (Table 349-2). Total bilirubin concentrations are typically between 34 and 85 µmol/L (2 and 5 mg/dL) but on occasion can be in the normal range or as high as 340–430 µmol/L TABLE 349-2 Principal Differential Characteristics of Inheritable Disorders of Bile Canalicular Function DJS ROTOR PFIC1 BRIC1 PFIC2 BRIC2 PFIC3 Gene Protein Cholestasis ABCCA MRP2 No SLCO1B1/SLCO1B3 OATP1B1/1B3 No ATP8B1 FIC1 Yes Serum GGT Serum bile acids Normal Normal Normal Normal Normal ↑↑ Clinical features Mild conjugated hyperbilirubinemia; otherwise, normal liver function; dark pigment in liver; characteristic pattern of urinary coproporphyrins Mild conjugated hyperbilirubinemia; otherwise, normal liver function; liver without abnormal pigmentation Severe cholestasis beginning in childhood Abbreviations: BRIC, benign recurrent intrahepatic cholestasis; BSEP, bile salt excretory protein; DJS, Dubin-Johnson syndrome; GGT, γ-glutamyl transferase; MRP2, multidrug resistance–associated protein 2; OATP1A/1B, organic anion transport proteins 1B1 and 1B3; PFIC, progressive familial intrahepatic cholestasis; ↑↑, increased.

(20–25 mg/dL) and can fluctuate widely in any given patient. The degree of hyperbilirubinemia may be increased by intercurrent illness, oral contraceptive use, and pregnancy. Because the hyperbilirubinemia is due to a predominant rise in conjugated bilirubin, bilirubinuria is characteristically present. Aside from elevated serum bilirubin levels, other routine laboratory tests are normal. Physical examination is usu ally normal except for jaundice, although an occasional patient may have hepatosplenomegaly.

Patients with DJS are usually asymptomatic, although some may have vague constitutional symptoms. These latter patients have usu ally undergone extensive diagnostic examinations for unexplained jaundice and have high levels of anxiety. In women, the condition may be subclinical until the patient becomes pregnant or receives oral contraceptives, at which time chemical hyperbilirubinemia becomes frank jaundice. Even in these situations, other routine liver function tests, including serum alkaline phosphatase and transaminase activi ties, are normal. A cardinal feature of DJS is the accumulation of dark, coarsely gran ular pigment in the lysosomes of centrilobular hepatocytes. As a result, the liver may be grossly black in appearance. This pigment is thought to be derived from epinephrine metabolites that are not excreted nor mally. The pigment may disappear during bouts of viral hepatitis, only to reaccumulate slowly after recovery. Biliary excretion of a number of anionic compounds is compro mised in DJS. These include various cholecystographic agents, as well as sulfobromophthalein (Bromsulphalein [BSP]), a synthetic dye formerly used in a test of liver function. In this test, the rate of disappearance of BSP from plasma was determined following bolus IV administration. BSP is conjugated with glutathione in the hepato

cyte; the resulting conjugate is normally excreted rapidly into the bile canaliculus. Patients with DJS exhibit characteristic rises in plasma concentrations at 90 min after injection, due to reflux of conjugated BSP into the circulation from the hepatocyte. Dyes such as ICG that are taken up by hepatocytes but are not further metabolized prior to bili ary excretion do not show this reflux phenomenon. Continuous BSP infusion studies suggest a reduction in the time to maximum plasma concentration (tmax) for biliary excretion. Bile acid disposition, includ ing hepatocellular uptake and biliary excretion, is normal in DJS. These patients have normal serum and biliary bile acid concentrations and do not have pruritus. CHAPTER 349 The Hyperbilirubinemias By analogy with findings in several mutant rat strains, the selective defect in biliary excretion of bilirubin conjugates and certain other classes of organic compounds, but not of bile acids, that characterizes DJS in humans was found to reflect defective expression of MRP2 (ABCC2), an ATP-dependent canalicular membrane transporter. Sev eral different mutations in the ABCC2 gene produce the Dubin-Johnson phenotype, which has an autosomal recessive pattern of inheritance. Although MRP2 is undoubtedly important in the biliary excretion of ATP8B1 FIC1 Episodic ABCB11 BSEP Yes ABCB11 BSEP Episodic ABCB4 MDR3 Yes Normal ↑↑ during episodes Normal ↑↑ Normal ↑↑ during episodes ↑↑ ↑↑ Recurrent episodes of cholestasis beginning at any age Severe cholestasis beginning in childhood Recurrent episodes of cholestasis beginning at any age Severe cholestasis beginning in childhood; decreased phospholipids in bile

