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Neonatal Jaundice and Liver Disease

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Bilirubin is one of three biologically active end-products of heme catabolism. Its clinical significance in the neonate relates to its propensity for deposition in the skin and mucous membranes, producing easily identifiable jaundice (French *jaune*, yellow) or icterus (Greek *ikteros*, jaundice). The yellow color, or the total serum (or plasma) bilirubin (TB) concentration at any point in time, represents the combined processes of bilirubin production and bilirubin elimination from the body, the latter process being comprised of bilirubin uptake into hepatocytes, bilirubin conjugation, and excretion of the conjugated product. As long as these functions remain in balance, a moderate degree of jaundice may develop but should not endanger an otherwise healthy, non-hemolyzing infant. However, an imbalance between bilirubin production and its elimination may result in increasing jaundice or hyperbilirubinemia. In rare cases, the degree of bilirubin production relative to its elimination may be so great that bilirubin may deposit in the brain, where it may cause dysfunction in the form of acute bilirubin encephalopathy (ABE). Although some cases of ABE may be transient and reversible, chronic bilirubin encephalopathy (CBE), known classically as kernicterus, with resultant permanent neuronal damage manifesting as a form of cerebral palsy, may ensue. As many as 80% of otherwise healthy, term newborns develop some degree of elevated TB levels. In contrast, severe hyperbilirubinemia (TB >25 mg/dL [428 μmol/L]) with its potentially devastating sequelae is rare. It is therefore nonetheless important to distinguish between and have a thorough understanding of the normal and pathologic processes of bilirubin physiology and the potential complications of severe hyperbilirubinemia.

Bilirubin Metabolism

Bilirubin Biochemistry

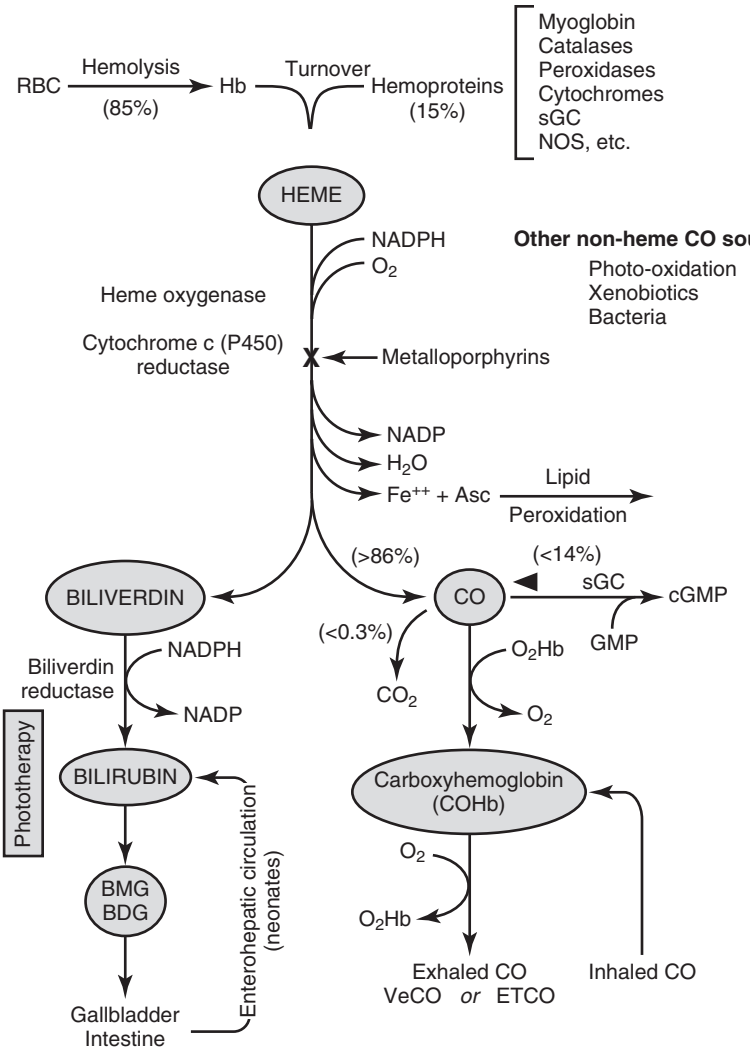
Overview

Throughout life, there is a continuum of bilirubin production and elimination from the body. Ongoing lysis of red blood cells (RBCs), whether physiologic or at increased rates (e.g., due to hemolysis), releases iron protoporphyrin (heme), the oxygen-carrying component of hemoglobin. Catalyzed by heme oxygenase (HO), heme is then converted to biliverdin and subsequently to bilirubin (Fig. 95.1), which in its unconjugated form is transported to the liver bound to albumin. Once in the hepatocyte, bilirubin is conjugated to glucuronic acid by the enzyme uridine diphosphate glucuronic acid (UDPGA)–glucuronosyltransferase 1A1 (UGT1A1). Water-soluble conjugated bilirubin can be now excreted into the bile from which it reaches the bowel and is ultimately eliminated from the body. This simplified overview of bilirubin biochemistry will be reviewed in greater detail later in this chapter.

Heme Degradation

Heme oxygenase-1 (HO-1), the inducible isoform of HO, is a membrane-bound enzyme found in cells of the liver and other organs that catalyzes the first step in the pathway by which heme is converted to biliverdin through oxidation of the α-methene bridge carbon of heme (Fig. 95.2). HO-1 is inducible by its substrate heme. This rate-limiting step produces biliverdin, free iron (which can be reutilized for hemoglobin synthesis), and carbon monoxide (CO), which is excreted in the lungs in equimolar amounts. This process occurs in all nucleated cells except for mature, anucleated RBCs. Biliverdin is a blue-green, water-soluble pigment that can be readily excreted by the liver and kidneys. In amphibians, reptiles, and certain avian species, the major pigmented end-product of heme catabolism is biliverdin. In mammals, however, biliverdin is converted to bilirubin by biliverdin reductase in the cytosol.

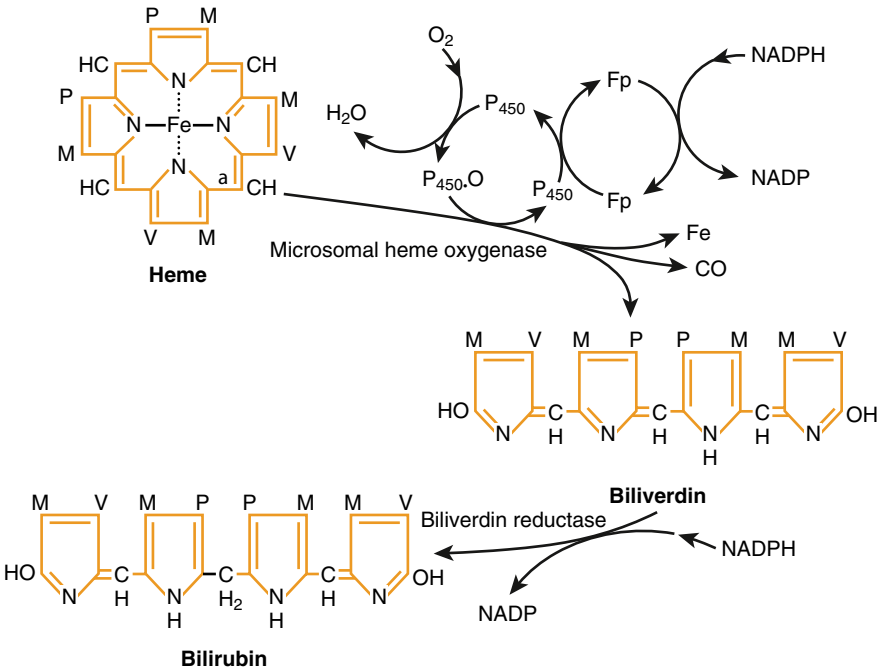
The degradation of 1 g of hemoglobin forms 34 mg of bilirubin. The isomeric form of bilirubin produced in this two-step process is IX-α (*Z,Z* isomer), defining the relative positions of the four pyrrole rings and the hydrogen molecules on the two linking lateral carbons. This form of



• **Fig. 95.1** Metabolic pathway of the degradation of heme and the formation of bilirubin. Heme released from the hemoglobin of red blood cells (*RBC*) or from other hemoproteins is degraded by heme oxygenase (*HO*), the first and rate-limiting enzyme in a two-step reaction requiring nicotinamide adenine dinucleotide phosphate (*NADPH*) and oxygen and resulting in the release of iron and the formation of carbon monoxide (*CO*) and biliverdin. Metalloporphyrins, synthetic heme analogs, can competitively inhibit HO activity (indicated by the *X*). Biliverdin is further reduced to bilirubin by the enzyme biliverdin reductase. CO can activate soluble guanylyl cyclase (*sGC*) and lead to the formation of cyclic guanosine monophosphate (*cGMP*). It can also displace oxygen from oxyhemoglobin or be exhaled. The bilirubin that is formed is taken up by the liver and conjugated with glucuronides to form bilirubin mono- or diglucuronide (*BMG* and *BDG*, respectively), in reactions catalyzed by uridine diphosphate glucuronic acid (UDPGA)-glucuronosyltransferase 1A1 (*UGT1A1*). The bilirubin glucuronides are then excreted into the intestinal lumen but can be deconjugated by bacteria so that the bilirubin is reabsorbed into the circulation, as shown. *COHb*, Carboxyhemoglobin ; *ETCO*, end-tidal CO; *Hb*, hemoglobin; *NOS*, nitric oxide synthase; *VeCO*, excretion rate of CO. (Modified from Vreman HJ, Wong RJ, Stevenson DK. Carbon monoxide in breath, blood, and other tissues. In Penney DG, ed. *Carbon Monoxide Toxicity*. Boca Raton, FL: CRC Press; 2000:22–30.)

bilirubin is water insoluble due to tertiary structural changes that internalize the keto and carboxy groups that would otherwise interact with water molecules. Intramolecular hydrogen bonding maintains the folded structure of bilirubin.

Because bilirubin is a weak acid and is neither water soluble nor readily excreted at pH 7.4, to facilitate its excretion the molecule must be conjugated to mono- and diglucuronic acids by the specific hepatic enzyme isozyme (UGT1A1).[1] The evolutionary advantage derived by mammalian species in the development of such an intricate energy-dependent system that first produces bilirubin from a water-soluble precursor and then converts it back to a water-soluble form for excretion is currently uncertain. The mammalian placenta is capable of removing unconjugated bilirubin but not biliverdin. Biliverdin accumulation in the mammalian fetus would presumably result in the accumulation of large amounts of potentially toxic metabolites. Evidence has shown that bilirubin[2] and even CO may be biologically useful molecules. The inducibility of HO-1 would appear to indicate that bilirubin production is helpful to cells when



• **Fig. 95.2** Catabolism of heme to bilirubin by microsomal heme oxygenase (*HO*) and biliverdin reductase. (From Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of hemoglobin to bilirubin. *Trans Assoc Am Physicians*. 1969;82:363–371.)

they are stressed. Bilirubin is a potent antioxidant that readily binds to membrane lipids and is capable of limiting membrane damage by preventing peroxidative injury. Biologic evidence of potentially beneficial effects of moderate concentrations of bilirubin is tempered by the association of high levels of unconjugated bilirubin with neuronal dysfunction and necrosis. Although cells may be potential beneficiaries of small amounts of bilirubin, in greater circulating quantities the same bilirubin molecule may be a causative factor of severe neuronal damage. The dilemma that faces the clinician is determining the desirable or "safe" level of bilirubin appropriate for a particular neonate.

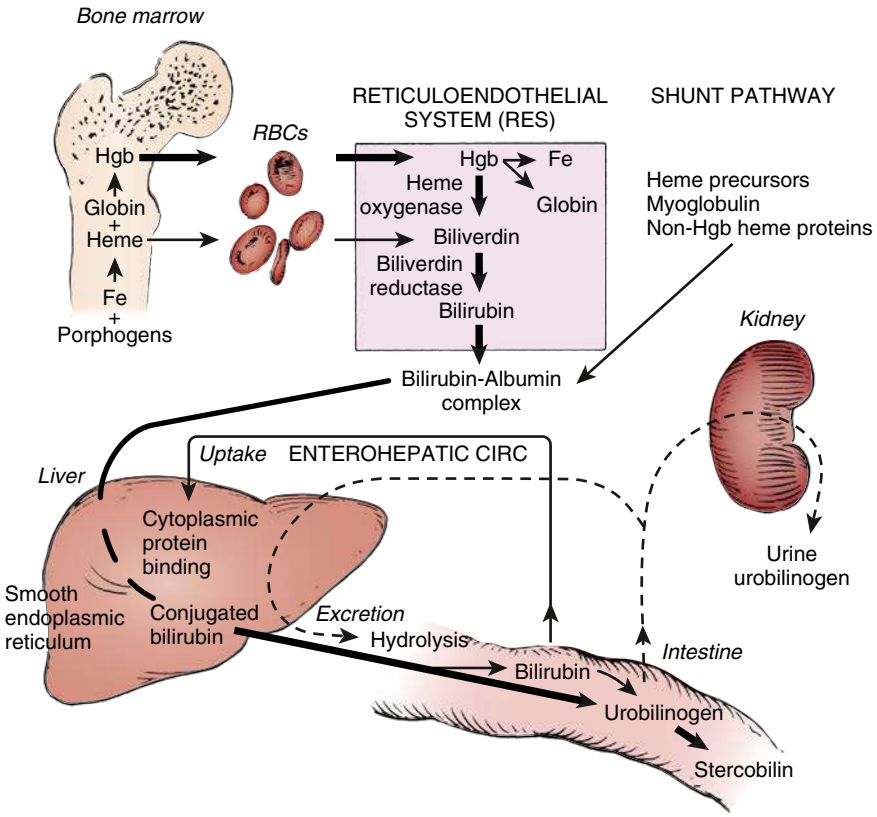
The CO formed by heme degradation binds to hemoglobin to form carboxyhemoglobin (COHb) and is then transported in the circulation to the lung. Here, the CO separates from hemoglobin and is exhaled in breath. Although there are other potential exogenous and endogenous sources of CO, such as lipid peroxidation and photo-oxidation, the main source of endogenous CO is derived from heme catabolism. Therefore quantitative estimation of its synthesis or excretion (in infants without significant lung disease or oxygen exposure) offers a reasonably accurate index of the rate of heme degradation from which the rate of bilirubin production can be derived.[3] It is believed that other hemoproteins undergo the same degradative process.

Bilirubin Production

The pathway of bilirubin synthesis, transport, and metabolism is summarized in Fig. 95.3. In the normal adult, bilirubin is derived primarily from the degradation of heme, which is released from senescing RBCs in the reticuloendothelial cells. Normally, about 20% of the bilirubin excreted into bile is derived from heme and other hemoproteins (mainly cytochromes, catalase). CO excretion in humans and more direct measurements in animals have demonstrated that, on the first day of life, bilirubin production is two to three times higher than the rate of adults, to an estimated average of 8 to 10 mg/kg body weight per day. Bilirubin production decreases rapidly during the first 2 postnatal days. Several factors may explain this increased production in the newborn. The circulating RBC life span is shortened to 70 to 90 days compared with 120 days in the adult. Increased heme degradation arises from the very large pool of hematopoietic tissue, essential to intrauterine well-being, but ceases to function shortly after birth. An additional factor may possibly include an increased turnover of cytochromes. Another major contributor to the bilirubin pool in the neonate is an increase in bilirubin absorbed from the bowel as part of the enterohepatic circulation. This mechanism results from both reformation of unconjugated bilirubin from conjugated bilirubin in the bowel and enhanced absorption of unconjugated bilirubin by the intestinal mucosa back to the circulation (see Enterohepatic Reabsorption of Bilirubin).

Transport of Bilirubin in Plasma

Unconjugated bilirubin is mostly insoluble in water at pH 7.4, with a solubility of less than 0.01 mg/dL (0.17 µmol/L), and, when released into the circulation by the reticuloendothelial cells, is rapidly bound to albumin. Each molecule of adult albumin is capable of binding at least two molecules of bilirubin; the first molecule is more tightly bound than the second. Additional binding sites with weaker affinities may also exist but are probably of little clinical importance. On average,



• **Fig. 95.3** The pathways of bilirubin synthesis, transport, and metabolism. *Hgb*, Hemoglobin; *RBCs*, red blood cells. (From Assali NS. *Pathophysiology of Gestation*. New York: Academic Press; 1972.)

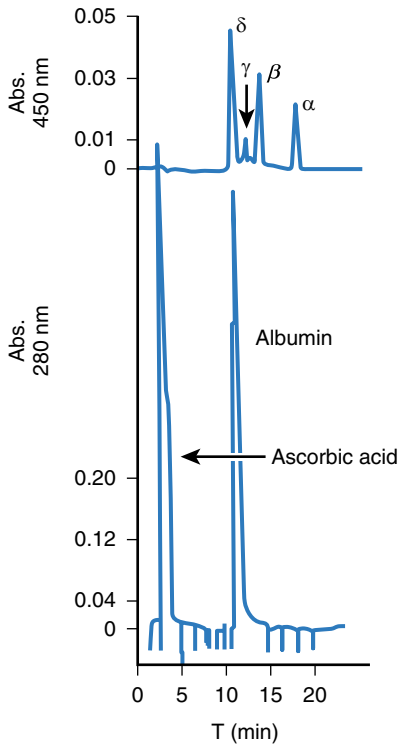
7 to 8 mg/dL (120–137 µmol/L) of unconjugated bilirubin can be bound to each gram of albumin. Physiologically, newborns have a lower bilirubin-binding capacity compared with adults or older children. This occurs because of reduced neonatal albumin concentrations and reduced molar binding capacities. Binding of unconjugated bilirubin by albumin is believed to be of importance in determining bilirubin neurotoxicity, because the unbound bilirubin fraction is thought to be the toxic form of bilirubin and a more sensitive predictor of bilirubin-induced neurologic disorders (BIND) than the TB used clinically.[4] However, except for in Japan, there is currently no reliable and clinically available device for determining unbound bilirubin concentrations that can serve as a useful clinical tool in evaluating a newborn's risk for developing BIND[4] or in the therapeutic decision-making process.

The TB concentration measured by conventional clinical laboratory methods is the basis for decision making for the management of hyperbilirubinemia. Bilirubin exists in four different forms in circulation: (1) unconjugated, which is reversibly bound to albumin and makes up the major portion; (2) unconjugated not bound to albumin (known as free or unbound bilirubin), which is present in a relatively minute quantities but potentially neurotoxic; (3) conjugated, which is comprised mainly of mono- and diglucuronides that have effluxed from the hepatocyte to the circulation and are readily excretable through the renal or biliary systems; and (4) conjugated covalently bound to albumin, known as δ-bilirubin. Indirect, or unconjugated, bilirubin may increase in the serum or plasma in the presence of exaggerated hemolysis or diminished bilirubin glucuronidation. Conjugated or direct bilirubin will increase in association with excretory immaturity or with cholestatic diseases in which bilirubin is conjugated but its excretion is impaired.[2] A similar effect may be seen following acute hemolytic episodes in which indirect bilirubin is conjugated, but the large amounts of conjugated bilirubin are unable to be excreted via the bile. δ-Bilirubin has a plasma disappearance rate similar to that of serum albumin (Fig. 95.4).

"Conjugated" Versus "Direct" Bilirubin

Conjugated bilirubin, but not δ-bilirubin, gives a "direct" reaction with standard diazo reagents, whereas bound or unbound unconjugated bilirubin yields an "indirect" reaction. The terms *indirect* and *direct bilirubin* tend to be used interchangeably with unconjugated and conjugated bilirubin, respectively. However, the terms are not identical and measure differing bilirubin components. Direct bilirubin assays measure all conjugated bilirubin including mono- and diglucuronides, as well as δ-bilirubin and some unconjugated bilirubin. Conjugated bilirubin implies measurement of mono- and diglucuronides only. Therefore conjugated bilirubin determinations may be lower than direct measurements performed on an identical serum or plasma sample.[5]

Conjugated bilirubin usually comprises a small fraction of the TB concentration. δ-Bilirubin can be measured only



• **Fig. 95.4** Separation of serum bilirubin fractions by high-performance liquid chromatography, showing bilirubin profiles at 450 nm (*upper tracing*) and 280 nm (*lower tracing*). α, Unconjugated bilirubin; β, monoconjugated bilirubin; δ, delta fraction bilirubin; γ, diconjugated bilirubin; *Abs*, absorption; *T*, time. (From Wu TW. Bilirubin analysis – the state of the art and future prospects. *Clin Biochem*. 1984;17(4):221–229.)

with newer techniques. It is found in detectable amounts in normal older neonates and children and at significantly increased concentrations in those with prolonged conjugated hyperbilirubinemia resulting from various liver disorders. However, it is virtually absent from the serum or plasma during the first 2 weeks of life.

Hepatic Uptake of Bilirubin

Bilirubin dissociates from circulating albumin before entering the liver cell. The latter process occurs partly by passive carrier-mediated diffusion and partly by organic anion transporter proteins. In the liver cell cytoplasm, the unconjugated bilirubin is bound to glutathione-*S*-transferase A, also known as ligandin, or with B-ligandin (Y protein). These are major intracellular transport proteins, and their bilirubin-binding ability helps keep the potentially toxic unbound bilirubin level low. Z protein, another hepatic cytoplasmic carrier, also binds bilirubin but with a lower affinity.