conjugated bilirubin, the fact that this pigment is still excreted in the absence of MRP2 suggests that other, as yet uncharacterized, transport proteins may serve in a secondary role in this process.

Patients with DJS also have a diagnostic abnormality in urinary coproporphyrin excretion. There are two naturally occurring copro porphyrin isomers, I and III. Normally, ~75% of the coproporphyrin in urine is isomer III. In urine from DJS patients, total coproporphy rin content is normal, but ≥80% is isomer I. Heterozygotes for the syndrome show an intermediate pattern. The molecular basis for this phenomenon remains unclear. Rotor Syndrome (RS) This benign, autosomal recessive disor der is clinically similar to DJS (Table 349-2), although it is seen even less frequently. A major phenotypic difference is that the liver in patients with RS has no increased pigmentation and appears totally normal. The only abnormality in routine laboratory tests is an eleva tion of total serum bilirubin, due to a predominant rise in conjugated bilirubin. This is accompanied by bilirubinuria. Several additional features differentiate RS from DJS. In RS, the gallbladder is usually visualized on oral cholecystography, in contrast to the nonvisualiza tion that is typical of DJS. The pattern of urinary coproporphyrin excretion also differs. The pattern in RS resembles that of many acquired disorders of hepatobiliary function, in which copropor phyrin I, the major coproporphyrin isomer in bile, refluxes from the hepatocyte back into the circulation and is excreted in urine. Thus, total urinary coproporphyrin excretion is substantially increased in RS, in contrast to the normal levels seen in DJS. Although the fraction of coproporphyrin I in urine is elevated, it is usually <70% of the total, compared with ≥80% in DJS. The disorders also can be distinguished by their patterns of BSP excretion. Although clearance of BSP from plasma is delayed in RS, there is no reflux of conjugated BSP back into the circulation as seen in DJS. Kinetic analysis of plasma BSP infusion studies suggests the presence of a defect in intrahepatocellular storage of this compound. This has never been demonstrated directly. Recent studies indicate that the molecular basis of RS results from simulta neous deficiency of the hepatocyte plasma membrane transporters OATP1B1 (SLCO1B1) and OATP1B3 (SLCO1B3). This results in reduced reuptake by these transporters of conjugated bilirubin that has been pumped out of the hepatocyte into the portal circulation by

MRP3 (ABCC3) (Fig. 349-1). It should be noted that these transport ers normally mediate uptake into the liver of a variety of drugs such as statins, and the possibility of drug toxicity resulting from their deficiency in RS must be considered. PART 10 Disorders of the Gastrointestinal System Benign Recurrent Intrahepatic Cholestasis (BRIC) This rare disorder is characterized by recurrent attacks of pruritus and jaundice. The typical episode begins with mild malaise and elevations in serum aminotransferase levels, followed rapidly by rises in alkaline phosphatase and conjugated bilirubin and onset of jaundice and itch ing. The first one or two episodes may be misdiagnosed as acute viral hepatitis. The cholestatic episodes, which may begin in childhood or adulthood, can vary in duration from several weeks to months, fol lowed by a complete clinical and biochemical resolution. Intervals between attacks may vary from several months to years. Between episodes, physical examination is normal, as are serum levels of bile acids, bilirubin, transaminases, and alkaline phosphatase. The disorder is familial and has an autosomal recessive pattern of inheritance. BRIC is considered a benign disorder in that it does not lead to cirrhosis or end-stage liver disease. However, the episodes of jaundice and pruritus can be prolonged and debilitating, and some patients have undergone liver transplantation to relieve the intractable and disabling symptoms. Treatment during the cholestatic episodes is symptomatic; there is no specific treatment to prevent or shorten the occurrence of episodes. A gene termed FIC1 was recently identified and found to be mutated in patients with BRIC. Curiously, this gene is expressed strongly in the small intestine but only weakly in the liver. The protein encoded by FIC1 shows little similarity to those that have been shown to play a role in bile canalicular excretion of various compounds. Rather, it