Equilibrium Between Bilirubin Production and Elimination Processes—The Major Determinant of TB Levels

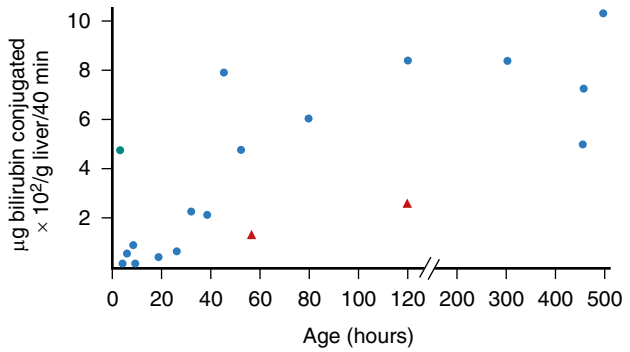
The equilibrium between the rates of bilirubin entry into the circulation (including de novo synthesis, enterohepatic circulation, and tissue shifts) and the bilirubin elimination process (including hepatic cell uptake and conjugation and excretion of bilirubin) determines the TB concentrations at any specific time. This concept is equally applicable under normal physiologic and pathologic circumstances alike.

A reduced capacity of net hepatic uptake of unconjugated bilirubin has been implicated in the development of physiologic jaundice. In the newborn monkey, deficiency of B-ligandin and reduced clearance of sulfobromophthalein were demonstrated in the first 3 days of life, the period during which this animal frequently has physiologic jaundice. Studies in the human indicate that a deficiency of bilirubin uptake is probably of less importance in the pathogenesis of unconjugated hyperbilirubinemia than an immaturity of the bilirubin conjugation system during the first 3 or 4 postnatal days. However, the relative contribution of uptake deficiency may be greater during the second week of life, when the rate of bilirubin conjugation increases and approaches that of normal adults.

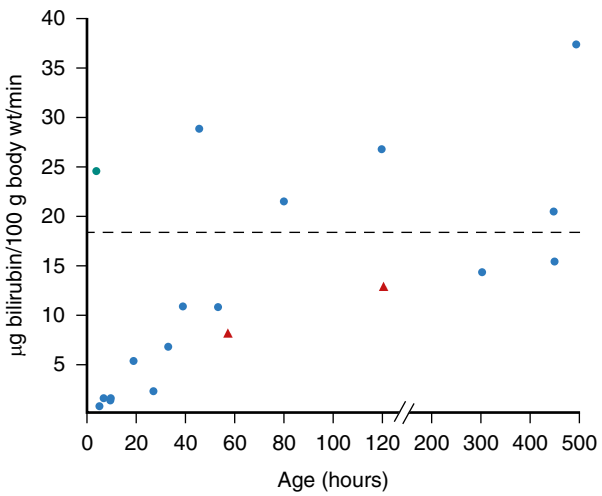
Conjugation of Bilirubin

In order for bilirubin to be excreted into the bile, the nonpolar, water-insoluble, unconjugated bilirubin must be converted to a more polar, water-soluble substance. The purpose of this process is to alter the bilirubin molecule by solubilizing bilirubin IX-α. Bilirubin is presumed to be transported by hepatic B-ligandin from the liver cell plasma membrane to the endoplasmic reticulum, where the conjugating enzyme UGT1A1 is situated. Conjugation is a two-step enzymatic process in which each molecule of bilirubin is conjugated with two molecules of glucuronic acid. Glucuronic acid is derived from activated UDPGA, itself synthesized by the soluble cytoplasmic enzyme uridine diphosphoglucose dehydrogenase from uridine diphosphoglucose, which is, in turn, synthesized from free glucose. The UGT1A1 enzyme first catalyzes the transfer of one glucuronic acid molecule from one of the two propionic acid side groups on one of the central pyrrole rings of bilirubin, in an ester linkage, to form bilirubin monoglucuronide. The physiologic reduction in enzyme activity in the newborn to less than 1% of normal may therefore result in unconjugated hyperbilirubinemia.

Although bilirubin monoglucuronide is water soluble and capable of being excreted into bile without further alteration, about two-thirds of the total bilirubin excreted into bile in the adult human is in the form of a diglucuronide. The second step of the enzymatic conjugation process involves the esterification of a second glucuronide molecule to the now monoconjugate. This process is catalyzed primarily by the same UGT1A1 enzyme on the endoplasmic reticulum, although a second enzyme, UDP–glucuronate glucuronosyltransferase (transglucuronidase), located in the canalicular portion of the hepatocyte plasma cell membrane, may also play a role. The substrate for the canalicular transglucuronidation is believed to be bilirubin monoglucuronide. The enzyme transfers one molecule of glucuronic



• **Fig. 95.5** Hepatic bilirubin uridine diphosphate glucuronic acid (UDPGA) glucuronosyltransferase 1A1 (UGT1A1) activity in term (*blue circles*), premature (*red triangles*), and post-mature (*green circle*) newborn rhesus monkeys. (From Gartner LM, Lee KS, Vaisman S, Zarafu I. Development of bilirubin transport and metabolism in the newborn rhesus monkey. *J Pediatr*. 1977;90(4):513–531.)



• **Fig. 95.6** Maximal hepatic bile bilirubin excretion in term (*blue circles*), premature (*red triangles*), and post-mature (*green circle*) newborn rhesus monkeys. The horizontal dashed line represents the mean normal hepatic bile bilirubin excretion for nine adult rhesus monkeys (18.2 ± 1.0 SEM). (From Gartner LM, Lee KS, Vaisman S, Zarafu I. Development of bilirubin transport and metabolism in the newborn rhesus monkey. *J Pediatr*. 1977;90(4):513–531.)

acid from one molecule of bilirubin monoglucuronide to another, resulting in the formation of one molecule of bilirubin diglucuronide. The latter molecule is excreted into the bile canaliculus, whereas the remaining molecule of now unconjugated bilirubin is returned to the endoplasmic reticulum for subsequent reconjugation. In circumstances such as in severe chronic hemolysis, increased loads of bilirubin are delivered to the liver, and a limited excretory ability may therefore result in retention of conjugated bilirubin in the form of bilirubin monoglucuronide.

The result of the esterification is to disrupt the intramolecular hydrogen bonds, thereby opening the molecule and rendering the conjugated bilirubin water soluble and hence excretable in the bowel. Water solubility also decreases the amount of bilirubin reabsorbed from the bowel, because hydrophilic agents do not pass through the intestinal wall easily.

In the normal adult, glucuronide conjugation accounts for the disposal of about 90% of all bilirubin. The remaining portion is converted to water-soluble substances by conjugation with substances other than glucuronic acid, or by oxidation, hydroxylation, or reduction. In humans, bilirubin forms a conjugate with glucose, xylose, possibly other carbohydrates, sulfates, and taurine. These non-glucuronide conjugates account for no more than 10% of the bilirubin conjugates excreted in the bile.

A number of in vitro studies have demonstrated the existence of deficiencies in hepatic UGT1A1 activity in newborns of many species, including humans. In newborn rhesus monkeys, hepatic bilirubin conjugating capacity is extremely low during the first hours of life and functions at about 5% of adult capacity (Fig. 95.5). However, by 24 hours of age, UGT1A1 activity increases sufficiently to process the bilirubin load presented to the liver, and the TB concentration begins to fall. In 1-day-old rats, the proportions of both xylose and glucose conjugates of bilirubin equal those of glucuronide conjugates. Total conjugating capacity increases to adult levels by the fourth day of life, when a mature pattern of glycoside distribution is present, with 75% of all conjugates being glucuronides. In human newborns, the monoglucuronide conjugate is the predominant bile pigment conjugate. UGT1A1 activity in term infants is about 1% of that of healthy adults (and even less in premature infants) and increases at an exponential rate until 3 months of age, when adult levels are reached. Non-glucuronide conjugates are insignificant in this period.

Excretion of Bilirubin

Excretion of the now polar, water-soluble bilirubin appears to be an energy-dependent concentrative process. The bilirubin conjugates are incorporated into mixed micelles along with bile acids, phospholipids, and cholesterol. The conjugates are excreted against a concentration gradient, and as a result the bile bilirubin concentration is about 100-fold that of the hepatocyte cytoplasm. Although the capacity for bilirubin excretion into bile is limited in newborn rhesus monkeys (Fig. 95.6), excretory deficiency is not a rate-limiting factor in the overall hepatic elimination of bilirubin in the human newborn. In newborn babies, bilirubin uptake into the hepatocyte and the enzyme-mediated conjugation processes are the more restrictive steps and may result in a "bottleneck" effect. However, a large bilirubin pool requiring elimination, such as in hemolytic disease of the newborn, may overwhelm the excretory capacity with efflux of backed up conjugated bilirubin into the circulation.[6] In contrast, in older humans and in the mature rhesus monkey and other mammals beyond the newborn period, hepatic excretion of conjugated bilirubin into bile predominates as the rate-limiting step in the presence of a large bilirubin load. At any age, in the presence of hepatic cell injury and biliary obstruction, hepatic excretory transport is the step most severely restricted, resulting in an efflux of conjugated bilirubin from the hepatocyte to the serum with resultant conjugated hyperbilirubinemia. Thus the hepatic excretory

step may have the least reserve capacity of all the processes contributing to bilirubin elimination.

Enterohepatic Reabsorption of Bilirubin

Conjugated bilirubin is not absorbed from the intestine; however, the mono- and diglucuronides of bilirubin are relatively unstable conjugates that are readily hydrolyzed to unconjugated bilirubin. Reverted unconjugated bilirubin may now be readily absorbed across the intestinal mucosa, contributing, through the enterohepatic circulation, to the circulating unconjugated bilirubin pool and again being presented to the liver for conjugation. Of importance in the mechanism of the enterohepatic circulation is the enteric mucosal enzyme β-glucuronidase, which is present in both term and premature neonates in high concentrations. Mild alkaline conditions present in the duodenum and jejunum contribute to the deconjugation process.

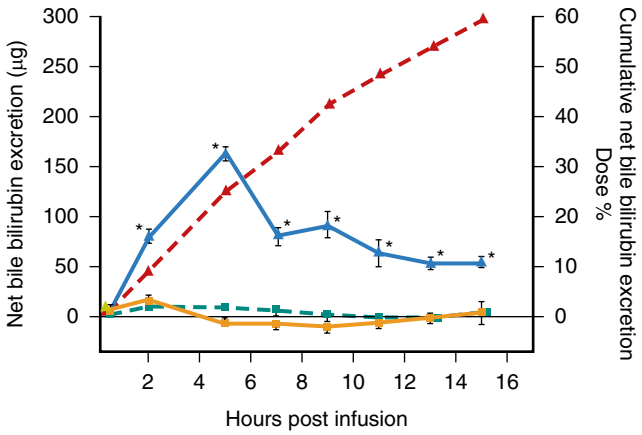
A study in adult rats demonstrated that enteric absorption of unconjugated bilirubin occurs predominantly in the duodenum and colon. The extent of absorption varies widely, depending on diet and caloric intake (Fig. 95.7). Although quantitative estimates of the disposal of bilirubin have been performed only for adult humans, these data do indicate that about 25% of the bilirubin excreted into the intestine is reabsorbed as unconjugated bilirubin. About 10% of the total is excreted in stool as unaltered bilirubin. The remaining pigment is converted to urobilinoids, most of which are excreted in stool, with a small portion being reabsorbed in the colon for subsequent excretion by both the liver and kidney.

Neonates have relatively high concentrations of unconjugated bilirubin in the intestine, which contribute to the enterohepatic circulation. Intestinal bilirubin is derived from increased bilirubin production, exaggerated hydrolysis of bilirubin glucuronide, and high concentrations of bilirubin found in meconium. The relative lack of bacterial flora in the newborn bowel to reduce bilirubin to urobilinogen further increases the intestinal bilirubin pool in comparison with that of the older child and adult. The increased hydrolysis of bilirubin conjugates in the newborn is enhanced by high mucosal β-glucuronidase activity and the excretion of predominantly monoglucuronide conjugates (in the newborn) rather than diglucuronides (in the adult). Oral administration of nonabsorbable bilirubin-binding substances, such as agar, activated charcoal, or a lipase inhibitor (e.g., Orlistat), may retain bilirubin in the bowel, thereby further increasing stool bilirubin content and reducing bilirubin reabsorption, thereby decreasing TB levels. Studies of intestinal bilirubin binding add to our understanding of the contribution of the enterohepatic circulation to unconjugated hyperbilirubinemia of the newborn.[1]

Genetic Control of Bilirubin Production

Heme Oxygenase-1

HO-1 is the rate-limiting enzyme that catabolizes heme to biliverdin and then to bilirubin, with the simultaneous release of equimolar quantities of ferrous iron (Fe^{2+}) and CO. Polymorphisms of the *HMOX1* gene promoter region may modulate its transcriptional activity, with increased HO-1 expression and total enzyme activity being associated with the overproduction of bilirubin.

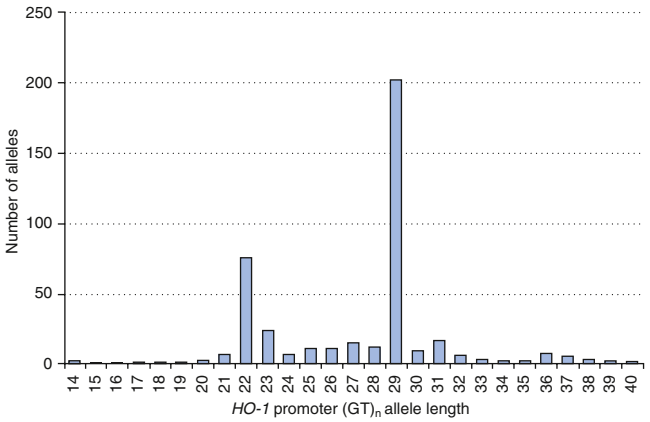


• **Fig. 95.7** Net rate of bilirubin excretion in adult rat bile over 15 hours after intraduodenal administration of 1000 mg unconjugated bilirubin in normal human milk at pH 8.6 (*orange squares*, mean ± SEM; *N* = 5) and human milk from mothers of infants with breast milk jaundice syndrome, pH 8.6 (*blue triangles*, mean ± SEM; *N* = 5). Cumulative net bilirubin excretion in bile for the same experiments expressed as a percentage of administered dose (*green squares*, bilirubin in normal human milk; *red triangles*, bilirubin in human milk from mothers of infants with breast milk jaundice syndrome). Asterisks indicate $P < .01$ for bilirubin in normal human milk versus bilirubin in human milk from mothers of infants with breast milk jaundice syndrome. (From Gartner LM, Lee KS, Vaisman S, Zarafu I. Development of bilirubin transport and metabolism in the newborn rhesus monkey. *J Pediatr*. 1977;90(4):513–531.)

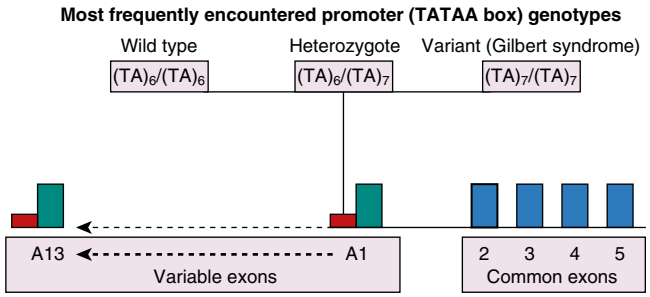
The *HMOX1* Gene and Promoter Polymorphisms

The *HMOX1* gene promoter region has a polymorphic glutathione thymidine, $(GT)_n$, repeat sequence with lengths ranging from 12 to 40 repeats. As can be seen in Fig. 95.8, the $(GT)_n$ repeat distribution is bimodal with the main alleles at or around 23 and 30 repeat lengths.[2] The number of $(GT)_n$ repeats can modulate the rate of transcriptional activity (hence, gene expression), with short sequences (less than 25) being associated with increased transcriptional activity compared with those with long (25 or greater) sequences.

The prevalence of the short $(GT)_n$ lengths is cardinal to the analyses of the contribution of *HMOX1* to the pathophysiology of hyperbilirubinemia. Higher HO activity associated with short alleles should lead to increased heme catabolism and hyperbilirubinemia. Newborn studies, however, have shown contradictory results. Most published studies have demonstrated no effect of *HMOX1* promoter polymorphisms on TB levels. In two studies, however, from Japan and Taiwan, a modulating effect of short $(GT)_n$ repeats in increasing TB has been reported.[7,8] A potential role of short $(GT)_n$ repeats to exacerbate hyperbilirubinemia in the presence of hemolysis in which large amounts of



• **Fig. 95.8** Distribution of *HMOX1* $(GT)_n$ repeats according to allele length of 199 newborns. Note the bimodal distribution common to other population groups. *HO-1*, heme oxygenase 1. (From Kaplan M, Renbaum P, Hammerman C, et al. Heme oxygenase-1 promoter polymorphisms and neonatal jaundice. *Neonatology*. 2014;106(4):323–329.)



• **Fig. 95.9** The human *UGT1* gene locus. Schematic representation of the genomic structure of the *UGT1* gene complex. Variable exon 1A1 and common exons 2 to 5 of the gene complex have been identified as those sites encoding the bilirubin conjugating enzyme, uridine diphosphate glucuronic acid (UDPGA) glucuronosyltransferase 1A1 (UGT1A1). Variable exons 1A2 to 1A13 do not participate in bilirubin metabolism. Genetic mutations associated with absent or decreased enzyme activity, which cause deficiencies of bilirubin conjugation, have been localized to this variable exon 1A1 (*green boxes*), its promoter (*red boxes*), or the common exons 2 to 5 (*blue boxes*). The upper section of the diagram demonstrates the common exon 1A1 promoter TATAA box genotypes. (Redrawn from Kaplan M, Hammerman C. Bilirubin and the genome: the hereditary basis of unconjugated neonatal hyperbilirubinemia. *Curr Pharmacogenom*. 2005;3(1):21–42.)

released hemoglobin result in *HMOX1* induction has been suggested.

Genetic Control of Uptake of Bilirubin into the Hepatocyte

This process by which unconjugated bilirubin is taken up from the hepatic sinusoids and crosses the hepatocyte membrane to enter that cell is facilitated by a carrier molecule, organic anion-transporting polypeptide-2 (*SLCO1B1*). In humans, this carrier may play an important role in the metabolism of bilirubin and in the prevention of hyperbilirubinemia by facilitating the entry of bilirubin into hepatocytes. A mutation in the gene leading to an impaired maturation of the protein with reduced membrane localization and abolished transport function has been described,[9] and a number of single-nucleotide polymorphisms (SNPs) have been identified, some of which are associated with an altered in vitro transport capability.[9]

Genetics of Diminished Bilirubin Conjugation

Perhaps one of the most important advances in our understanding of the genomics of bilirubin metabolism is the elucidation of the *UGT1A1* gene encoding the bilirubin-conjugating enzyme, UGT1A1. It is becoming more apparent that the modulation of bilirubin metabolism and whether TB levels remain within physiologic or hyperbilirubinemic ranges lie within genetic control. Although not limited to the neonates, Skierka et al. demonstrated that 146/181 neonatal- and pediatric-aged patients referred for hyperbilirubinemia had at least one heterozygous *UGT1A1* variant, indicating that many cases of unconjugated hyperbilirubinemia could be attributed to variations at the *UGT1A1* locus.[10] Below is a short overview to help the reader comprehend mutations of this gene and interactions of these mutations with genetic or environmental factors in the mechanism of jaundice.

The *UGT* Gene

The *UGT* gene is a superfamily of genes whose function is to encode a biochemical reaction leading to the conjugation of glucuronic acid to certain target substrates to facilitate their elimination from the body. The *UGT2* genes are located on chromosomes 4q13 and 4q28. The enzymes encoded by this family preferentially conjugate endobiotic substances, such as steroids and bile acids, and, although of physiologic and pharmacologic importance, they are of little relevance to bilirubin metabolism. In contrast, the *UGT1A1* isoform, which belongs to the *UGT1* family, is of major importance to the conjugation and therefore elimination of bilirubin. This gene isoform has been mapped to chromosome 2q37. *UGT1A1* was cloned by Ritter and associates in 1991.[10a] The gene consists of four common exons (exons 2, 3, 4, and 5) and 13 variable exons, of which only variable exon A1 is of any importance regarding bilirubin conjugation; the remaining exons play a role in the detoxification of a diverse range of chemical substances (Fig. 95.9). The variable exon A1 functions in conjunction with common exons 2 to 5: in response to a specific signal, transcription processing splices mRNA from the variable exon to the common exons. This process provides a template for the synthesis of an individual enzyme isoform. Upstream of each variable exon is a regulatory noncoding promoter that contains a box sequence of thymidine-adenine (TA) repeat (TATAA) of nucleic acids. Mutations of variable exon A1, its promoter, or the common exons 2 to 5 may result in deficiencies of bilirubin conjugation. SNPs of the noncoding promoter area

affect bilirubin conjugation by diminishing expression of a normally structured enzyme, whereas mutations of the gene coding area may affect enzyme function by altering the structure of the enzyme molecule itself. Further information can be found in the section on Conjugated Hyperbilirubinemia.

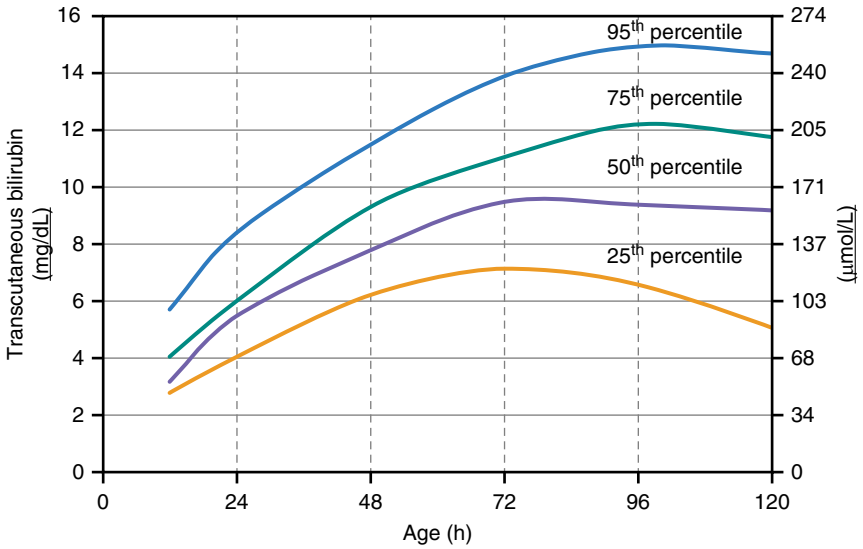
Co-Expression of Genes Modulating the Risk of Neonatal Hyperbilirubinemia

In a genome-wide study performed on adults in Sardinia, three loci were associated with the modulation of TB levels: *UGT1A1*, glucose-6-phosphate dehydrogenase (*G6PD*), and *SLCO1B3*, the latter a member of the SLC family implicated in bilirubin uptake into the hepatocyte.[9] Pursuing this finding in relation to neonatal hyperbilirubinemia, Lin et al.[10b] studied the allele frequencies of mutations and polymorphisms of *UGT1A1*, *G6PD*, and *SLCO1B1* in DNA samples obtained from the DNA Polymorphism Discovery Resource of the National Human Genome Research Institute, which had been studied and were thought to be representative of the current US population. Although no clinical information was available for the individuals whose DNA was included in the sampling, a high rate of gene co-expression does suggest a potentially important role for genetic polymorphism co-inheritance in neonatal hyperbilirubinemia. Co-expression of genes with another, with mutations or polymorphisms, or with environmental factors may potentiate their role and exacerbate the pathophysiology of neonatal hyperbilirubinemia to a greater extent than each gene individually. Results of three genome-wide association studies ($N = 9464$) were pooled to identify genetic contributors to variability in TB levels in adults. The substantial genetic influence of *UGT1A1* variants, consistent with past studies, was confirmed, along with strong evidence of a role for allelic variations in *SLCO1B1*.

Nonpathologic Unconjugated Hyperbilirubinemia

Elevations in unconjugated bilirubin levels occur ubiquitously in the human neonatal population. In a sense, this "normal" increase in TB levels is not true hyperbilirubinemia when compared with a reference group of all newborns. A more appropriate term that would add to our understanding of the phenomenon and distinguish the normal or physiologic state from the pathologic entity implied in the term *hyperbilirubinemia* may be *physiologic bilirubinemia*. An hour-specific bilirubin nomogram developed by Bhutani et al.,[10c,11] although useful for clinical evaluation of TB levels, does not reflect the natural history of bilirubin dynamics during the first postnatal days because of selection bias. The natural increase in TB levels during the first days of life of a newborn is, however, reflected in the recently constructed, global transcutaneous bilirubin (TcB) nomogram. This nomogram included over 119,000 measurements from 44,392 apparently normal, predominantly breastfed newborns ≥35 weeks of gestation. As seen in Fig. 95.10, the pooled TcB trajectories increased during the first 3 postnatal days and peaked or plateaued between the third and fourth days.[12]

Unconjugated hyperbilirubinemia in the human, regardless of age, is defined as an indirect-reacting bilirubin concentration of 2.0 mg/dL (34 µmol/L) or greater, depending on the standard used in the calibration of the reaction. Nearly all adults and older children normally have indirect-reacting bilirubin concentrations in circulation of less than



• **Fig. 95.10** Nomogram constructed from pooled transcutaneous bilirubin (*TcB*) readings derived from 20 nomograms from 12 different countries, depicting the approximate natural history of postnatal bilirubinemia in normal, predominantly breastfed newborns ≥35 weeks of gestation. (From Kaplan M, Maisels MJ. Natural history of early neonatal bilirubinemia: a global perspective. *J Perinatol*. 2021;41(4):873–878.)

0.8 mg/dL (14 μmol/L) and δ-bilirubin of 0.2 to 0.3 mg/dL (3–5 μmol/L). Conjugated hyperbilirubinemia is defined as an elevation of the direct-reacting fraction in the van den Bergh diazo reaction of greater than 1.5 mg/dL (26 μmol/L) provided it comprises more than 10% of the TB concentration. The latter portion of the definition is added to guard against over-interpretation of direct reactions in newborns with markedly elevated indirect-reacting bilirubin concentrations, because up to 10% of the unconjugated pigment behaves as direct-reacting pigment in the van den Bergh–type methods.

Clinical situations in which the direct-reacting bilirubin concentration is equal to or close to the TB concentration are extremely rare, especially in the newborn period. In the neonate with conjugated hyperbilirubinemia, the hyperbilirubinemia is usually "mixed," the elevated direct-reacting fraction accounting for 20% to 70% of the total pigment. Thus a neonate with mixed hyperbilirubinemia should be considered primarily to have *conjugated* hyperbilirubinemia. Except in cases of extreme hemolysis, such as in hemolytic disease of the newborn, pathology resulting from interference with hepatic cell excretion and bile transport, rather than from abnormalities of increased bilirubin production or deficient hepatic bilirubin uptake or conjugation, should be sought.

Fetal Bilirubin

During the last stages of human gestation, the normal degradation of erythrocytes formed earlier in fetal life results in about a 150% increase in bilirubin production per unit of body weight compared with adults. The mammalian fetus of all species appears to be capable of degrading heme without limitation through the two enzymatic steps responsible for the formation of unconjugated bilirubin IX-α, CO, and biliverdin (see Fig. 95.2). However, notable species differences exist in the pattern of development of hepatic bilirubin conjugation. Marked deficiency in UGT activity is noted in rat, rabbit, guinea pig, sheep, dog, monkey, and human fetuses. At term, UGT activity in the rhesus monkey is only 1% to 5% of that in the adult. In the human fetus, UGT activity is extremely low before 30 weeks of gestation, at about 0.1% of adult activity, and gradually increases to about 1% at term. Diminished UGT activity is the central rate-limiting step that, in conjunction with additional processes, including increased bilirubin production, enhanced enterohepatic circulation, and diminished uptake into the hepatocyte, manifests as physiologic jaundice in monkeys and humans.

Significant hyperbilirubinemia is unusual in the human fetus, because the placenta transports unconjugated bilirubin from the fetus to the mother. Administration of radioactive unconjugated bilirubin into the fetal circulation of a dog, guinea pig, or monkey shows a rapid disappearance from the fetal side and recovery in the maternal bile. Even in states of severe intrauterine hemolysis from conditions such as Rh or other isoimmunizations, the degree of anemia by far exceeds the level of hyperbilirubinemia, and clinical jaundice is usually mild at birth.[2] Indeed, intrauterine therapeutic interventions in this condition are indicated by fetal anemia rather than fetal hyperbilirubinemia. After delivery and separation of the placenta from the infant, increases in TB levels may be expected and excessive in the face of hemolytic disorders. By contrast, the placenta is barely permeable to conjugated bilirubin. Thus in the absence of evidence of hemolytic disease, if clinical jaundice is present at birth, a conjugated hyperbilirubinemia caused by intrauterine hepatic pathology should be suspected.

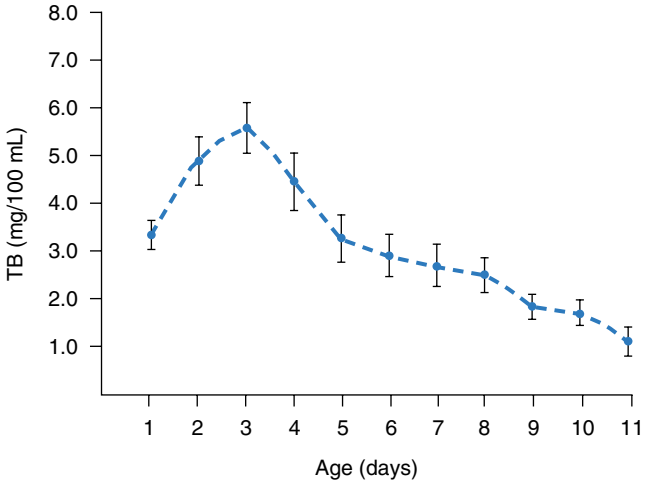
A large amount of bilirubin is found in meconium, indicating appreciable activity of fetal hepatic bilirubin conjugation. A significant level of β-glucuronidase activity is found in meconium, suggesting that conjugated bilirubin in the fetal intestine can be hydrolyzed back to unconjugated bilirubin and then absorbed from the bowel and into the portal circulation. This absorbed bilirubin may reenter the hepatocyte for subsequent reconjugation and re-excretion or may be transferred through the placenta into the maternal circulation. The efficiency of this process is protective to the fetus against severe hyperbilirubinemia, even when hemolysis is severe.

Conjugated hyperbilirubinemia in the mother, which may occur in hepatitis or recurrent jaundice of pregnancy, is not reflected in the cord blood. Severe hemolytic disease in the fetus results in small but significant increases in amniotic fluid bilirubin concentrations. How bilirubin enters the amniotic fluid pool is not known, but suggestions have included direct transfer across the placenta from the maternal circulation, transudation of pigment across the amniotic membranes or cord vessels, and secretion of bilirubin in the pulmonary fluids flowing from the fetal lung into the fetal pharynx and oral cavity and then into the amniotic fluid. Although to a great extent replaced by noninvasive measurements of anterior cerebral artery flow as an index of fetal anemia in recent decades, measurement of amniotic fluid bilirubin concentrations by spectrophotometry, combined with percutaneous umbilical blood sampling allowing for serial hematocrit determinations and fetal intravascular transfusions, have resulted in markedly improved outcomes for the now-rare fetus and infant with Rh erythroblastosis (see Chapter 22).

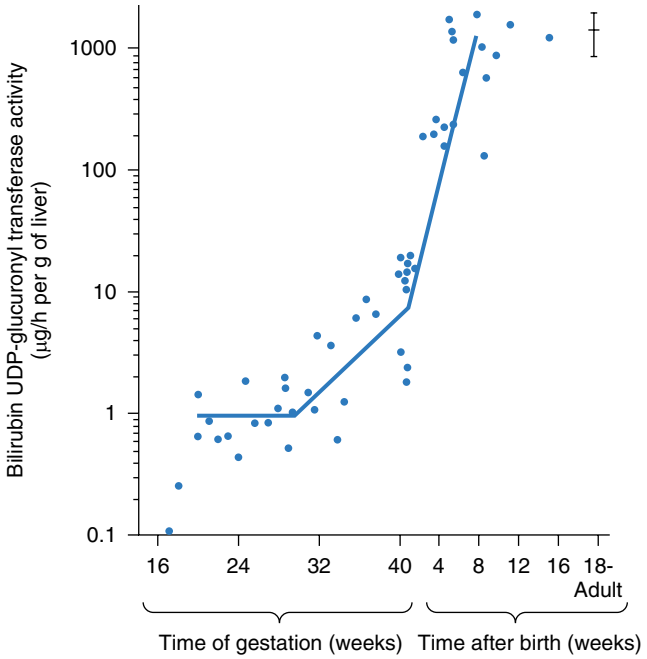
Neonatal Hyperbilirubinemia

Term Neonate

In the full-term newborn, physiologic jaundice is characterized by a progressive rise in TB concentration from about 2 mg/dL (34 μmol/L) in cord blood to a mean peak of 5 to 6 mg/dL (86–103 μmol/L) between 48 and 120 hours of age in Caucasian and African American infants, with most TB levels peaking at 72 to 96 hours of age, and 10 to 14 mg/dL (171–239 μmol/L) between 72 and 120 hours of age in Asian-American infants. This is followed by a rapid decline to about 3 mg/dL (51 μmol/L) by the fifth day of life (Fig. 95.11) in Caucasian and African American neonates and by the seventh to tenth day of life in Asian-American neonates.



• **Fig. 95.11** Mean total bilirubin (*TB*) concentrations in 22 full-term normal Caucasian and African American infants during the first 11 days of life. Vertical bars represent standard errors of the mean. (From Gartner LM, Lee KS, Vaisman S, Zarafu I. Development of bilirubin transport and metabolism in the newborn rhesus monkey. *J Pediatr*. 1977;90(4):513–531.)



• **Fig. 95.12** Developmental pattern of hepatic bilirubin uridine diphosphate glucuronic acid (UDPGA) glucuronosyltransferase 1A1 (UGT1A1) activity in humans. (From Kawade N, Onishi S. The prenatal and postnatal development of UGT activity towards bilirubin and the effect of premature birth on this activity in the human liver. *Biochem J*. 1981;196(1):257–260. Reprinted by permission of the Biochemical Society, London.)

This early period of physiologic jaundice has been designated *phase 1* physiologic jaundice. During the period from the fifth to the tenth days of life in Caucasian and African American infants, TB concentrations decline slowly, reaching the normal adult value of less than 2 mg/dL (34 μmol/L) by the end of that period. This late neonatal period of minimal, slowly declining hyperbilirubinemia, has been designated *phase 2* physiologic jaundice. The epidemiology is dependent, in part, on the prevalence of breastfeeding in a population, because lower peak TB values will be found among predominantly formula-fed infants.

Studies in the newborn rhesus monkey, an animal with a pattern of physiologic jaundice of the newborn that is similar to that in humans, show that phase 1 results from the combination of a sixfold postnatal increase in the load of bilirubin presented to the liver combined with a markedly diminished UGT activity. The presence of either of these factors alone would result in retention of unconjugated bilirubin to a lesser extent than when in combination. Hepatic uptake and excretion of bilirubin are also decreased during this period, although their function as rate-limiting steps in the transport of bilirubin from plasma into bile is dwarfed by the combination of increased bilirubin load to the liver and diminished conjugative capacity. The very large increase in bilirubin load appears to result from both increased de novo bilirubin synthesis and enteric reabsorption of unconjugated bilirubin. In the newborn monkey, the markedly increased load persists for 3 to 6 weeks, primarily because of enhanced intestinal bilirubin absorption. Similar data are not yet available for the human neonate.

In the human, UGT1A1 activity is extremely low in the fetal period. After birth, UGT1A1 activity increases at an exponential rate, reaching the adult level by 6 to 12 weeks of age (Fig. 95.12). The early deficiency in enzyme activity may result from insufficient enzyme synthesis, inhibition of enzymatic activity by naturally occurring substances, deficient synthesis of the glucuronide donor UDPGA, or a combination of these factors. Phase 2 physiologic jaundice appears to result from an imbalance in which hepatic uptake of bilirubin remains diminished while an increased bilirubin load presented to the liver persists. Developmental deficiency of B-ligandin may contribute to deficient uptake of bilirubin.

Up to 80% of term newborns become visibly jaundiced during the first 3 days of life. A greater proportion of exclusively breastfed infants can be expected to display some degree of jaundice. Cutaneous icterus in the newborn will not become evident until TB concentrations exceed 5 to 6 mg/dL (86–103 μmol/L). This situation contrasts with that of the older child and adult, in whom jaundice may be noticeable in the conjunctiva and skin at TB concentrations as low as 2 mg/dL (34 μmol/L). Variations in the duration of hyperbilirubinemia, in skin color, and in perfusion may account for these differences. As the intensity of jaundice increases, clinical icterus progresses in a caudal direction. At lower levels of TB, only the head and conjunctiva may be affected, with the chest, abdomen, legs, and feet becoming jaundiced in parallel to increasing TB concentrations. Because routine daily TB determinations are not usually performed on full-term or even premature newborns, in the past, careful scrutiny of the nursery population several times a day by experienced personnel was essential in detecting infants who were becoming jaundiced, as some of these

may subsequently develop significant to severe hyperbilirubinemia and require further TB testing. Visual assessment of jaundice, however, is largely subjective, inaccurate, and dependent on the observer's experience. More recent developments of TcB monitoring devices intended to measure the skin color objectively and noninvasively and convert this color reading to a bilirubin estimation may improve on the reliability of visual estimation. Daily noninvasive TcB determinations may enhance the predictive value of the technique by allowing the actual trajectory to be plotted against those of the hour-specific TB or TcB nomograms (see Transcutaneous Bilirubinometry). This is important in the pre-discharge assessment of newborns, especially those discharged before 72 hours of age.

Preterm Neonate

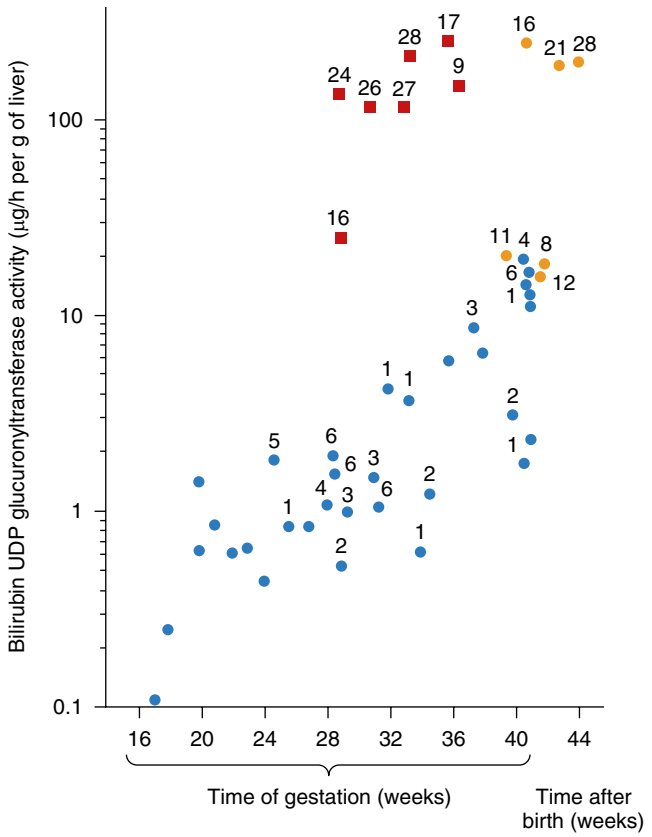
Physiologic jaundice in premature neonates is more severe than in term neonates. In larger preterm infants, mean peak TB concentrations may reach 10 to 12 mg/dL (171–205 µmol/L) by the fifth day of life. This delay in reaching the maximal concentration compared with term neonates primarily reflects the delay in the maturation of hepatic UGT1A1 activity. Because mean peak unconjugated bilirubin concentrations as low as 10 to 12 mg/dL (171–205 µmol/L) may be associated with ABE or kernicterus in certain high-risk, low birth weight neonates, all degrees of visible jaundice in premature neonates should be monitored closely and investigated fully. Small premature infants cared for in an intensive care nursery will rarely be allowed to reach the TB levels mentioned, but treatment with phototherapy will be initiated at much lower levels. The natural peak TB level in small premature infants is therefore mainly unknown.

Despite lower UGT1A1 activity in premature neonates than in term neonates at birth, UGT1A1 activity increases rapidly, far exceeding the expected maturational rate noted in utero (Fig. 95.13). This observation indicates that there are two components in the maturational process of hepatic UGT1A1 activity: (1) chronologic maturation, and (2) accelerated maturation related to birth. Nevertheless, normal TB concentrations in premature neonates may not be reached in many cases until the end of the first month of life.

Jegathesan et al.[13] recently constructed a nomogram from 6143 pre-phototherapy TB results from 2549 preterm infants born between 29 and 35 weeks of gestation in Toronto, Ontario, Canada (Fig. 95.14). The nomogram provides some clinical information about hourly trends in TB levels in moderately preterm newborns.[13]

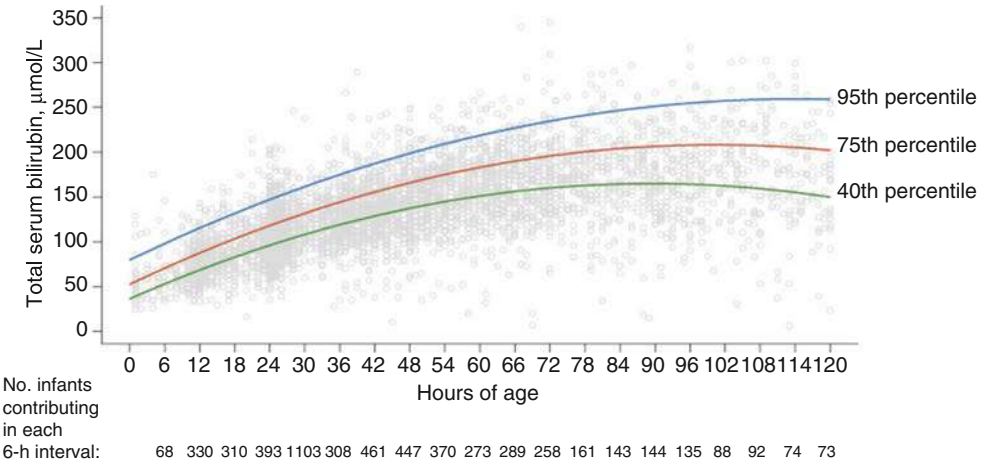
Late Preterm Neonate

Late preterm gestation (34 0/7–36 6/7 weeks) is an important risk factor for the development of severe neonatal hyperbilirubinemia and kernicterus. These infants are physiologically immature and have limited compensatory responses compared with term infants. They are at greater risk of morbidity and mortality than term counterparts.



• **Fig. 95.13** Effect of premature birth on development of hepatic uridine diphosphate glucuronic acid (UDPGA) glucuronosyltransferase 1A1 (UGT1A1) activity in humans. Numbers beside the symbols represent age (days) at which activities were measured. Symbols represent enzyme activities for premature (*red squares*) and full-term (*orange circles*) infants who lived more than 8 days after birth, and for fetuses and premature and full-term infants who died within 7 days of delivery (*blue circles*). (From Kawade N, Onishi S. The prenatal and postnatal development of UGT activity towards bilirubin and the effect of premature birth on this activity in the human liver. *Biochem J*. 1981;196(1):257–260. Reprinted by permission of the Biochemical Society, London.)