appears to be a member of a P-type ATPase family that transports aminophospholipids from the outer to the inner leaflet of a variety of cell membranes. Its relationship to the pathobiology of this disorder remains unclear. A second phenotypically identical form of BRIC, termed BRIC type 2, has been described resulting from mutations in the bile salt excretory protein (BSEP), the protein that is defective in progressive familial intrahepatic cholestasis (PFIC) type 2 (Table 349-2). How some mutations in this protein result in the episodic BRIC phe notype is unknown. Progressive Familial Intrahepatic Cholestasis This name is applied to three phenotypically related syndromes (Table 349-2). PFIC type 1 (Byler’s disease) presents in early infancy as cholestasis that may be initially episodic. However, in contrast to BRIC, Byler’s disease progresses to malnutrition, growth retardation, and end-stage liver disease during childhood. This disorder is also a consequence of an FIC1 mutation. The functional relationship of the FIC1 protein to the pathogenesis of cholestasis in these disorders is unknown. Two other types of PFIC (types 2 and 3) have been described. PFIC type 2 is associated with a mutation in the protein originally named sis ter of P-glycoprotein, now known as bile salt excretory protein (BSEP, ABCB11), which is the major bile canalicular exporter of bile acids. As noted above, some mutations of this protein are associated with BRIC type 2, rather than the PFIC type 2 phenotype. PFIC type 3 has been associated with a mutation of MDR3 (ABCB4), a protein that is essential for normal hepatocellular excretion of phospholipids across the bile canaliculus. Although all three types of PFIC have similar clinical phenotypes, only type 3 is associated with high serum levels of γ-glutamyl transferase (GGT) activity. In contrast, activity of this enzyme is normal or only mildly elevated in symptomatic BRIC and PFIC types 1 and 2. Interestingly, mutations in FIC1 or BSEP are not found in approximately one-third of patients with clinical PFIC and normal GGT. Recent studies have shown that patients with mutations in NR1H4, the gene encoding the farnesoid X receptor (FXR), a nuclear hormone receptor activated by bile acids, have a syndrome identical to PFIC2 with absent expression of BSEP. Mutations in tight junction protein 2 (TJP2) as well as ubiquitin-specific protease 53 (USP53), a protein that interacts

with TJP2, have also been associated with severe cholestasis with normal GGT levels, likely due to disruption of tight junctions at the bile canaliculus. ■ ■FURTHER READING Bull LN, Thompson RJ: Progressive familial intrahepatic cholestasis. Clin Liver Dis 22:657, 2018. Canu G et al: Gilbert and Crigler Najjar syndromes: An update of the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene mutation data base. Blood Cells Mol Dis 50:273, 2013. Hansen TW: Biology of bilirubin photoisomers. Clin Perinatol 43:277, 2016. Hassan S, Hertel P: Overview of progressive familial intrahepatic cholestasis. Clin Liver Dis 26:371, 2022. Kavallar AM et al: Management and outcomes after liver transplantation for progressive familial intrahepatic cholestasis: A systematic review and meta-analysis. Hepatology Communications 7:e0286, 2023. Lamola AA: A pharmacologic view of phototherapy. Clin Perinatol 43:259, 2016. Memon N et al: Inherited disorders of bilirubin clearance. Pediatr Res 79:378, 2016. Soroka CJ, Boyer JL: Biosynthesis and trafficking of the bile salt export pump, BSEP: Therapeutic implications of BSEP mutations. Mol Aspects Med 37:3, 2014. van de Steeg E et al: Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. J Clin Invest 122:519, 2012. van Wessel DBE et al: Genotype correlates with the natural history of severe bile salt export pump deficiency. J Hepatol 73:84, 2020. Wolkoff AW: Organic anion uptake by hepatocytes. Compr Physiol 4:1715, 2014.

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