Among the infants registered in the voluntary US-based Kernicterus Registry, late preterm infants were disproportionately represented compared with those born at term. At this point of gestation, hepatic conjugative capacity is still immature and may contribute to the greater prevalence, severity, and duration of neonatal hyperbilirubinemia in these infants. Additional risk factors increasing the incidence of severe hyperbilirubinemia in these infants include feeding with human breast milk, large-for-gestational-age status, male sex, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and others. Suck-swallow immaturity may also contribute to the risk of hyperbilirubinemia. These infants are at increased risk for readmission, primarily for hyperbilirubinemia. In a US survey, late prematurity increased the risk for neonatal hyperbilirubinemia more than fivefold.[14] In a nationwide population-based cohort study including greater than 1.6 million newborns born at 35 to 41 weeks of gestation between 1998 and 2016 in Sweden, Mitha et al.[15] demonstrated a progressively increasing incidence of neonatal jaundice as gestational age decreased from 41 through



• **Fig. 95.14** Total serum/plasma bilirubin (*TB*) nomogram for preterm infants 29 to 35 weeks of gestation. To convert to metric units, divide by 17.1. (From Jegathesan T, Ray JG, Bhutani VK, et al. Hour-specific total serum bilirubin percentiles for infants born at 29-35 weeks' gestation. *Neonatology*. 2021;118(6):710–719.)

35 weeks of gestation. Compared with neonates born at 39 to 40 weeks of gestation, the relative risk (RR) of developing hyperbilirubinemia was 2.77 (95% confidence interval [CI], 2.70–2.83) for those born at 37 to 38 weeks of gestation (see following paragraph) and increased to 12.85 (95% CI, 12.51–13.20) for those born at 35 to 36 weeks of gestation.[15] Scrupulous attention to screening for jaundice in the newborn nursery, adequate lactation support, parental education, and appropriate post-discharge follow-up should facilitate institution of treatment when clinically indicated.

Early-Term Neonate

Although within the definition of term, newborns born at the early end of term (37–38 weeks of gestation) may be, as a result of relative immaturity, at higher risk for neonatal hyperbilirubinemia than full-term counterparts, and unexplained jaundice may be twofold greater.[16] In a Canadian study, neonates born at 37 and 38 weeks of gestation had a higher risk of readmission for hyperbilirubinemia than those born at ≥39 weeks.[17] Similarly, in Utah, late-term and early-term neonates had higher rates of readmission for hyperbilirubinemia than those born at term.[18]

Term Infant

Neonatal hyperbilirubinemia is the most common cause for readmission in otherwise healthy term infants. Many readmissions can be avoided by discharging the mother-infant dyad when both are ready, when the mother has recovered sufficiently and is able to care for her newborn. Jaundice, if present, must be evaluated, and appropriate treatment or follow-up arranged according to the 2022 American Academy of Pediatrics (AAP) Clinical Practice Guideline.[19]

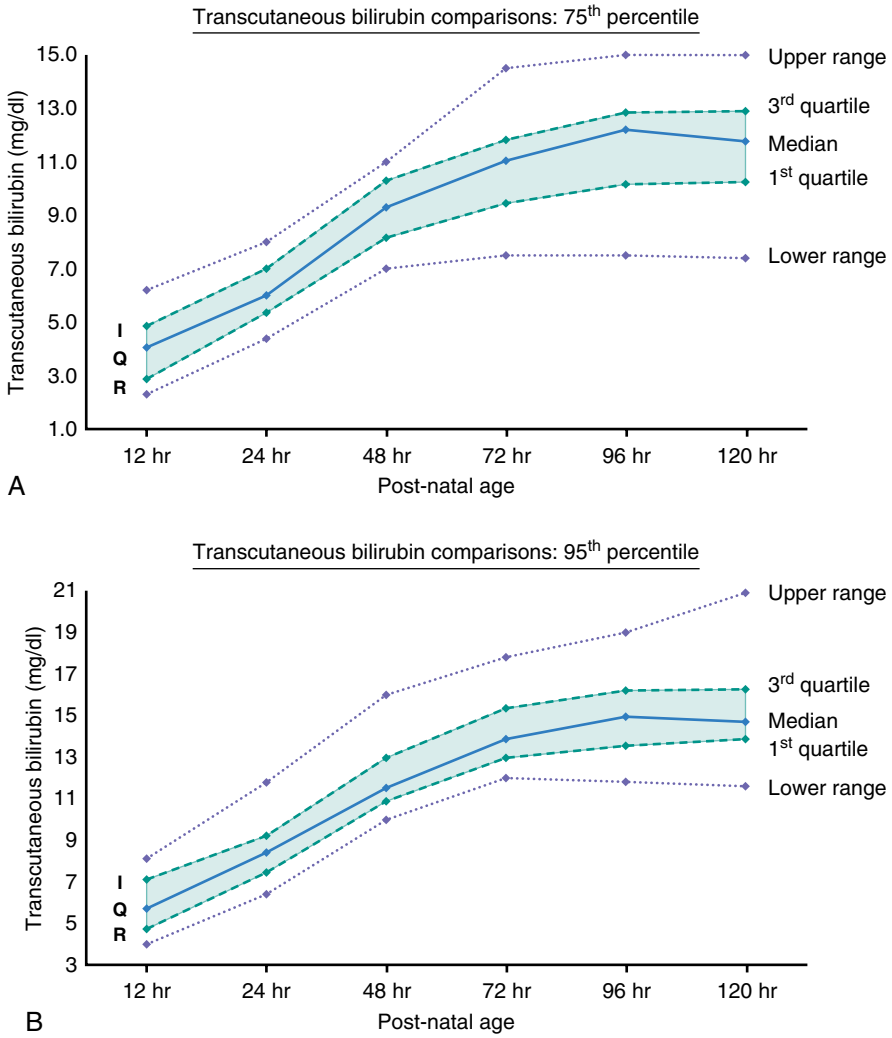
Post-Term Neonate

Nearly all post-term neonates and about half of all term neonates who are small for gestational age may be expected to have little or no physiologic jaundice, with peak TB concentrations of less than 2.5 mg/dL (43 µmol/L). The mechanism for this acceleration of hepatic maturation is unknown. Similarly, neonates of mothers treated with phenobarbital, a drug known to stimulate hepatic UGT activity and the concentration of ligandin, and neonates of heroin users have less than the anticipated severity of physiologic jaundice. Other drugs, less well investigated, also may have similar "maturing" effects.

Genetic, Ethnic, and Cultural Effects

The severity of physiologic jaundice varies significantly among different ethnic populations. Mean maximal TB concentrations in Chinese, Japanese, Korean, Native-American, and other Asian term newborns are 10 to 14 mg/dL (171–239 µmol/L), about double those of the Caucasian and African American populations. The incidence of bilirubin toxicity as defined by autopsy-proven kernicterus is also increased significantly in Asian newborns. There is no clinical evidence for increased hemolysis in Asian newborns to account for these dramatic differences, although some studies of CO production have suggested that bilirubin synthesis may be slightly increased compared with that in Caucasian or African American neonates. A mutation (Gly71Arg) known as *UGT1A1*6* in the *UGT* gene frequently found in Japanese, Korean, and Chinese neonates, but rare in Caucasians, is associated with Gilbert syndrome in Asian populations and has been shown to be associated with an increased incidence of neonatal hyperbilirubinemia in these groups.[2] In contrast, variations in the number of TA repeats in the promoter for the *UGT1A1* gene are commonly encountered in Caucasians and are associated with Gilbert syndrome in that population group (see previous section, Genetics of Diminished Bilirubin Conjugation). The promoter polymorphism is rare in Asian communities.[2] Thus there is mounting evidence that the phenotypic variability in neonatal TB levels seen in different populations results in part from genotypic heterogeneity.

The ease of modern, painless TcB measurements has facilitated construction of TcB nomograms that demonstrate differences in bilirubin trajectories among population



• **Fig. 95.15** Median transcutaneous bilirubin (*TcB*) values, interquartile ranges (*IQRs*), and upper and lower values for the ranges at each point studied for the 75th (A) and 95th (B) percentiles of the 19 nomogram studies analyzed. (From Kaplan M, Bromiker R. Variation in transcutaneous bilirubin nomograms across population groups. *J Pediatr*. 2019;208:273–278.e1.6.)

groups. This phenomenon was first recognized by de Luca, but more recently was again shown by Kaplan and Bromiker.[20] The 95th and 75th percentiles of 19 published TcB nomograms derived from 12 different countries were analyzed. As seen in Fig. 95.15, a wide range of TcB levels was found through the first 5 postnatal days for both percentiles studied. The highest levels for the 95th percentile analysis (of greatest clinical importance) at 48 hours came from Greece, Taiwan, Italy, and Mongolia and at 96 hours from Greece, Taiwan, Italy, and India. The lowest levels at 48 hours were in Israel, China, the United States, and Thailand, and at 96 hours the lowest levels came from the United States, Israel, Brazil, and Thailand. The authors speculated that the differences are reflective of dissimilarities in bilirubin metabolism, combined with differences in feeding practices and breastfeeding rates, and between distinctive ethnic and/or racial groups. Because of the wide range of TcB levels found between population groups, caution and clinical judgment should be used when using a TcB nomogram constructed from a population group different from that of the specific newborn being tested.

In contrast to Asians, the African American race is often considered protective against hyperbilirubinemia, with a lower risk of developing TB levels greater than 20 mg/dL (342 µmol/L) than Caucasian infants.[11] However, African American infants are over-represented, relative to population statistics, in both the US-based Kernicterus Registry and a British and Irish series of severe hyperbilirubinemia. They also appear at greater risk of developing TB levels greater than 30 mg/dL (513 µmol/L) with resulting increased risk of kernicterus than Caucasian counterparts. Okolie et al.[21] reported that African American neonates accounted for more than 25% of kernicterus cases in the United States, despite making up only approximately 14% of all births. This phenomenon may be explained in part by the high incidence of G6PD deficiency within this ethnic group. Symptomatic ABO hemolytic disease, especially O-B incompatibility, is common in African American

newborns, whereas late preterm delivery is disproportionately more common in African American mothers. Dark skin pigmentation may increase the unreliability of visual inspection for neonatal jaundice; also, reports of low risk for severe hyperbilirubinemia and AAP classification of African American race associated with decreased risk of severe hyperbilirubinemia may all be factors contributing to the African American kernicterus health disparity.[21] In a national dataset for the years 2002 to 2017 including almost 58 million newborns born in the United States, Qattea et al.[22] found that, although African-American neonates were less likely to be diagnosed with hyperbilirubinemia than Caucasian neonates (adjusted odds ratio [aOR] = 0.77; 95% CI, 0.77–0.78; $P < .001$), they were more likely to develop bilirubin neurotoxicity than Caucasian counterparts (aOR = 3.05; 95% CI, 2.13–4.36; $P < .001$).

Certain geographically distinct populations may demonstrate a markedly increased incidence of neonatal unconjugated hyperbilirubinemia without associated hemolysis. The most dramatic of these are from certain Greek islands, especially the islands of Lesbos and Rhodes. Although the incidence of G6PD deficiency in these populations is markedly increased compared with the remainder of the Greek population and the world, the incidence of hyperbilirubinemia was not directly correlated with the frequency of G6PD deficiency, suggesting interaction of additional icterogenic factors. Unless aggressively treated with phenobarbital prophylaxis, phototherapy, and/or exchange transfusion, the incidence of kernicterus was also much greater in the newborns from these Greek islands than in those of the mainland population.

It has been speculated that the increased incidence of neonatal unconjugated hyperbilirubinemia in Asian and geographically identifiable populations may result either from environmental influences, such as the maternal ingestion of certain ethnically characteristic herbal medications or foods, or from a genetic predisposition to slower maturation of bilirubin metabolism and transport. Asian-origin infants born in the United States and Greek newborns born in Australia appear to be at similar risk for neonatal jaundice as natives of Asia and Greece, respectively, suggesting that geographic factors alone are not determinants. Differentiating the influence of drugs, foods, or traditional practices from that of genetic factors requires further investigation. Severe hyperbilirubinemia can result from hemolysis associated with sepsis or, if genetically vulnerable (e.g., in G6PD deficiency), exposure to chemicals (such as naphtha in mothballs) or pharmaceutical agents (such as antimalarials, sulfonamides, sulfones, antipyretics, and analgesics). In some societies with a high incidence of G6PD deficiency, application of henna to the skin or use of menthol-containing umbilical potions may precipitate severe hyperbilirubinemia and potentiate bilirubin encephalopathy. Even though some of these agents and stressors have received public attention, others represent generally unsuspected dangers, such as the intramuscular (IM) injection of vitamin K_3 (menadione) or the inhalation of paradichlorobenzene, which is used in moth repellents, air fresheners, and bathroom deodorizers. In addition, newborn exposure to a hemolytic agent, especially in the presence of G6PD deficiency, can occur transplacentally or through breast milk, as in the case of maternal ingestion of fava beans, or directly.

Pathologic Unconjugated Hyperbilirubinemia

Elevated concentrations of unconjugated bilirubin are of concern because of the danger of developing bilirubin encephalopathy or neuropathy associated with this fraction of bilirubin. Although there have been some reports of bilirubin encephalopathy associated with elevated levels of conjugated bilirubin, the role of conjugated hyperbilirubinemia in the mechanism of bilirubin encephalopathy is not clear. Most studies of kernicterus have been related to the TB concentration, of which the conjugated fraction usually comprises only a small fraction. Elevated levels of conjugated bilirubin frequently indicate disease processes of hepatic origin. The following discussion therefore relates primarily to unconjugated, or indirect, hyperbilirubinemia and is followed by a section on Conjugated Hyperbilirubinemia.

The TB level at any point in time reflects a multiplicity of forces in delicate balance. Processes including bilirubin production, transport, uptake, conjugation, excretion, and reabsorption are not only interdependent but also influenced by tremendous physiologic flux present in this complex system in the first few days of neonatal life. Examples of such changes include differences in the rate of heme catabolism and progressive maturation of the bilirubin conjugation system. Physiologically, the net result is an increase in TB levels up to about the fifth day of life, after which point TB values level off and then gradually decrease. Superimposed on these physiologic alterations of bilirubin metabolism may be specific disorders that may further exaggerate or prolong the normal pattern of an elevated TB level. These conditions may affect the entire spectrum of bilirubin metabolism and include disorders of bilirubin production, as well as bilirubin conjugation and elimination.

Causes of Unconjugated Hyperbilirubinemia

Disorders of Bilirubin Production

Although increased bilirubin production could result from pathologic states in which degradation of nonhemoglobin heme (i.e., hemoproteins such as cytochromes and catalase) and erythrocyte hemoglobin precursor heme are increased, such disorders in fact have not been identified in the newborn period. The most common pathologic hemolytic causes of unconjugated hyperbilirubinemia in the newborn include isoimmune hemolytic disease, caused by blood group incompatibility between mother and fetus, and G6PD deficiency. Disorders associated with increased erythrocyte destruction are listed in Box 95.1 (see Chapter 22).

Neonates who are acutely hemolyzing appear to be at a higher risk for developing bilirubin-induced brain damage

• BOX 95.1 Conditions Associated With Increased Erythrocyte Destruction

Isoimmunization

- Rh incompatibility
- ABO incompatibility
- Other blood group incompatibilities

Erythrocyte Biochemical Defects

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency
- Pyruvate kinase deficiency
- Hexokinase deficiency
- Congenital erythropoietic porphyria
- Other biochemical defects

Structural Abnormalities of Erythrocytes

- Hereditary spherocytosis
- Hereditary elliptocytosis
- Infantile pyknocytosis
- Other

Infection

- Bacterial
- Viral
- Protozoal

Sequestered Blood

- Subdural hematoma and cephalohematoma
- Ecchymoses
- Hemangiomas

compared with those without hemolysis. Indeed, the first association to be recognized between increasing TB levels and the risk for kernicterus was made in newborns with Rh isoimmunization. Some reports suggested that kernicterus in hyperbilirubinemic newborns with hemolytic disease may occur more frequently than that in their counterparts without evidence of hemolytic disease. Surveying the literature up to 1983, Watchko and Oski[22a] reinforced the concept that hyperbilirubinemia among neonates without hemolytic disease was less dangerous with regard to the development of kernicterus than in cases in which hemolysis was present; however, there are few data to substantiate this view. A study shedding some light on this question was performed by Ozmert and colleagues[22b] in 1996. In 102 children 8 to 13 years old, indirect hyperbilirubinemia ranging from 17 to 48 mg/dL (291–821 µmol/L) associated with a positive direct Coombs' test (also known as the direct antiglobulin test [DAT]), presumed to reflect ongoing hemolysis, was associated with lower intelligence quotient (IQ) scores and a higher incidence of neurologic abnormalities. In these same children, the incidence of detected neurologic abnormalities increased as the time of exposure to high TB levels became more prolonged. Similarly, in Norway, Nilsen and co-workers[22c] found that, of males born in the early 1960s who developed neonatal hyperbilirubinemia, those with a positive Coombs' test and hyperbilirubinemia for greater than 5 days had significantly lower IQ scores than average for that country.[2]

In a reanalysis of the data from the Collaborative Perinatal Project, no relationship was found between maximum TB levels and IQ scores; however, in those children who had had a positive DAT, a TB of greater than 25 mg/dL (428 µmol/L) was associated with a decrease in IQ scores. A study of severe neonatal hyperbilirubinemia from Egypt reported that the threshold TB level in identifying infants with bilirubin encephalopathy was lowered in those with identifiable risk factors associated with hemolysis, including Rh isoimmunization, ABO blood group incompatibility, and sepsis.[2]

Although there is to date no hard evidence demonstrating higher levels of unbound bilirubin in hemolyzing neonates, many believe that hemolysis is a potential factor that increases the risk for bilirubin-related brain damage. Although a TB concentration of 20 to 24 mg/dL (342–410 µmol/L) may be associated with kernicterus in a neonate with Rh isoimmunization, a healthy, term infant without an obvious hemolytic condition will rarely be endangered in this TB range. Conditions associated with hemolysis, including direct Coombs'-positive Rh and ABO immunization or other isoimmunizations and G6PD deficiency, may pose an increased threat to an otherwise healthy newborn. The Subcommittee on Hyperbilirubinemia of the AAP includes jaundice developing within the first 24 hours, blood group incompatibility with a positive DAT, and other known conditions including G6PD deficiency, all associated with increased hemolysis, as major risk factors for the development of severe hyperbilirubinemia.[19] The AAP recommends initiating phototherapy or performing exchange transfusions at lower levels of TB in neonates with hemolytic conditions than in apparently non-hemolyzing counterparts. However, it is not proposed that a hyperbilirubinemic newborn without an obvious hemolytic condition will be unaffected by bilirubin encephalopathy. Patients with kernicterus have been reported in whom no evidence of hemolysis was evident.[2] Crigler-Najjar syndrome, a condition not associated with increased hemolysis, is frequently complicated by bilirubin encephalopathy.

One consequence of hemolysis is the development of a "bilirubin load" considerably larger than normal. This is because heme is metabolized to bilirubin. The bilirubin load is the amount of bilirubin that must be taken up by hepatocytes, conjugated, and excreted. When hemolysis produces a bilirubin load too large to be cleared efficiently by normally functioning bilirubin metabolic mechanisms, TB levels rise, producing *hemolytic jaundice*. If the hemolytic rate exceeds the capacity of the bone marrow (and liver) to increase RBC production in compensation, the condition also proceeds to *hemolytic anemia*.

When neonatal hyperbilirubinemia is found to be the result of hemolysis, caregivers should be aware that: (1) the TB level can rise rapidly, (2) the hyperbilirubinemia might be slow to fall even with intensive phototherapy, and (3) the hyperbilirubinemia is likely to rebound after phototherapy is discontinued. These consequences explain, in part, why neonates with *hemolytic* jaundice are at risk for developing ABE or kernicterus.[23]

The absence of defined diagnostic markers associated with hemolysis should not lead to a state of complacency or belief that hemolysis is not, in fact, taking place. Blood counts are notoriously unreliable indicators of hemolysis in newborns. Studies utilizing the endogenous production of CO, an accurate index of heme catabolism, have demonstrated increased hemolysis in many jaundiced newborns, even in the absence of evidence of a specific hemolytic disorder. The term *non-hemolytic jaundice* should be used cautiously so as not to unwittingly potentiate bilirubin neurotoxicity in a possibly hemolyzing infant in whom it could have been prevented.[3] Hemolytic conditions in the newborn are generally divided into two major etiologic groups: immune and non-immune.

Isoimmunization

The hallmark of isoimmunization is a positive DAT (also known as the Coombs' test). This is indicative of the presence of a maternally produced antibody that has traversed the placenta and is now found within the fetus. The test is termed *direct* if the antiglobulin is adhered to the RBCs. An *indirect* test refers to the antibody being detected in the serum.

Rh Disease. In past decades, Rh hemolytic disease was the most common cause of severe hemolytic hyperbilirubinemia and a frequent cause of kernicterus. However, maternal prophylaxis with high-titer anti-D immunoglobulin G (RhoGAM), combined with aggressive fetal surveillance and intrauterine blood transfusions, has greatly reduced the incidence and severity of this disease. Mothers sensitized before the development of immune serum prophylaxis are no longer commonly encountered in industrialized countries. Additional technologies used antenatally, which have led to improvements in outcomes, include antenatal blood group genotyping by polymerase chain reaction (PCR) from fetal cells obtained by amniocentesis or even from maternal blood samples.[24] Assessment of the degree of fetal anemia and determination of the need for intrauterine transfusion noninvasively by determining middle cerebral artery peak systolic velocity by the Doppler technique have reduced the need for invasive procedures.[25]

Those without access to preventive treatment, immigrants from countries in which prophylaxis is not widely available, or those who did not receive prophylaxis following abortion or invasive procedures may continue to deliver affected infants. The problem continues to be rife in developing countries. Although the incidence of Rh negativity is lower in countries such as India, Nigeria, Pakistan, Kenya, and Thailand than in North America or in Europe because of their large populations, large numbers of women from these countries may be at risk. Zipursky and Paul[26] estimated that in these low-income countries, more than 1 million women annually do not receive anti-D prophylaxis and that more than 100,000 children are born with Rh disease.[27] Pegoraro et al.[28] estimated that ~50% of the women around the world who require this type of immunoprophylaxis do not receive it, presumably due to a lack of awareness, availability, and/or affordability. Hundreds of thousands of fetuses and neonates are therefore at risk for Rh disease each year, contributing to an enormous, continuing burden of fetal and neonatal disease globally.

The Rh blood group proteins are highly antigenic and capable of causing severe isoimmunization with a high risk for fetal hydrops and death. Although several systems of nomenclature exist, the CDE system is the most common. These three loci each contain two major alleles (C,c; D,d; E,e) and several minor alleles. The D antigen may produce maternal sensitization with a fetomaternal hemorrhage as small as 0.1 mL. Whereas C and E alleles are relatively uncommon causes of isoimmunization, they can, on occasion, lead to severe hemolysis and hyperbilirubinemia. Indeed, Rh C disease may be as severe as Rh D isoimmunization.[29] Furthermore, women with multiple RBC antibodies may develop significant hemolytic disease of the fetus and newborn to a greater extent than those with a single antibody, especially in the presence of anti-(Rh)D. The pathophysiology of this phenomenon may represent a more aggressive immune response in those with more than one RBC antibody.[30] Rh disease in pregnancy is highly associated with both intrauterine hemolysis and severe hemolytic disease following delivery. Untreated, the condition can lead to intrauterine anemia and severe hydrops fetalis, with rapid postnatal evolvement of hyperbilirubinemia with the potential of kernicterus.

The immunization process may begin if a Rh-negative woman, usually D negative, is exposed to a D antigen. This usually occurs by ante- or intrapartum transplacental fetomaternal transfusion of fetal RBCs containing a D antigen, or by transfusion of Rh-positive RBCs during abortion, blood administration, or procedures including amniocentesis, chorionic villus sampling, or fetal blood sampling. Following exposure to the D antigen on the fetal RBCs, the mother's immune system responds by forming anti-D immunoglobulin G (IgG) antibodies. The IgG then crosses the placenta and adheres to fetal RBCs containing the D antigen. The subsequent antigen-antibody interaction leads to hemolysis and anemia. The immune response may become more severe and more rapid with progressive pregnancies. Resultant anemia causes bone marrow stimulation, with increased numbers of immature RBCs appearing in the circulation (*erythroblastosis*) and extramedullary hematopoiesis. *Fetal hydrops*, a condition characterized by generalized tissue edema and pleural, pericardial, and peritoneal effusions, may result from a combination of hypoproteinemia, tissue hypoxia, and capillary leak. Anemia with resultant poor myocardial function may further exacerbate the hydrops by causing congestive cardiac failure and venous congestion.

Elevated COHb levels detected in blood obtained by cordocentesis in affected fetuses of non-smoking isoimmunized mothers confirm that destruction of erythrocytes begins in utero. However, the primary manifestation of the in utero hemolysis is that of anemia. Although large amounts of bilirubin are produced concomitantly, erythroblastotic infants

are not severely icteric at birth. Concentrations of TB are usually below 5 mg/dL (86 μmol/L) by transfer of unconjugated bilirubin across the placenta. Jaundice may appear, however, shortly after delivery. Classically, in the initial stages, the bilirubin is all indirect reacting, although small amounts of conjugated bilirubin have been noted. After some days of excessive bilirubin accumulation, the excretory system may become overwhelmed with the efflux of conjugated bilirubin into the serum, and an increasing conjugated bilirubin fraction is not uncommonly seen.[6] Hepatic conjugation may mature more rapidly than excretory function as a result of stimulation by chronic exposure to high concentrations of bilirubin in utero. Furthermore, hepatic excretory function may also be adversely affected by development of hepatic congestion secondary to heart failure and swelling caused by extramedullary hepatic hematopoiesis, anemia, and poor hepatic perfusion.

ABO Heterospecificity. With the reduction of the incidence of Rh isoimmunization by immune prophylaxis, DAT-positive ABO incompatibility in industrialized countries with functional medical systems is now the single most prominent cause of immune hemolytic disease in the neonate. The clinical picture is usually milder than that of Rh disease, although infrequently severe hemolysis with hyperbilirubinemia may occur. In a recent series of infants with either bilirubin encephalopathy/kernicterus or extreme hyperbilirubinemia reported from diverse countries including the United States, Canada, United Kingdom, Ireland, Denmark, Switzerland, China, and Nigeria, in whom the etiology of the hyperbilirubinemia was determined, infants with blood group A or B born to group O mothers comprised 19% to 55%.[31,32]

ABO blood group heterospecificity is the situation in which a blood group A or B infant is born to a group O mother, a setup occurring in about 12% of pregnancies. In some instances, women with blood group O have a high titer of naturally occurring anti-A or anti-B antibodies. High titers of anti-A or anti-B antibodies can sometimes be found in blood group O women even before their first pregnancy. This contrasts with Rh isoimmunization, in which immune sensitization occurs progressively with subsequent pregnancies. In contradistinction to blood group A or B individuals, in whom their respective anti-B or anti-A antibodies are IgM molecules with limited ability to cross the placenta, the respective antibodies of blood group O individuals are predominantly smaller IgG molecules and may cross the placenta. Attachment to corresponding fetal RBCs may follow, provided these cells have the A or B antigen. Extravascular hemolysis of the IgG-coated RBCs is thought to be mediated within the reticuloendothelial system by Fc-receptor-bearing cells. As with Rh isoimmunization, the immune process may commence in utero; however, unlike Rh disease, there is little danger of severe hyperbilirubinemia, anemia, or hydrops in utero, and prenatal intervention is not indicated. Infants may sometimes be born with moderate anemia. After delivery, there is a potential danger of hyperbilirubinemia.

About one-third of blood group A or B neonates born to a blood group O mother will have a positive direct Coombs' test or DAT. Measurements of endogenous formation of CO, reflective of heme catabolism, have demonstrated, overall, an increased rate of heme catabolism in affected infants compared with controls.[2,33] In one study, those infants who developed hyperbilirubinemia (TB >95th percentile on the Bhutani nomogram) had even higher COHb values than the already high values of those who were non-hyperbilirubinemic. Strength of the DAT may also be predictive, as ABO-heterospecific neonates with ++DAT had a higher incidence of hyperbilirubinemia and higher COHbc values than those with ± or +DAT.[34] Not all DAT-positive neonates, however, develop severe hyperbilirubinemia. In one study, only 20% of DAT-positive neonates actually developed TB levels >12.8 mg/dL (219 μmol/L), whereas, in another study, only 19.6% required phototherapy. Despite this apparent clinical mildness, newborns with severe hyperbilirubinemia of early onset who do not respond to phototherapy and require intravenous immune globulin (IVIG) therapy or exchange transfusion are occasionally encountered. In contrast to the above-mentioned studies, a study from Israel found that 52% of 164 DAT-positive, ABO-incompatible newborns developed a TB >95th percentile, many of these within the first 24 hours. At the extreme end of the spectrum, as already mentioned, kernicterus has been described. ABO blood group incompatibility with a negative DAT, not usually predictive of hemolysis or hyperbilirubinemia, may sometimes be associated with early and rapidly progressing jaundice, reminiscent of DAT-positive hemolytic disease. In these cases, another cause of the hyperbilirubinemia should be sought.

Paucity of A and B antigenic sites on neonatal RBCs or weak expression of these antigens in neonates compared with adults may explain, in part, an absence of clinical disease in many DAT-positive newborns. A or B antigenic sites situated in sites other than the RBCs may bind with transplacentally acquired antibodies, limiting their availability to the RBCs.

Because many ABO-incompatible, DAT-positive neonates have no evidence of ongoing hemolysis and do not develop early jaundice or hyperbilirubinemia, ABO heterospecificity with a positive DAT does not necessarily indicate ABO hemolytic disease. Some or all of the following criteria are necessary to support the diagnosis of ABO hemolytic disease:

1. Indirect hyperbilirubinemia, especially during the first 24 hours of life
2. Mother with blood group O; infant with blood group A or B
3. Spherocytosis on blood smear
4. Increased reticulocyte count
5. Evidence of hemolysis based on increased endogenous production of CO as assessed using end-tidal CO measurements, corrected for ambient CO (ETCOc) levels

In DAT-negative, ABO-heterospecific newborns, an interaction with a polymorphism for the $(TA)_7$ sequence in the promoter of the gene encoding UGT1A1 significantly increases the incidence of TB to at least 15 mg/dL (257 μmol/L) compared with controls and has been described.

It is essential to closely observe any newborn born to a blood group O mother and to perform a TcB or TB measurement at the first appearance of jaundice. Routine blood group and DAT determination on umbilical cord blood is an option that may allow for additional risk determination.

Isoimmunization Caused by Antibodies Other Than RhD. More than 50 RBC antigens may cause hemolytic disease of the newborn. The most important of these with regard to prenatal hemolysis include anti-C, anti-Kell, and anti-E, although others may also infrequently be problematic. Alloimmunization caused by these autobodies can sometimes cause severe hemolytic disease of the fetus requiring prenatal intervention. Fetal surveillance protocols and clinical strategies developed for RhD alloimmunization are useful in monitoring all alloimmunized pregnancies. Similarly, the postnatal management should be based on the principles outlined in the management of the RhD-immunized newborn (see Therapy for Unconjugated Hyperbilirubinemia). Anti-Kell isoimmunization warrants special mention because fetal anemia, rather than hyperbilirubinemia, often predominates the clinical picture. This may be due to erythropoietic suppression in addition to a hemolytic process.

Non-Immune Hemolysis

Erythrocyte Enzymatic Defects. The mature human erythrocyte lacks a nucleus and the organelles necessary for protein and lipid syntheses. Most of the protein present within its cell membranes is hemoglobin. Because the uptake and release of oxygen and carbon dioxide by hemoglobin in the tissues does not require energy, the erythrocyte relies on glycolysis (through the anaerobic Embden-Meyerhof pathway and the aerobic pentose phosphate pathway) and not on mitochondrial oxidative phosphorylation to generate adenosine triphosphate (ATP). Thus defects in the glycolytic enzymatic machinery may have profound effects on erythrocyte function and life span.

G6PD Deficiency. An entity that is highly associated with extreme neonatal hyperbilirubinemia and bilirubin encephalopathy is G6PD deficiency. Because G6PD deficiency has major neonatal public health implications, it is discussed in some detail. G6PD deficiency is a common enzyme deficiency estimated to affect hundreds of millions of people worldwide. From its original indigenous distribution, including areas in south Europe, Africa, the Middle East, and Asia, immigration patterns have transformed it into a condition that may now be encountered virtually in any corner of the globe. It is not surprising that, in low- and middle-income countries with a high frequency of G6PD deficiency, the condition is associated with a high incidence of neonatal mortality and neurodevelopmental disorders.[35] It is remarkable, though, that the condition has been over-represented in reports of neonates with extreme hyperbilirubinemia and bilirubin encephalopathy relative to the background frequencies of this condition among the populations of the United States, Canada, United Kingdom, and Ireland. In the US-based Pilot Kernicterus Registry, more than 20% of reported neonates were diagnosed with G6PD deficiency, whereas its overall frequency in the military of that country was only 2.2%. Recent immigration from African and Middle Eastern countries to Europe may increase the frequency of this condition in that continent, as demonstrated by detection of G6PD A– and G6PD-Mediterranean mutations among African and Middle Eastern immigrants to Denmark.[36] A (non-comprehensive) list of some population subgroups in North America and Europe at high risk for G6PD deficiency is shown in Box 95.2.

Function of G6PD. G6PD plays a major part in stabilization of the RBC membrane against oxidative damage. The enzyme catalyzes the first step in the hexose monophosphate pathway, oxidizing glucose-6-phosphate to 6-phosphogluconolactone, thereby reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. NADPH is essential for the regeneration of reduced glutathione from oxidized glutathione, a substance that plays an integral part in the body's antioxidative mechanisms. The pathway is also instrumental in stimulating catalase, another important antioxidant. In the absence of G6PD, NADPH will not become available, reduced glutathione will not be regenerated, and cells may be rendered susceptible to oxidative stress. Unlike other body cells, no alternative source of NADPH is available in the RBC, which explains the extreme vulnerability of the G6PD-deficient RBC to oxidative damage. Oxidative membrane damage incurred to the cell membrane may manifest as hemolysis.

Genetics of G6PD Deficiency. Because G6PD deficiency is an X-linked condition, males may be normal hemizygotes or deficient hemizygotes, whereas females may be either normal or deficient homozygotes or heterozygotes. Because of X-inactivation, heterozygotes have two RBC populations: one G6PD deficient, the other G6PD normal. Because X-inactivation may be non-random, unequal ratios of normal

• BOX 95.2 Some Population Subgroups in North America and Europe at High Risk for Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

North America	Europe
African Americans	Indigenous African ancestry
Italians, especially Sardinian ancestry	Italians
Greeks	Greeks
Turks	Turks
Southeast Asians	Middle Eastern origin
Chinese	Chinese
Asian ancestry	Refugees and migrants from the Middle East
Sephardic Jews	
Mexicans (some regions)	
Latin Americans (varying prevalence rates between countries and regions)	

Adapted and updated from Kaplan M, Hammerman C. The need for neonatal glucose-6-phosphate dehydrogenase screening: a global perspective. *J Perinatol*. 2009;29(Suppl 1):S46–S52.

and enzyme-deficient RBCs may coexist. Heterozygotes may, as a result, have a normal, intermediate, or deficient phenotype. It was previously thought that heterozygotes had sufficient enzyme activity to protect them from the dangers of G6PD deficiency. However, reports suggest that heterozygotes may not be without risk, and fatal kernicterus has been described in a heterozygote.[36,37] The most commonly encountered mutation is G6PD A–, found in Africa and southern Europe and in African Americans. G6PD-Mediterranean, regarded as a more severe type than G6PD A–, is found in Mediterranean countries, the Middle East, and India. Another variant encountered primarily in Asia is G6PD Canton. The number of mutations discovered is continually increasing, and they total 217 at the time of this writing.[37]

G6PD Deficiency and Hemolysis. Most G6PD-deficient individuals lead perfectly normal lives and will, for the most part, be unaware of their inherited condition. However, G6PD deficiency may be associated with severe hemolytic episodes with resultant jaundice and anemia, following exposure to a hemolytic trigger. Classically, these episodes often occur after ingestion of or contact with the fava bean (favism). Medications and chemical substances may be suspected, but sometimes no offending trigger is identified. Infection may play a role in the pathogenesis of acute hemolysis.

In neonates, extreme hemolytic hyperbilirubinemia may develop suddenly and without previous warning. Some identifiable substances associated with neonatal hemolysis include naphthalene (moth balls used when storing clothes), herbal medicines, henna applications, or menthol-containing umbilical potions. Frequently, however, the trigger cannot be identified and the hemoglobin concentration may not drop. This, in combination with the finding of diminished bilirubin conjugation due to the *UGT1A1*28* polymorphism, has led some to the conclusion that hemolysis is not occurring. However, a recent summary of several studies[38] and a new report by Bahr et al.[39] have demonstrated elevated endogenous CO production in G6PD-deficient neonates with extreme hyperbilirubinemia, confirming the role of hemolysis as an important pathogenetic factor in severe G6PD deficiency-associated neonatal hyperbilirubinemia. There can, however, be no other viable explanation for the exponential increase in TB to dangerous levels. G6PD deficiency may therefore be the one reason that kernicterus may not be completely preventable. Exchange transfusion may be the only recourse. Early hospital discharge with delayed follow-up may place these patients at risk for severe sequelae.

In a Nigerian neonatal cohort, G6PD-deficient and -intermediate (presumable heterozygotes) infants had higher TB, lower hematocrit values, and a greater need for phototherapy during the first postnatal week than G6PD-normal counterparts, suggestive of increased hemolysis.[40] Frequently, hematologic indices typical of hemolysis in older children and adults, including falling hemoglobin and hematocrit values and increasing reticulocyte counts, may be absent despite a clinical picture of hemolysis. However, studies of endogenous CO formation, reflective of the rate of heme catabolism, have demonstrated an important role of increased hemolysis in association with this condition. Significantly higher levels of COHb have been reported in Nigerian G6PD-deficient neonates who developed kernicterus compared with neonates who were hyperbilirubinemic but did not develop signs of kernicterus.

More frequently and less life-threatening, G6PD-deficient neonates may have a moderate form of jaundice, which occurs at a rate several-fold higher than that of controls. The jaundice usually responds to phototherapy, although exchange transfusion may also be necessary. These infants have a low-grade hemolysis that cannot be implicated as the primary icterogenic factor. Diminished bilirubin conjugation has been shown to be of major importance in the pathogenesis of the hyperbilirubinemia. An intriguing interaction has been noted between G6PD deficiency and a non-coding area $(TA)_7$ promoter polymorphism in the gene encoding UGT1A1.[1] This polymorphism, also known as *UGT1A1*28*, is associated with Gilbert syndrome. The incidence of a TB of at least 15 mg/dL (257 μmol/L) increased in a stepwise, dose-dependent fashion in G6PD-deficient neonates who were heterozygous or homozygous, respectively, for the polymorphism. This effect was not seen in the G6PD-normal control group. Furthermore, G6PD deficiency alone, in the absence of the promoter polymorphism, did not increase the incidence of hyperbilirubinemia over and above that of G6PD-normal counterparts. In Asians, in whom the $(TA)_7$ promoter polymorphism is rare, a similar interaction was observed between G6PD deficiency and coding area mutations of *UGT1A1*.[1] In a recent study of African American neonates, although 20.6% had variations in both *UGT1A1* and *SLCO1B1*, these genetic variants did not have an effect on the incidence of hyperbilirubinemia.[41] This may be because very few neonates in that cohort were G6PD deficient, confirming the concept of a gene interaction necessary to influence the incidence of hyperbilirubinemia.

The apparent mildness of this form of hyperbilirubinemia may be deceiving. Inherent to these infants is increased hemolysis, as demonstrated by endogenous CO production studies, in combination with a predilection for diminished bilirubin conjugation related to *UGT1A1*28*. Any further hemolysis, or additional conjugation decrease such as associated with prematurity, may upset the equilibrium between bilirubin production and elimination, thereby precipitating severe hyperbilirubinemia. Unlike the acute hemolytic form of jaundice, this milder form of jaundice can be predicted by pre-discharge bilirubin testing. G6PD-deficient neonates who had a pre-discharge TB concentration below the 50th percentile were unlikely to develop subsequent hyperbilirubinemia. However, as the pre-discharge TB increased progressively above the 50th percentile, the risk for subsequent hyperbilirubinemia increased in tandem. Confirming this apparent potential severity of mild hyperbilirubinemia is the experience in Cleveland, Ohio. Approximately 50% of infants readmitted for hyperbilirubinemia had G6PD deficiency, of whom about half had received phototherapy during birth hospitalization. Okolie et al.[21] warned that this finding underscores that a positive response to phototherapy "should not result in complacency with regard to potential danger in these infants" and emphasized the

importance of post-discharge TB surveillance in neonates with G6PD deficiency, including those who previously received phototherapy.

Testing and Screening for G6PD Deficiency. Many qualitative or quantitative screening tests are available that should accurately determine the hemizygous state in males or the homozygous state in females. Because many heterozygotes may have intermediate to normal G6PD enzyme activity, the result of non-random X-chromosome inactivation, the heterozygote state is difficult to determine using standard biochemical tests. Females of high-risk groups (Mediterranean origin, African American, African, Middle Eastern, or Asian, as well as Sephardic Jews) should have close follow-up to detect the development of jaundice despite a normal screening result. Also, biochemical tests may give a false normal result if performed during an acute hemolytic episode. The reason is that older RBCs, depleted in G6PD enzyme activity, may be destroyed, leaving younger cells with higher enzyme activity intact. In such cases, G6PD testing should be performed several weeks after the acute hemolysis has subsided. An alternative method is to analyze DNA for the specific suspected mutation. Some countries have introduced neonatal screening for G6PD deficiency combined with parental education in the hope that identification of an infant with G6PD deficiency should lead to avoidance of known triggers of hemolysis and speed the process of evaluation and treatment should an affected neonate become jaundiced. Reports have demonstrated a decrease in the number of cases of kernicterus following introduction of screening programs, as reviewed.[42] G6PD screening with rapid turnaround time is feasible.[43,44] Discussions regarding neonatal screening for G6PD deficiency have started in the United States, and New York recently (2021) became the first state to pass a bill (#S4316) to expand their newborn screening to include adrenoleukodystrphy and G6PD deficiency. The treatment of neonatal hyperbilirubinemia associated with G6PD deficiency should follow the 2022 AAP Clinical Practice Guideline[19] for neonates with hemolytic risk factors.

Pyruvate Kinase Deficiency. Pyruvate kinase catalyzes the conversion of phosphoenolpyruvate to pyruvate and the formation of ATP from adenosine diphosphate in the Embden-Meyerhof pathway. Deficiency of this enzyme results in a lack of ATP for erythrocytic metabolic activity and in chronic anemia. Pyruvate kinase deficiency, a condition prevalent in northern Europeans and inherited in an autosomal recessive manner, results in a lack of ATP, an important source of energy for RBC metabolism. In the newborn period, anemia, reticulocytosis, and severe, early, hemolytic jaundice may ensue. Exchange transfusion may be required, and kernicterus has been reported.

Four isozymes are encoded by two genes, among which 250 mutations and six polymorphisms have been described.[45] Diagnosis is determined by an enzyme assay, which should be performed in cases of hemolysis and hyperbilirubinemia not associated with a positive direct Coombs' test or spherocytosis. Molecular studies may also confirm the diagnosis.[46]

Hexokinase catalyzes the conversion of glucose to glucose-6-phosphate, the initial step in glycolysis. Hexokinase deficiency predisposes the erythrocyte to oxidant damage and thus is another cause of hemolysis and neonatal hyperbilirubinemia. Inheritance is autosomal recessive, and the gene has been localized to chromosome 10.

Congenital erythropoietic porphyria is an extremely rare, recessively inherited disorder of heme metabolism in which deficient uroporphyrinogen III cosynthase activity affects the conversion of hydroxymethyl bilane to type III uroporphyrinogen. Normal heme synthesis can occur only in the presence of greatly elevated levels of type I uroporphyrinogen and type I coproporphyrinogen. These porphyrins are deposited in massive quantities throughout the cells of the body, including the erythrocytes. The disease may present at birth with anemia, jaundice, and splenomegaly. Pink to brown staining of diapers soaked with porphyrin-rich urine is an early clue to the diagnosis. Because porphyrins are photoreactive, the diapers readily fluoresce under ultraviolet light. The same photoreactive properties of porphyrins lead to hemolysis, hyperbilirubinemia, and cutaneous photosensitivity with subepidermal bullae formation. Deficiencies of other enzymes in the glycolytic pathway, including glucose phosphate isomerase, can produce severe hemolysis and hyperbilirubinemia in the neonatal period.

Erythrocyte Structural Defects. Defects in erythrocyte membrane and cytoskeletal structure (see Chapter 81) alter the shape and deformability of the cell and result in sequestration within the narrow splenic sinusoids. Hemolysis, hyperbilirubinemia, and splenomegaly are the clinical hallmarks of these disorders.

Hereditary Spherocytosis. Of the hereditary RBC membrane defects that may lead to acute hemolysis and hyperbilirubinemia in the newborn, hereditary spherocytosis is probably the most common. In spherocytosis, the normal biconcave shape of the erythrocyte is altered such that the cell assumes a spherical shape—the shape with the smallest possible diameter for its volume. In addition to a reduction in surface area with consequential diminished oxygen uptake and delivery, the limitation in deformability may result in massive splenic sequestration. This condition may be inherited in both an autosomal dominant and recessive fashion, and frequently there may be a history of acute hyperbilirubinemia in a sibling or parent. The condition should be suspected in individuals of Northern European ancestry. Microvesiculization of the RBC membrane results from a deficiency of proteins, including ankyrin, band 3, α-spectrin, β-spectrin, and protein 4.2 in that membrane. These osmotically fragile RBCs are trapped in the spleen, the microvesicles are aspirated by macrophages, and the cell is destroyed. The diagnosis can be made microscopically by identifying spherocytes in the peripheral blood smear, with confirmation by the osmotic fragility test. The latter test may be especially important in differentiating infants with hereditary spherocytosis from those with DAT-positive ABO isoimmunization, a condition that may also result in microspherocytosis. Mutations of at least five genes encoding the previously mentioned proteins have been recognized.

Hereditary spherocytosis is frequently associated with neonatal hyperbilirubinemia. Of 178 affected Italian term, predominantly breastfed newborns, 112 (63%) developed neonatal hyperbilirubinemia requiring phototherapy. The incidence of hyperbilirubinemia was even higher in those who also had a genetic variation of the *UGT1A1*28* promoter, similar to that described in G6PD deficiency. Kernicterus has been described. A mean corpuscular hemoglobin concentration (MCHC) of 36.0 g/dL or greater had an 82% sensitivity and a 98% specificity for identifying hereditary spherocytosis and should alert caregivers to the possibility of this diagnosis.[47]

Hereditary Elliptocytosis, Hereditary Pyropoikilocytosis, Hereditary Ovalocytosis, and Hereditary Stomatocytosis. These are rare conditions affecting the erythrocyte membrane. The diagnosis may be made by microscopic examination of the peripheral blood smear. Hemolysis may occur in the neonatal period and result in anemia and hyperbilirubinemia. Infantile pyknocytosis is a transient abnormality of erythrocyte morphology associated with hemolysis and neonatal jaundice. Small, irregular, dense RBCs with spiny projections are seen in the peripheral blood smear and account for more than 5% of the total RBC population. Anemia and hemolysis persist throughout the first month of life and often into the second and third months. Jaundice is most severe during the first 2 weeks of life.

Blood Transfusion. In preterm infants, increases in TB levels have been documented, sometimes warranting phototherapy following blood transfusion. This may be because of a large bilirubin load caused by lysis of the transfused RBCs prior to, during, or following the transfusion.[48]

Infection. Bacterial infection is a known cause of hemolysis and hyperbilirubinemia. Sepsis causes hyperbilirubinemia by increasing TB concentrations through hemolysis or by impairing conjugation, thereby resulting in decreased excretion of bilirubin. Several theories have been proposed for the mechanism of hyperbilirubinemia in the septic neonate. Neonatal erythrocytes are particularly susceptible to cell injury and Heinz body formation in response to oxidative stress. In addition, *HMOX1* can be induced by oxidants, which could lead to increased catabolism of heme to bilirubin. Because bilirubin is a protective antioxidant, initially in infection TB levels may decrease as a result of its consumption. However, the frequent manifestation of hyperbilirubinemia associated with sepsis suggests that this protective mechanism may be overwhelmed in septicemia. Furthermore, disseminated intravascular coagulation resulting from sepsis may produce hemolysis as erythrocytes traverse the deposition of fibrin within the microvasculature. In addition, conjugated hyperbilirubinemia may result from hepatitis secondary to bacterial, viral, fungal, or protozoal infections.

Sequestration. Sequestration of blood within body cavities can result in increased bilirubin production as the body metabolizes and recycles the heme released as erythrocytes are catabolized. Birth trauma resulting in the collection of RBCs within the layers of tissue covering the skull and brain (cephalohematoma, subdural hematoma, subgaleal hematoma) or elsewhere (bruising associated with precipitous or instrument-assisted deliveries) has the potential to produce hyperbilirubinemia (see Chapter 28). Large hemangiomas, as in Kasabach-Merritt syndrome, may be associated with hemolysis and hyperbilirubinemia, in addition to thrombocytopenia and depletion of fibrinogen and other clotting factors.

Polycythemia. An increase in RBC mass with resultant increased breakdown of these cells has the potential to overload the already immature capacity of the newborn to eliminate heme degradation products. Polycythemia may be associated with delayed cord clamping, maternal-fetal transfusion, and twin-twin transfusion. Infants of diabetic mothers, especially those who are large for gestational age, are known to be at risk for polycythemia. Although the mechanisms underlying the polycythemia are unclear, CO excretion studies have shown that increased RBC breakdown, even in the absence of polycythemia, is the source of the hyperbilirubinemia in these neonates (see Chapter 81).[3]

Disorders of Hepatic Uptake and Conjugation

Uptake into hepatocytes may be affected by polymorphisms of the *SLCO* gene. Liu et al.[49] reported that *SLCO1B1 388G>A* was associated with an increased risk of neonatal hyperbilirubinemia in Chinese but not in Caucasian, Thai, Malaysian, or Latin-American populations. Gilbert syndrome is a benign disorder that affects about 6% of the population and produces a chronic unconjugated hyperbilirubinemia. Both defective hepatic uptake of bilirubin and decreased hepatic UGT1A1 activity have been demonstrated. The basis of the reduced activity of UGT1A1 lies in the presence of additional TA repeats in the TATAA box in the promoter region of the gene. The mutation is also known as *UGT1A1*28*. Because the noncoding, rather than coding, area of the gene is affected, individuals with Gilbert syndrome have a normally structured UGT1A1 enzyme but with diminished expression. The latter leads to a decrease in UGT1A1 enzyme activity. Although this disease usually does not manifest until after the second decade of life, some neonates with Gilbert syndrome may exhibit hyperbilirubinemia secondary to diminished uptake of bilirubin. When mutations in both the gene for G6PD and the promoter for *UGT1A1* occur, the degree of neonatal hyperbilirubinemia has been shown to be dose dependent.[1] In addition, in G6PD-deficient infants who had the wild-type $(TA)_6$ (normal) gene promoter, the incidence of hyperbilirubinemia was similar to that in infants who were G6PD normal, with the wild-type promoter (9.7% vs. 9.9%). The variant *UGT1A1* promoter, rather than the rate of heme catabolism as measured by blood COHb, was subsequently shown to be the crucial factor in determining the TB level and had a similar incidence of hyperbilirubinemia (>15 mg/dL [257 μmol/L]) to those infants with and without G6PD deficiency. However, in G6PD-deficient infants who were both hetero- and homozygous for the Gilbert variant, hyperbilirubinemia was more frequent in a stepwise,

dose-dependent manner (32% vs. 50%, respectively).[1] Polymorphisms of the *UGT1A1* promoter TATAA box, even in the heterozygotic form, may also play roles in the generation of extreme hyperbilirubinemia in G6PD deficiency–associated hemolytic episodes. Several studies have shown that *UGT1A1*28* in and of itself, in the absence of G6PD deficiency, is not associated with significant hyperbilirubinemia.[50] As described earlier, UGT1A1 catalyzes the conjugation of bilirubin in the liver. Disorders of conjugation include both those in which there is a primary mutation in *UGT1A1* and those in which UGT1A1 function is secondarily altered.

Crigler-Najjar Syndrome Type I

Crigler-Najjar syndrome type I is a rare autosomal recessive disease characterized by an almost complete absence of hepatic UGT1A1 activity. Because the coding area of *UGT1A1* is mutated, the enzyme produced is structurally abnormal, with no bilirubin conjugating capacity. In the homozygous form, severe unconjugated hyperbilirubinemia develops during the first 3 days of life and progresses in an unremitting fashion, with TB concentrations reaching 25 to 35 mg/dL (428–599 µmol/L) during the first month of life. Kernicterus often occurs in the neonatal period, especially when the etiology of the disease is unsuspected and aggressive treatment is not initiated. Stools are pale yellow, and bile bilirubin concentrations are less than 10 mg/dL (171 µmol/L), with normal being 50 to 100 mg/dL (855–1710 µmol/L); there is a total absence of bilirubin glucuronide in the bile. Bilirubin glucuronide formation measured in vitro with liver obtained by biopsy is absent. Formation of most non-bilirubin glucuronides is either severely reduced or absent.

With either direct hepatic enzymatic assay or indirect measurement of glucuronide formation, both parents are found to have partial defects (about 50% normal). Enzyme activity reserve should be sufficient to keep TB concentrations within normal limits. Unless a family is known to be affected by the condition, the recognition of this disorder during the first week of life may be difficult because of confusion with other types of conditions causing exaggerated unconjugated hyperbilirubinemia. Persistence of unconjugated hyperbilirubinemia at TB concentrations of greater than 20 mg/dL (342 µmol/L) beyond the first week of life, or repeated need for phototherapy in the absence of obvious hemolysis, should prompt concern for the existence of this syndrome. Strauss et al.[51] warned that there is no need to wait for the final diagnosis in order to institute treatment. Routine monitoring for hyperbilirubinemia and treatment according to established guidelines (below) should control hyperbilirubinemia and prevent neurotoxicity while a diagnosis is being pursued.

Indirect methods of diagnosis, including microassay of UGT1A1 activity from a percutaneous liver biopsy specimen or analysis of bile conjugates, have been largely replaced by gene analysis. An update of the genetic mutations known to date (Fig. 95.9) includes many of the mutations known to be associated with the condition.[52] Occurrence of the identical mutation in both parents of an affected homozygous infant suggests parental consanguinity.

The management of these neonates requires maintenance of TB concentrations to less than 20 mg/dL (342 µmol/L) during at least the first 2 to 4 weeks of life. The risk for kernicterus persists into adulthood, but aggressive management may diminish this risk while awaiting liver transplantation.[51] Today, nearly all neonates with this disorder are treated with phototherapy as initial therapy or after one or more exchange transfusions. Phototherapy is generally continued throughout the early years of life in the hope that this will prevent the development of kernicterus. Despite attempts to expose older children to phototherapy at the highest intensities and longest durations possible, the response to phototherapy progressively decreases with years of use. This may result from increased skin thickness or a changing distribution of the bilirubin pool. Prompt management of all intercurrent infections, febrile episodes, and other types of illness may help prevent later development of kernicterus. Inducers of *UGT1A1*, such as phenobarbital, are not effective in Crigler-Najjar syndrome type I disease.

Liver transplantation offers the only definitive treatment for the disease. This procedure should not be delayed indefinitely, because phototherapy may become less effective with the passage of time, and intercurrent illnesses may precipitate high levels of TB, with the potential of kernicterus, even in children whose TB concentrations had appeared to be under control and who had appeared to be neurologically intact. In a multicenter report of a world survey, seven of 21 (33%) transplanted children had already developed some form of brain damage at the time of their transplantation. Average age at transplantation was 9.1 ± 6.9 years (range, 1–23). Hepatocyte transplantation has been used. In a recent study, liver transplantation normalized unconjugated bilirubin and eliminated phototherapy dependence.[51] Gene therapy may also have promise for these patients in the future. Stem cell therapy using hepatocyte-like cells differentiated from human-induced pluripotent stem cells reduced TB levels in Gunn rats, indicating that the transplanted cells expressed UGT1A1 activity.[53]

Crigler-Najjar Syndrome Type II

Crigler-Najjar syndrome type II (also known as Arias disease) is more common than type I and typically benign. Although unconjugated hyperbilirubinemia occurs in the first days of life, TB levels generally do not exceed 20 mg/dL (342 µmol/L). Fasting, illness, and anesthesia may cause temporary increases in bilirubin to above baseline. The occurrence of kernicterus is rare. Evidence of hemolytic disease is absent (although it may occur coincidentally), stool color is normal, and neonates are otherwise healthy.

Unconjugated hyperbilirubinemia persists into adulthood. Biochemically, hepatic UGT1A1 activity is nonexistent and indistinguishable from that found in type I disease. Less than 50% of the daily bilirubin production is excreted in bile, and the monoglucuronide is the predominant form.

Another difference between type II and type I diseases lies in the response to phenobarbital. Jaundiced neonates and adults with type II disease respond readily to oral administration of phenobarbital with a sharp decline in TB levels, whereas individuals with type I disease demonstrate no such change. Phenobarbital may be used as a simple clinical tool to differentiate the two syndrome types. Beyond the neonatal period, there should be no long-term risk for kernicterus unless there is coincidental hemolytic disease.

Crigler-Najjar syndrome type II occurs as both an autosomal recessive and dominant inheritance. The range of expression in one or both parents can be from an asymptomatic defect in conjugation on testing to severe icterus. Other members of the family also may either appear icteric or have detectable low-grade unconjugated hyperbilirubinemia. Screening of the parents and other close relatives for hyperbilirubinemia is a useful method for supporting the diagnosis when it is suspected. Testing of the neonate and the parents for the capacity to form glucuronides of bilirubin was used diagnostically in the past, but these methods have been largely replaced by sequencing of *UGT1A1*.[2]

Transient Familial Neonatal Hyperbilirubinemia (Lucey-Driscoll Syndrome)

Lucey-Driscoll syndrome is a rare familial disorder in which neonates of certain mothers may develop severe unconjugated hyperbilirubinemia during the first 48 hours of life. Kernicterus has been reported in untreated newborns. The sera of these neonates and their mothers contain high concentrations of an inhibitor of UGT1A1 when tested in vitro. The serum inhibitory effect gradually declines after delivery coincident with gradual decline in TB levels.

Pyloric Stenosis

Pyloric stenosis may be associated with unconjugated hyperbilirubinemia at the time vomiting begins. Hepatic UGT1A1 activity is markedly depressed in the jaundiced neonates. The mechanism of diminished UGT1A1 activity may be due to the presence of the variant $(TA)_7$ *UGT1A1* promoter, which is associated in adults with Gilbert syndrome. Duodenal and jejunal obstructions are also associated with exaggerated unconjugated hyperbilirubinemia. Surgical relief of the obstruction results in a decline of TB levels to normal within 2 to 3 days. Lower intestinal obstruction, as in Hirschsprung disease, also may result in unconjugated hyperbilirubinemia, although usually of a milder degree than with upper intestinal tract disease. In this situation, as well as when there is upper intestinal tract obstruction, hyperbilirubinemia may result from increased reabsorption of unconjugated bilirubin from the intestine due to stasis of the intestinal contents (see Chapter 88).

Hypothyroidism

UGT1A1 activity in congenital hypothyroidism is deficient and may remain suboptimal for weeks or months. Because about 10% of congenitally hypothyroid neonates may develop prolonged, exaggerated jaundice, testing for thyroid function should be performed in these cases. Treatment with thyroid hormone promptly alleviates the hyperbilirubinemia. The mechanism of this association in the human newborn is unknown, but in rats, hypothyroidism impairs hepatic uptake and reduces hepatic ligandin concentrations. Thyroid hormone is also instrumental in many maturational processes. Its absence may delay hepatic bilirubin enzyme and transport development (see Chapter 92). It has also been suggested that thyroid hormone can cause changes in UGT1A1 protein expression.

Disorders of Excretion

Impaired hepatic excretion of bilirubin from disorders such as hepatocyte injury results in conjugated hyperbilirubinemia and is discussed later in this chapter.

Disorders of Enterohepatic Circulation

Jaundice Associated with Breastfeeding. Jaundice associated with breastfeeding is common, and breastfed newborns are more likely to develop prolonged hyperbilirubinemia than those fed formula. Two pathophysiologic mechanisms have been suggested for the early-onset association of jaundice with breastfeeding, although this differentiation is not clear, and overlap may exist between these suggested entities. Breastfeeding failure jaundice (or breastfeeding-associated jaundice) has been so labeled to distinguish it from breast milk jaundice. Breastfeeding failure jaundice appears to be associated with poor feeding practices and not with any change in milk composition. In contrast, breast milk jaundice is apparently related to a change in the composition or physical structure of the milk. Both types result in an exaggerated enterohepatic circulation of bilirubin—one through "starvation" and the other through altered milk chemistry.

Breastfeeding Failure Jaundice. Breastfeeding failure jaundice occurs in the first weeks of life in breastfed newborns. Establishing effective breastfeeding may be difficult, especially in first-time mothers, who may find lactation to be an intricate process. Maternal factors, such as lack of proper technique, engorgement, cracked nipples, and fatigue may impair effective breastfeeding. Neonatal factors such as ineffective suck also may hamper attempts at breastfeeding. Even if the mother is experienced in breastfeeding and her baby is interested, her milk supply is usually limited to small amounts of colostrum in the first 24 to 48 hours after birth. A genetic predisposition including a $(TA)_7$ promoter polymorphism or G71R mutation, both of *UGT1A1*, can lead to the development of hyperbilirubinemia in breastfed infants and may contribute to the development of prolonged jaundice.[2,54] Breastfed newborns are more likely to develop prolonged hyperbilirubinemia than those fed formula.[55]

All of these factors may act in combination, resulting in infrequent or ineffective breastfeeding. As a result, there may be little stimulus for milk production. Formula supplementation may further impair successful lactation. Exclusively breastfed neonates are therefore at risk for being relatively underhydrated and less well-nourished than formula-fed

counterparts. Poor enteral intake may lead to a state of relative starvation with delayed meconium passage. Intestinal content stasis may lead to increased enterohepatic reuptake of bilirubin, thus increasing the bilirubin load presented to the liver and leading to unconjugated hyperbilirubinemia. This process is similar to "starvation jaundice" seen in older human patients, as well as other animal species, fasted for more than 24 hours.

Prevention of breastfeeding failure jaundice includes encouraging frequent breastfeeding (at least 8–12 times per day for the first several weeks),[56] avoiding supplementation with water or glucose solutions,[2] and providing access to maternal lactation counseling. Intensive support of the breastfeeding mother is necessary, especially in view of early discharge policies in place at many hospitals. Both during birth hospitalization and after hospital discharge, the newborn should be closely monitored for weight gain, adequate urination, stool formation, and the development of jaundice.[57]

Breast Milk Jaundice. Late breast milk jaundice occurs after the first 3 to 5 days of life and may last into the third week of life or beyond. Epidemiologic studies report that 10% to 30% of breastfed infants in the second to sixth weeks of life are affected, with some having hyperbilirubinemia into the third month. In a recent study of predominantly breastfeeding North American Caucasian neonates at 3 to 4 weeks of age, 34% to 43% had TcB levels >5.0 mg/dL, and many were clinically jaundiced.[2] The presence of the variant $(TA)_7$ *UGT1A1* (*UGT1A1*28*) promoter may be associated with prolonged breast milk jaundice in Caucasian populations and G71R (*UGT1A1*6*) in Japanese.[2]

Typically, the TB level rises steadily, peaking at 5 to 10 mg/dL (86–171 μmol/L) at about 2 weeks of age, with a gradual decline over the first several months of life. More severely affected neonates may achieve peak levels as high as 20 to 30 mg/dL (342–513 μmol/L). There is no evidence of hemolysis, nor do these neonates appear ill; weight gain and intestinal function are normal. Pregnane-3-α,20-β-diol, a progesterone metabolite found in the breast milk fed to affected neonates, was historically thought to be the cause of this disorder, because this substance was shown to be a competitive inhibitor of UGT1A1 in vitro. Although the milk and urine of mothers of these neonates contain this pregnanediol isomer, the inhibitory effect of this hormone has been questioned. Studies have indicated that the milk associated with this syndrome also contains high concentrations of non-esterified long-chain fatty acids. This suggests that certain of these fatty acids act as inhibitors of hepatic UGT1A1, causing retention of unconjugated bilirubin. It is unlikely that the non-esterified long-chain fatty acids would reach the sites of conjugation in smooth endoplasmic reticulum of hepatocytes without prior esterification. Triglycerides of these long-chain fatty acids do not inhibit in vitro activity of UGT1A1. Neither the pregnanediol nor the fatty acids have ever been substantiated as an inhibitor of hepatic conjugation in vivo, and their role in the cause of the breast milk jaundice syndrome remains questionable.

Studies of the enterohepatic circulation of bilirubin in the rat suggest that milk from mothers of neonates with this syndrome contains β-glucuronidase, an enzyme that could deconjugate bilirubin and, consequently, enhance the enteric reabsorption of bilirubin, thereby increasing the hepatic bilirubin load. The presence of this enhancer of intestinal bilirubin absorption in human milk strongly correlates with the presence of mild to moderate unconjugated hyperbilirubinemia in neonates during the second and third weeks of life. With more than 50% of all breastfed neonates manifesting this effect of breast milk, this phenomenon may be a normal physiologic development comparable to, and an extension of, physiologic jaundice of the early newborn period.

Usually, other than being jaundiced, the neonates appear healthy, and no abnormal findings are noted. Although not recommended unless TB concentrations reach phototherapy levels or those that might be of danger to the infant, interruption of nursing and substitution with formula feeding for 1 to 3 days frequently causes a prompt decline of TB levels. On resumption of nursing, TB levels do not usually increase substantially. Brief interruption of nursing may be useful to confirm the diagnosis, thereby allaying parental anxiety. Failure to respond in this manner indicates that the neonate's jaundice may be unrelated to breastfeeding, and other causes should be sought. Supplementation with milk formula may have an effect similar to complete cessation of nursing. An alternative to temporary cessation of breastfeeding would be to confirm that the bilirubin is primarily unconjugated, that thyroid function tests are normal, and that there is no evidence of urinary infection. In the situation where the infant is thriving and the TB does not reach levels indicating the need for phototherapy, it may be prudent to just observe the infant. It should not be forgotten, however, that rare disorders of bilirubin conjugation may occur in breastfeeding infants and may lead to erroneous diagnoses. Effective nursing practices that prevent early "starvation" in breastfed newborns may reduce not only the incidence of breastfeeding failure jaundice but also the severity of breast milk jaundice.[2]

Sequelae of Unconjugated Hyperbilirubinemia

The recognition that unconjugated bilirubin may penetrate the brain cell under certain circumstances and its association with neuronal dysfunction and death are reasons for carefully managing newborn infants with significant hyperbilirubinemia.[58] There is some evidence that, despite the publication of national guidelines for the prevention and management of hyperbilirubinemia in several countries (United States, Canada, South Africa, United Kingdom, Norway, Israel, Japan, and the Netherlands), kernicterus continues to occur in industrialized countries in which the condition was thought to have been "extinct."[59,60] ABE (defined as a syndrome of the acute manifestations of bilirubin neurotoxicity)[4] and its resultant sequelae, kernicterus

(defined as chronic and permanent sequelae of bilirubin neurotoxicity), should, for the most part, be preventable conditions. Concerns that risks for ABE or kernicterus have been exaggerated with regard to term, otherwise healthy neonates have been outweighed by the perpetuation of cases up to present times.[19]

There is no single TB concentration that can be regarded as safe or categorically dangerous. The TB level, although used clinically to determine the need for phototherapy and exchange transfusion, is a poor predictor of subsequent neurodevelopmental outcome. In a study of 140 newborns with TB values >25 mg/dL (428 µmol/L) who were treated with phototherapy or exchange transfusion, 5-year outcomes were not significantly different from those of randomly selected controls. Re-analyzing data from the Collaborative Perinatal Project, Kuzniewicz et al.[60a] and Newman et al.[60b] found no relationship between maximum TB levels and IQ scores. However, in both of the aforementioned studies, the presence of a positive DAT did result in a poorer prognosis than the general population studied. Of 249 newborns admitted to a children's hospital in Cairo, Egypt, with TB values of 25 mg/dL (428 µmol/L) or greater, Gamaleldin et al.[61] found little correlation between admission TB levels and ABE.

It is unlikely that, in an otherwise healthy term infant with no obvious hemolytic condition, bilirubin neurotoxicity will occur at TB concentrations below 25 mg/dL (428 µmol/L), or even higher.[62] In the presence of hemolysis, prematurity, or other risk factors (see later) or in the presence of poor relative health, the danger point may be reached at lower levels of TB. In a term infant thought to be actively hemolyzing, a TB concentration of 18 to 20 mg/dL (308–343 µmol/L) should probably not be exceeded. In California, any TB >30 mg/dL (513 µmol/L) is rare (8.6 per 100,000 live births), and chronic bilirubin-induced neurotoxicity is uncommon and occurs only in the setting of additional risk factors and TB values substantially above the 2004 AAP exchange transfusion thresholds[63] (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Until definitive scientific data indicate otherwise, hyperbilirubinemia should be seen as being capable of producing a spectrum of neurologic dysfunction in the newborn, ranging from transient mild encephalopathy to permanent severe neurologic impairment secondary to neuronal necrosis (kernicterus). In addition, it is important to understand that bilirubin metabolism is a dynamic process influenced by many factors. An isolated TB level obtained at one point in time is inadequate to fully assess the risk for sequelae for a particular neonate. Many other factors, including the gestational age and relative health of the newborn, need to be carefully evaluated.

Clinical Spectrum of Bilirubin Neurotoxicity

Rather than being limited to the classic form of kernicterus characterized predominantly by choreoathetotic cerebral palsy, it is now recognized that there are many and varied clinical manifestations of bilirubin neurotoxicity.[64] The significant clinical features persist permanently despite resolution of the extreme neonatal hyperbilirubinemia. Classic kernicterus, known as choreoathetotic cerebral palsy, presents as the persisting tetrad of signs and symptoms, including (1) choreoathetosis, (2) auditory impairments, (3) paralysis of upward gaze, and (4) dental enamel dysplasia, in individuals who, as newborns, had been extremely hyperbilirubinemic. However, as the various components can also exist independently, and not as a tetrad, the etiologic role of prior hyperbilirubinemia may be evasive. Auditory manifestations of bilirubin neurotoxicity can manifest as varying degrees of hearing loss in combination with auditory-processing disturbance. Auditory neuropathy spectrum disorder (ANSD) includes absent or abnormal auditory brainstem response (ABR), in the presence of normal or giant cochlear microphonic responses, with or without normal otoacoustic emissions (OAEs). The term is not synonymous with hearing loss but involves abnormal processing of auditory signals. Subtle kernicterus includes neurodevelopmental disabilities for which other etiologies have been excluded and therefore appear to be due to bilirubin neurotoxicity. This includes isolated hearing loss not meeting the criteria of ANSD, mild motor involvement including awkwardness or clumsiness, and other manifestations, also known as BIND. Kernicterus with additional neurologic dysfunctions not usually regarded as the result of bilirubin neurotoxicity is known as "kernicterus plus," implying kernicterus plus some other condition. Additional associated neurologic manifestations not regarded as the direct result of bilirubin neurotoxicity include spasticity, microcephaly, and magnetic resonance imaging (MRI) abnormalities not typically associated with kernicterus.

Bilirubin Neurotoxicity: A New Definition

Because of this wide spectrum of clinical manifestations of bilirubin neurotoxicity, Le Pichon et al.[64] proposed using the term kernicterus spectrum disorder (KSD) as an all-encompassing term including the many and varied clinical expressions and severity of bilirubin neurotoxicity. Motor-predominant and auditory-predominant subtypes have been described as part of a continuum of KSD.

Epidemiology of Kernicterus and Extreme Hyperbilirubinemia

Did Kernicterus Really Disappear and Then Resurface?

Some authorities refer to a disappearance and resurgence of kernicterus in Westernized countries during the last decades. Others claim that the condition never completely disappeared and as a result is still being seen. Undoubtedly, phototherapy and exchange transfusion, along with immune prophylaxis of Rh isoimmunization, have prevented many cases of kernicterus. The disappearance theory cannot explain cases of kernicterus still occurring due to G6PD deficiency and DAT-positive ABO immunization, both common to this day.

The evidence for and against the disappearance/resurgence theory is inconsistent. In favor is a dearth of reported cases followed by several case reports and culminating in the US Kernicterus Registry report.[2] In Denmark, cases of bilirubin encephalopathy were found between 1994 and 2001 but not during the 20 years preceding that period. However, in the United States, there was a 70% decrease in the number of hospitalizations between 1988 and 2005 for kernicterus. There was a constant incidence of kernicterus in California occurring during two time epochs: 1988 to 1993 and 1994 to 1997, whereas on a national US basis, mortality data due to kernicterus from the Centers for Disease Control and Prevention databases remained consistent between 1979 and 2006. Overshadowing this debate is the fact that cases of kernicterus with major public health implications are still occurring.

How Frequent Is Kernicterus?

The incidence of kernicterus varies from country to country, as well as between industrialized countries and countries with developing medical systems. Estimates in developed countries range from about 0.4 to 2 per 100,000 live births.[65] Table 95.1 summarizes the frequency of the condition in some Westernized countries. In addition to those listed, cases of kernicterus have been reported from Italy and Germany. The problem continues well into the 21st century, as evidenced by two recent reports from Denmark[66] and Sweden.[67] The current incidence in the United States is estimated to be 82 new cases annually.[68]

These reports shared several common epidemiologic and etiologic features, including ABO heterospecificity, G6PD deficiency, other isoimmunizations, late prematurity, breastfeeding, sepsis, male sex, and discharge prior to 48 hours. It is remarkable that many of the infants were discharged as healthy from birth hospitalization but were subsequently readmitted for extreme hyperbilirubinemia. African American ethnicity and minority groups were over-represented, relative to the baseline population, in the United States and United Kingdom/Ireland reports.

TABLE 95.1 Incidence (per Live Births) of Kernicterus in Westernized Countries

Country/State	Years of Birth	Incidence of Kernicterus
Denmark	1994–1998	1/64,000
Denmark	1994–2003	1/79,000
United Kingdom	2003–2005	1/150,000
Canada	2007–2008	1/43,000
California[a]	1988–1997	0.44/100,000

[a]California data obtained from Brooks JC, Fisher-Owens SA, Wu YW, et al. Evidence suggests there was not a "resurgence" of kernicterus in the 1990s. *Pediatrics*. 2011;127(4):672-679.
Adapted in part from Maisels MJ. Neonatal hyperbilirubinemia and kernicterus—not gone but sometimes forgotten. *Early Hum Dev*. 2009;85(11):727–732.

Kernicterus in Developing Countries

Kernicterus, with a high incidence of bilirubin-attributable neonatal mortality, continues to occur in countries where G6PD deficiency is indigenous, as well as in developing countries with underdeveloped health services or in war zones, as illustrated in a recent report from Baghdad, Iraq.[69] Other recent reports derive from Nigeria, Oman, Turkey, Kuwait, and Egypt.[2]

Surrogates for Assessing the Incidence of Kernicterus: Extreme Hyperbilirubinemia and Readmission

Although a devastating condition, kernicterus is in fact rare, and it is difficult to assess its incidence in any specific population group. As we hardly ever now encounter cases of KSD, we need to have clinical surrogates by which we judge the success of its prevention.[70] Surrogates for the potential to develop bilirubin neurotoxicity have been sought, including the incidence of extreme hyperbilirubinemia—TB >25 mg/dL (428 µmol/L) or 30 mg/dL (513 µmol/L)—or the incidence of readmission for hyperbilirubinemia. Severe hyperbilirubinemia may be the most plausible surrogate indicator for kernicterus. Overall, the rate of extreme hyperbilirubinemia or one of the alternatives should be low, provided surveillance for hyperbilirubinemia both during birth hospitalization and post-discharge is effective, although a zero rate will be difficult to obtain. The modern-day incidence of extreme hyperbilirubinemia per 100,000 live births in Western countries and/or regions, demonstrates a wide range in incidence (Table 95.2).

The reported range for readmission for hyperbilirubinemia lies between 0.17% and 3.2%.[71] The main reasons for readmission include lower gestational age, late prematurity, early discharge, unsuccessful breastfeeding, and lack of pre-discharge assessment of the risk for subsequent hyperbilirubinemia. The high risk for readmission of early term newborns in combination with early discharge was recently documented in Australia: Neonates born at 37 weeks of gestation and who were discharged at ≤48 hours were readmitted for hyperbilirubinemia at a ninefold higher frequency than those born at 39 weeks of gestation and remained in hospital for 3 to 4 days.[72] In Utah, late preterm and early term newborns were readmitted at higher rates than term counterparts, emphasizing the need to assess readiness for discharge and to vigilantly follow these infants for the development of hyperbilirubinemia after discharge.[18] Extreme hyperbilirubinemia, despite the close surveillance and ready availability of treatment in an organized health system in California, occurred in 0.14% of newborns (TB ≥25 mg/dL [428 µmol/L]); 0.01% had TB values ranging from 30.5 to 45.5 mg/dL (522–778 µmol/L). Within a health system in Detroit, Michigan, only 0.6% of infants compared with 2% in the California survey developed a TB of 20 mg/dL (342 µmol/L) or greater. This difference was attributed to rigorous bilirubin screening, follow-up, and treatment.

Again, in California, 0.12% of 18,089 newborns 35 weeks of gestation or greater developed TB levels that exceeded the

TABLE 95.2 Modern-Day Incidence of Extreme Hyperbilirubinemia per 100,000 Live Births in Western, Industrialized Countries/Regions, Demonstrating a Wide Range in Incidence

Country/Region	Author/Year of Publication	Number of Newborns With TB >25 mg/dL per 100,000 Live Births	Number of Newborns With TB >30 mg/dL per 100,000 Live Births	Comment
Canada	Sgro et al., 2006[152]	40	—	TB ≥25 mg/dL or ET
United Kingdom and Ireland	Manning et al., 2007[153]	—	7.1	—
Denmark	Bjerre et al., 2008[154]	45	—	TB ≥26 mg/dL
Switzerland	Zoubir et al., 2011[32]	17	—	—
Netherlands	Gotink et al., 2013[155]		10.4	TB ≥29 mg/dL or ≥20 mg/dL + ET
Utah, US	Christensen et al., 2013[156]	47.6	10.6	—
California, US	Kuzniewicz et al., 2014[63]	—	8.6	—
Australia	McGillivray et al., 2015[157]	9.4	—	TB ≥26 mg/dL
California	Bhutani et al., 2016[86]	21.3	4.2	—
Canada	Sgro et al., 2016[95]	11.6	—	—
Sweden	Alken et al., 2019[67]	50 (TB 25.0-29.9 mg/dL)	6.8	—
Denmark	Donneborg et al., 2020[66]	42	—	—

Note that all series included above were published after the turn of the millennium (year 2000). List is ordered by publication date.
ET, Exchange transfusion; *TB*, total serum/plasma bilirubin.
From Hammerman C, Kaplan M. Hyperbilirubinemia in the term infant: Re-evaluating what we think we know. *Clin Perinatol*. 2021;48(3):533–554.

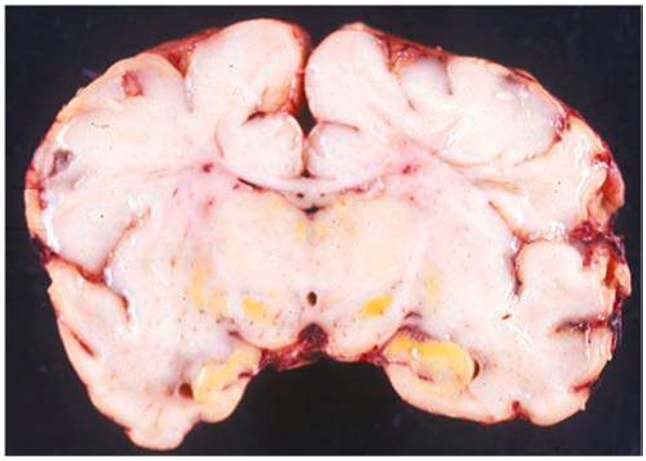
2004 AAP[11] indications for exchange transfusion (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). It is remarkable that the majority of the affected infants were less than 38 weeks of gestation.[73]

Transient Encephalopathy or Acute Bilirubin Encephalopathy

ABE is characterized by lethargy, poor feeding, hypotonia, and a high-pitched cry in a severely jaundiced infant. Hyperextension of the extensor muscles and back arching may ensue.[74] Early bilirubin toxicity may be transient and reversible, and not all infants displaying signs of ABE will necessarily go on to the full-blown clinical picture of kernicterus.[2] This is suggested by clinical observations of increasing lethargy and other signs in tandem with rising TB levels, with reversal of the symptoms after exchange transfusion. In Egyptian newborns with severe hyperbilirubinemia, a BIND score, based on mental status, muscle tone, and cry patterns, was used to predict residual neurologic and hearing dysfunction at 3 to 5 months of age. Those with high scores either died or were left with residual neurologic and auditory impairments. Low BIND scores were associated with a high rate of normalcy at follow-up after aggressive intervention. However, the BIND score was not an absolute predictor; some infants with low BIND scores but very high TB levels did have neurologic residua at follow-up. Moreover, extreme hyperbilirubinemia in the absence of clinical evidence of ABE may be indicative of a favorable prognosis. In a 10-year Danish study, no evidence was found of an increased risk of deficits in motor development, executive function, or hearing in children who had had extreme neonatal hyperbilirubinemia but did not have intermediate or advanced bilirubin encephalopathy.[75]

Brainstem auditory-evoked responses (BAERs) may show changes in the wave latency and magnitude, characteristic of early signs of ABE. BAER signs typically encountered in neonates with moderate unconjugated hyperbilirubinemia (10–20 mg/dL [171–342 μmol/L]) include prolongation of latencies of waves III and IV-V, and interpeak I-III and I-V, compared with neonates of similar gestational and postnatal ages without hyperbilirubinemia. Prolonged latencies in peak IV-V and interpeak I-V suggest interference with brainstem conduction. These changes in evoked responses reverse with either an exchange transfusion or a spontaneous decline in TB levels.

Long-term follow-up of children with neonatal abnormalities in brainstem responses believed to be caused by hyperbilirubinemia is not yet available, and the significance



• **Fig. 95.16** Acute bilirubin encephalopathy (*ABE*). Coronal section through parietotemporal lobes. Note selective symmetric yellow discoloration in the hippocampus and subthalamic nuclei. Thalamus and globus pallidus are focally stained. (From Zangen S, Kidron D, Gelbart T, et al. Fatal kernicterus in a girl deficient in glucose-6-phosphate dehydrogenase: a paradigm of synergistic heterozygosity. *J Pediatr*. 2009;154(4):616–619.)

of abnormal BAER findings remains unknown. An abnormal BAER suggests an injury of the VIII cranial nerve. Bilirubin neurotoxicity affects the neural tissues of the auditory center and nerve but not the cochlea. Therefore bilirubin-induced auditory neuropathy may occur even in the context of normal cochlear function as measured by OAEs. This emphasizes the need to perform BAER testing, and not to rely on OAEs, in neonates who are suspected of having auditory damage due to hyperbilirubinemia. During the early stages of bilirubin neurotoxicity, a characteristic signal signature can be seen by MRI using T1- and T2-weighted imaging. Consequently, the presence of an abnormal BAERs, normal OAEs, and focal changes in the globus pallidus and medial lobe of the hippocampus by MRI is highly suggestive of ABE (Fig. 95.16).

Kernicterus

If early ABE is unrecognized or untreated, it may progress to permanent neurologic impairment. The term *kernicterus* (German *kern*, kernel or nucleus, and Greek *ikteros*, jaundice) has been traditionally used to describe the pathologic findings of bilirubin toxicity within the brain: staining and necrosis of neurons in the basal ganglia, hippocampal cortex, subthalamic nuclei, and cerebellum, followed by gliosis of these areas in survivors (Fig. 95.17). The cerebral cortex is generally spared. About half of all infants with kernicterus observed at autopsy also have extraneural lesions of bilirubin toxicity. These include necrosis of renal tubular cells, intestinal mucosa, and pancreatic cells in association with intracellular crystals of bilirubin. Gastrointestinal hemorrhage may accompany these lesions.

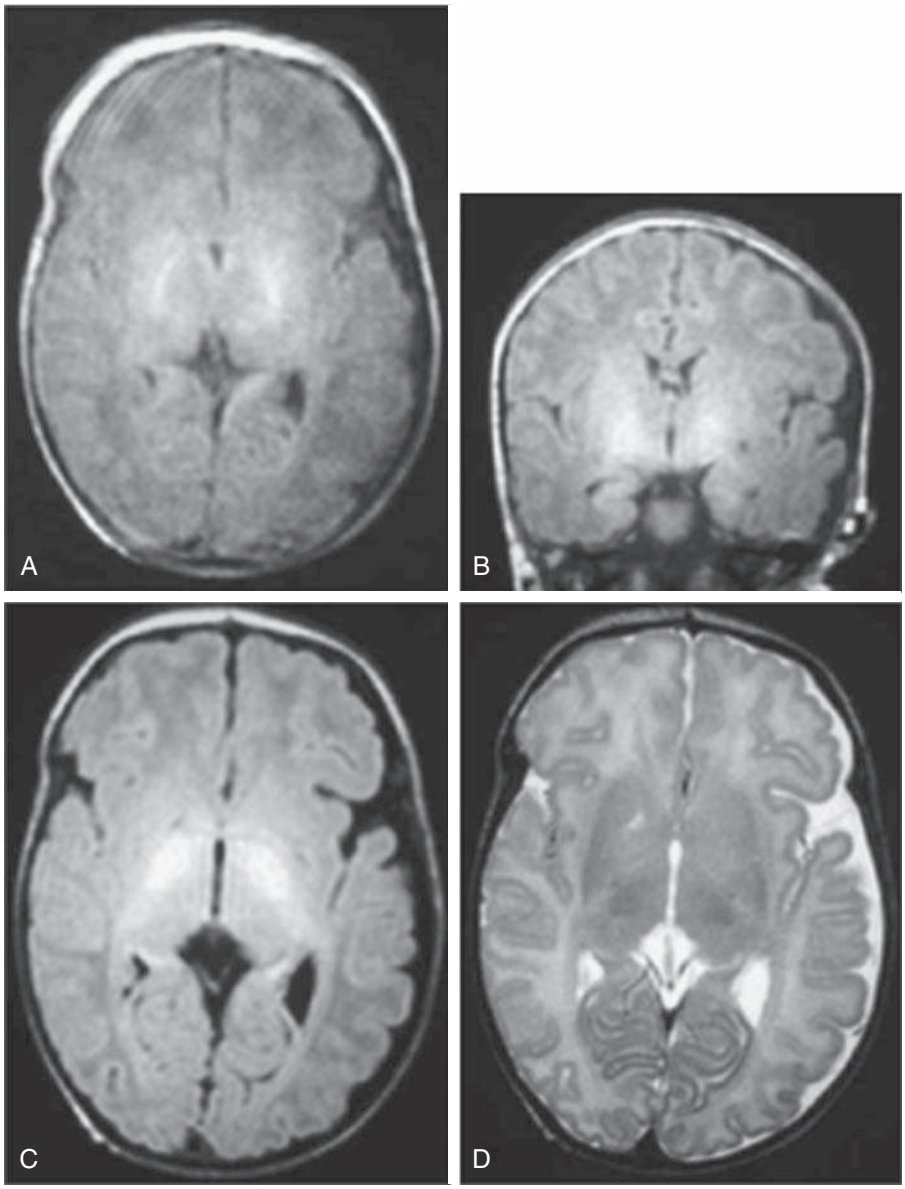
Kernicterus is also used to describe the clinical presentation of worsening encephalopathy. In the term newborn, several phases have been classically described, progressing from ABE in the early stages to chronic athetoid cerebral palsy later. Phase 1 is marked by poor sucking, hypotonia, and depressed sensorium. Fever, retrocollis, and hypertonia that may progress to frank opisthotonos are seen in phase 2. The hypertonia becomes less pronounced in phase 3, but high-pitched cries, hearing and visual abnormalities, poor feeding, and athetosis are manifest. Seizures may also occur. The usual time course for progression of the disease is about 24 hours. Long-term survivors often demonstrate the classic signs of kernicterus. These include a tetrad of (1) choreoathetoid cerebral palsy, (2) upward gaze palsy, (3) sensorineural hearing loss, and (4) dental dysplasia during later infancy and childhood.[2] The intellect may be spared, and mental retardation is not universally encountered. However, children with normal intelligence frequently have severe physical handicaps, making rehabilitation, education, and independent living unlikely. These sequelae of bilirubin toxicity may also develop in neonates who never manifested clinical signs of ABE during the newborn period. In addition, some neonates may have sequelae of subclinical bilirubin encephalopathy characterized only by the later development of mild disorders of motor function or abnormal cognitive function, or both.

A report of 25 California cases of strictly defined kernicterus illustrates the dismal picture of these unfortunate children. At a mean age of 7.8 ± 3.9 years old, 60% did not walk at all, and only 16% were able to walk unaided. Self-feeding was possible in only 52%, and a feeding tube was in place in 12%. Severe or profound mental retardation or severe disabilities were found in 36%, with only 32% having no evidence of mental retardation. Epilepsy and severe, profound, or untestable visual and auditory impairments were common, with only 36% having normal hearing. Motor spasticity, ataxia and dyskinesis, and hypotonia completed the clinical picture in many others.

Kernicterus in Preterm Infants and Low-Bilirubin Kernicterus

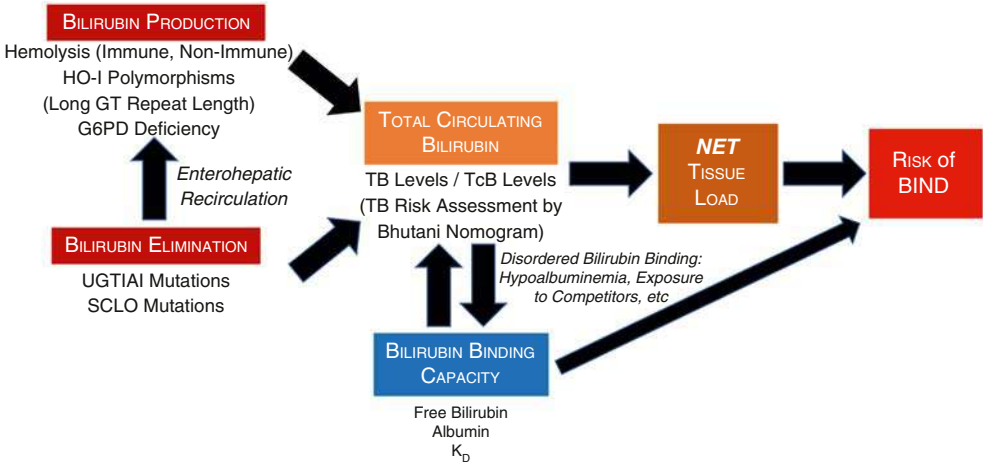
Presentation in preterm neonates is less stereotypical, and these patients may simply appear ill without signs specific for kernicterus. Bilirubin may enter the brain at lower levels of TB than would be expected in term infants. Moreover, bilirubin staining of central nervous system (CNS) structures in premature neonates may not be indicative of overt kernicterus and may result from developmental differences in CNS permeability to bilirubin and in situ bilirubin metabolism. Mortality occurs in about 50% of term newborns but may occur more frequently in the preterm population with kernicterus. Based on a Japanese survey, it was estimated that 2/1000 preterm infants <30 weeks of gestation developed kernicterus. Many did not have TB levels in the hyperbilirubinemic range, suggesting that the unbound bilirubin component may have been high in those affected.[76]

Low-bilirubin kernicterus can and does occur in premature infants at TB levels lower than what would be expected to be associated with neurotoxicity and at levels lower than those indicating phototherapy or exchange transfusion. Low-bilirubin kernicterus is a condition that was encountered in



• **Fig. 95.17** Magnetic resonance images of a 21-day-old, preterm male neonate with acute kernicterus. Axial (A) and coronal (B) T1-weighted and axial fluid-attenuated inversion recovery (FLAIR) (C) images at the level of the basal ganglia show symmetric, hyperintense globus pallidus involvement. This is not apparent on the axial T2-weighted (D) image. (From Coskun A, Yikilmaz A, Kumandas S, et al. Hyperintense globus pallidus on T1-weighted MR imaging in acute kernicterus: is it common or rare? *Eur Radiol*. 2005;15(6):1263–1267.)

the past during autopsy examinations of premature infants in whom TB did not reach excessive levels that were thought to be neurotoxic. It is still encountered today in premature infant survivors who did not have very high TB levels but who have clinical and MRI evidence of kernicterus. Low-bilirubin kernicterus has been defined as the occurrence of kernicterus at TB levels below commonly recommended exchange transfusion thresholds.[2] Because of the low nature of the TB in this situation, in the range not necessarily obligating phototherapy, the condition is unpredictable and the consequences refractory. Adherence to an accepted guideline is not a guarantee that some infants will not be unscathed. No guideline can be expected to prevent illness in every individual. It is not known what factors potentiate bilirubin neurotoxicity at low TB levels. Some factors that have been implicated include low serum albumin (almost ubiquitous in sick, unstable premature infants) and co-morbid CNS injury, including intraventricular hemorrhage, periventricular leukomalacia, and infection. Given the occurrence of the condition at low TB levels, it is unlikely that the condition will be eliminated barring a significant lowering of exchange transfusion thresholds, with the potential of bringing in its wake a number of complications (infections, hemorrhage, blood pressure instability, necrotizing enterocolitis, complications related to blood transfusion in general) associated with this procedure.



• **Fig. 95.18** Processes that affect an infant's risk for developing bilirubin-induced neurologic disorders (*BIND*). *HO-1*, Heme oxygenase-1; *TB*, total serum/plasma bilirubin. (From Wong RJ, Bhutani VK, Stevenson DK. The importance of hemolysis and its clinical detection in neonates with hyperbilirubinemia. *Curr Pediatr Rev*. 2017;13(3):193–198.)

Bilirubin Entry Into the Brain

Bilirubin is thought to enter the brain through multiple mechanisms (Fig. 95.18), including (1) bilirubin production that overwhelms the normal buffering capacity of the blood and tissues, (2) alterations in the bilirubin-binding capacity of albumin and other proteins resulting in the presence of unconjugated bilirubin in the circulation, (3) increased CNS permeability to bilirubin secondary to disruption of the blood-brain barrier, and (4) other factors that may affect those previously mentioned or act through novel independent mechanisms.

Unconjugated bilirubin is nonpolar and lipid soluble, and its aqueous solubility in plasma water is extremely small, mandating its binding in plasma primarily to albumin. Binding to other components of blood (β-globulin, RBC membranes, and platelets) may also occur when the albumin-binding capacity for bilirubin is exceeded. It has long been believed that bilirubin toxicity occurs when the albumin-binding capacity for bilirubin is saturated and the unbound or "free" bilirubin (bilirubin in aqueous phase) concentration rises in blood. Whether this is, in fact, the basic pathophysiologic mechanism of kernicterus remains unresolved.

The human albumin molecule is capable of binding at least two molecules of bilirubin, with the first molecule more tightly bound than the second. Additional classes of binding sites, if operative in vivo, have much lower affinities than the first two. At a molar ratio of 1, each gram of human albumin binds 8.2 mg of bilirubin. Thus, at an average albumin concentration of 3 g/dL, the first binding site should be capable of binding 25 mg of bilirubin/dL of serum or plasma. The second binding site should be capable of binding an additional 25 mg/dL for a total binding capacity of 50 mg/dL.

Various techniques have been proposed to measure albumin binding of bilirubin, but their application and interpretation in clinical management are not generally accepted. The dye-binding methods using 2-(4′-hydroxybenzeneazo) benzoic acid and Direct Yellow 7 are based on the measurement of reserve binding sites on the albumin molecule and should be capable of indicating impending risk. The column chromatographic methods (Sephadex G-25), the salicylate displacement spectrophotometric method (saturation index), the RBC uptake method, and the oxidation technique (peroxidase method) should all be capable of detecting small increases in unbound bilirubin or "loosely bound" bilirubin, in theory, denoting increased risk for developing kernicterus.[2] However, current laboratory and clinical data are still insufficient to permit a recommendation for the use of either a single method or a combination of methods to guide the clinical management of infants with neonatal unconjugated hyperbilirubinemia. Furthermore, it may be falsely reassuring to assume that in vitro binding capacities will remain reliably static in an in vivo system that changes so dynamically during the first postnatal days.

The bilirubin-binding capacity of albumin is thought to be decreased in sick term and premature human neonates. In addition, the serum albumin concentration is often lower in these patients than in healthy, term counterparts. Theoretically, both of these factors may act to place the sick term or premature neonate at higher risk for kernicterus at lower TB levels than seen in the healthy term newborn. There is still debate about whether the bilirubin-binding capacity of albumin decreases as pH drops below 7.4, despite the known solubility decrease of bilirubin with increasing acidity. Numerous agents compete with bilirubin for binding sites on albumin, acting to displace bilirubin and increase the ratio of free to bound bilirubin. Free fatty acids, which are elevated in sepsis and hypoxemia, are capable of displacing bilirubin. Similarly, IV lipid emulsions rarely can contribute to elevated free fatty acids and thus displacement of bilirubin. Sulfisoxazole and other sulfa drugs, indomethacin, and salicylates readily displace bilirubin. Even ampicillin, when injected rapidly, has the potential to act in a similar manner. Benzyl alcohol, once used as a preservative in various medications,

has been shown to competitively inhibit bilirubin binding. Finally, certain substances used in the preparation of albumin solutions may act to decrease its bilirubin-binding capacity.

Disruption of the blood-brain barrier allows the passage of molecules otherwise prevented from entering the CNS. Hypertonicity of the serum, meningitis, and hypoxemia all increase CNS permeability to bilirubin.[2] It has been reported that unconjugated bilirubin is a substrate for phosphorylated glycoprotein (P-GP) and that P-GP in the blood-brain barrier may play a role in limiting the entry of bilirubin into the CNS. P-GP is an integral plasma membrane transport protein, dependent on ATP, which can transport a wide variety of substrates across biologic membranes.

Many other factors may play a role in regulating bilirubin entry into the brain. Kernicterus has been reported to occur in adults with Crigler-Najjar syndrome type I, but only when TB levels have reached 45 to 55 mg/dL (770–941 μmol/L). This contrasts with the situation in the full-term newborn in which kernicterus may be anticipated at somewhat lower concentrations, suggesting the presence of a maturational process in the blood-brain barrier integrity.

In addition to bilirubin produced within the reticuloendothelial system, bilirubin may be produced within the brain. The mammalian brain has two isoforms of HO, HO-1 and HO-2, which convert heme to biliverdin. HO-1 normally shows little baseline activity in the brain but is capable of being rapidly upregulated in response to stress. Most HO activity in the brain is the result of the constitutive isoform HO-2. HO-1 and HO-2 are distributed in selected areas of the brain, many of which play roles in motor and auditory function. Biliverdin reductase is also found in the brain, catalyzing the conversion of biliverdin to bilirubin. Although bilirubin thus formed is normally rapidly cleared by bilirubin oxidase, it is possible that this fraction of the bilirubin pool does contribute to the development of kernicterus. These enzyme systems are developmentally regulated and may also be influenced by any of the disease states previously mentioned. Because the bilirubin produced and metabolized in situ must be transported out of the brain, interference with this transport mechanism may be another potential mechanism of contributing to kernicterus.

Once bilirubin has gained access to the CNS, there are several postulated mechanisms of neuronal injury: (1) passage through lipid moieties of cell membranes into the lipids of subcellular organelles such as mitochondria, interfering with critical steps in energy metabolism; (2) binding to specific membrane, organelle, or cytoplasmic proteins and inhibiting their function; and (3) damage and direct interference with the function of DNA.

The neurotoxicity of bilirubin is currently being debated. As described earlier, HO catalyzes the conversion of heme to biliverdin, releasing equimolar amounts of CO. CO may function as a neurotransmitter within the CNS and has been implicated as playing a role in memory. However, CO may also act as a neurotoxin and have deleterious effects, including neuronal necrosis. The deposition of bilirubin in the brain of patients with kernicterus may not be the primary insult but rather may be a relatively innocuous marker of neuronal damage produced by other means. Bilirubin has antioxidant properties and may, at physiologic levels, provide protection from oxidative injury. Whatever the mechanism of bilirubin neurotoxicity, clinical decisions regarding the management of hyperbilirubinemia and the institution of therapy are based on the TB level, and, given its apparent effectiveness in reducing the incidence of kernicterus, it would be unwise to alter the current approach to therapy.

Bilirubin-Induced Neurologic Disorders

BIND represents a syndrome of neurologic disorders less severe than classic choreoathetotic cerebral palsy but nevertheless attributable to bilirubin neurotoxicity. Neonates are usually exposed to TB levels of lesser severity than those encountered in classic kernicterus, but the clinical picture may manifest as a range of subtle processing disorders including disturbances of vision, motor ability, auditory and speech function, cognition, and language.[2,4]

Diagnosis of Unconjugated Hyperbilirubinemia

About two-thirds of the more than 4 million neonates born annually in the United States become clinically jaundiced. Clearly, the number of jaundiced neonates who will develop sequelae of hyperbilirubinemia is substantially less than this. The challenge to the pediatrician is to determine which newborns may become or are already abnormally jaundiced and therefore are at risk for severe sequelae. The 2004 recommendations by the AAP[11] for the laboratory evaluation of the jaundiced infant at 35 weeks of gestation or later are listed in Table 95.3 (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]).

Total Bilirubin Measurements

When the clinical screening examination detects a jaundiced newborn, the mainstay of clinical management includes determination of TB concentrations in serum or plasma. Direct bilirubin measurements will not contribute much information in the early stages of jaundice but should be performed in cases of prolonged hyperbilirubinemia or of hyperbilirubinemia not responding to therapy, or if a disease process is suspected. In most patients, repeat determinations will be necessary in the acute stage of jaundice to determine the trajectory, the peak TB level, and whether indications for instituting therapy have been reached. Following that, an at least daily determination should be performed until a clear pattern of decline is observed. Clinical judgment is necessary to determine whether TB concentrations may be monitored on an ambulatory basis, thereby eliminating the need for prolonged hospitalization, or whether the risk for severe hyperbilirubinemia warrants in-hospital observation.

TABLE 95.3 Laboratory Evaluation of Jaundiced Infants ≥35 Weeks of Gestation

Indications	Assessments
Jaundice in first 24 hours	TcB and/or TB
Jaundice appears excessive for age	TcB and/or TB
Infant receiving phototherapy or TB rising rapidly (i.e., crossing percentiles; see Fig. 95.21) and unexplained by history and physical examination	Blood type and DAT or Coombs' test, if not obtained with cord blood CBC and smear Direct or conjugated bilirubin Optional: reticulocyte count, G6PD, ETCOc (if available) Repeat TB in 4–24 hours, depending on infant age and TB level
TB concentration approaching exchange levels or not responding to phototherapy	Reticulocyte count, G6PD, albumin, ETCOc (if available)
Elevated direct (or conjugated) bilirubin level	Urinalysis and urine culture Evaluate for sepsis indicated by history and physical examination
Jaundice present at or beyond age 3 weeks, or if the infant is sick	Total and direct (conjugated) bilirubin level If direct bilirubin is elevated, evaluate for causes of cholestasis. Check results of newborn thyroid and galactosemia screen; evaluate for signs and symptoms of hypothyroidism.

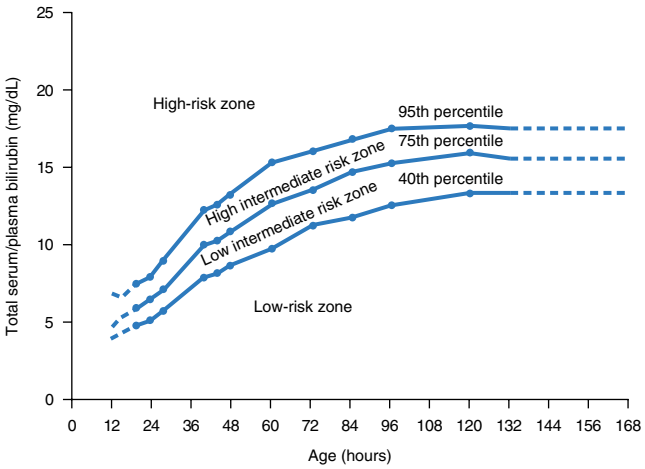
Refer to the 2022 AAP Clinical Practice Guideline for updated recommendations.[19]

CBC, Complete blood count; *DAT*, direct antiglobulin test; *ETCOc*, end-tidal carbon monoxide, corrected for inhaled CO; *G6PD*, glucose-6-phosphate dehydrogenase; *TB*, total serum/plasma bilirubin; *TcB*, transcutaneous bilirubin.

From American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114(1):297–316.

Thus the clinician must determine whether any individual neonate is at high or low risk for developing severe hyperbilirubinemia or kernicterus. Neonates at high risk for kernicterus include those presenting with jaundice in the first 24 hours of life, with pallor or hepatosplenomegaly, and with documented immune or nonimmune hemolytic conditions.

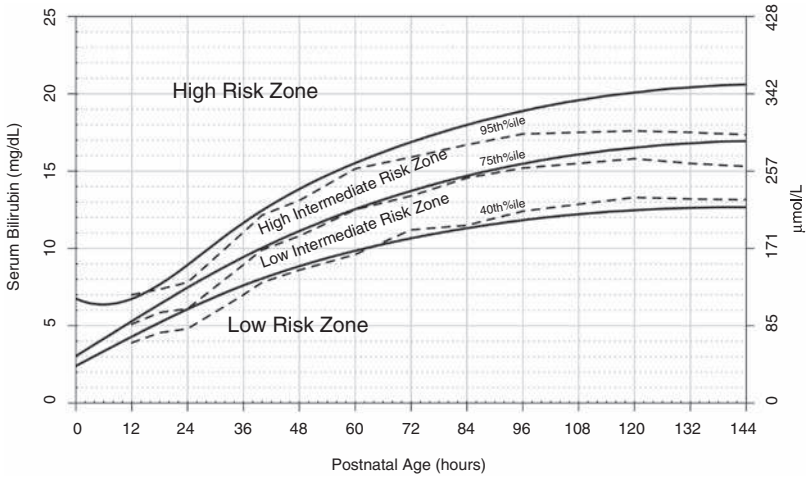
Bhutani and colleagues,[10c] in a study performed in 1999 on a racially diverse population of term healthy newborns, established that a percentile-based, hour-specific bilirubin nomogram of pre-discharge TB levels can accurately predict an infant's level of risk for developing hyperbilirubinemia (see Fig. 95.19).[11] Infants with a pre-discharge TB in the 95th percentile or higher (high-risk zone) had a 57% risk for developing severe hyperbilirubinemia (TB of 17 mg/dL [291 µmol/L] or greater). Risk was 13% for infants in the high-intermediate zone (TB between the 75th and 95th percentiles). Infants with TB levels between the 40th and 75th percentiles (low to intermediate risk) had a risk of 2.1%. Infants with TB in the 40th percentile or less had no risk. However, an analysis of pre-discharge TB values in 143 neonates who were readmitted for hyperbilirubinemia demonstrated a false-negative pre-discharge bilirubin screen in six (4.2%) in the low-risk zone (<40th percentile) and 40 (28%) in the intermediate low-risk zone (41st–75th percentiles).[71] The results of this and another study suggest that pre-discharge TB values in the low-risk zones may not be benign, confirming the 2004 AAP recommendations[11] for follow-up for all neonates within a few days of discharge (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]).



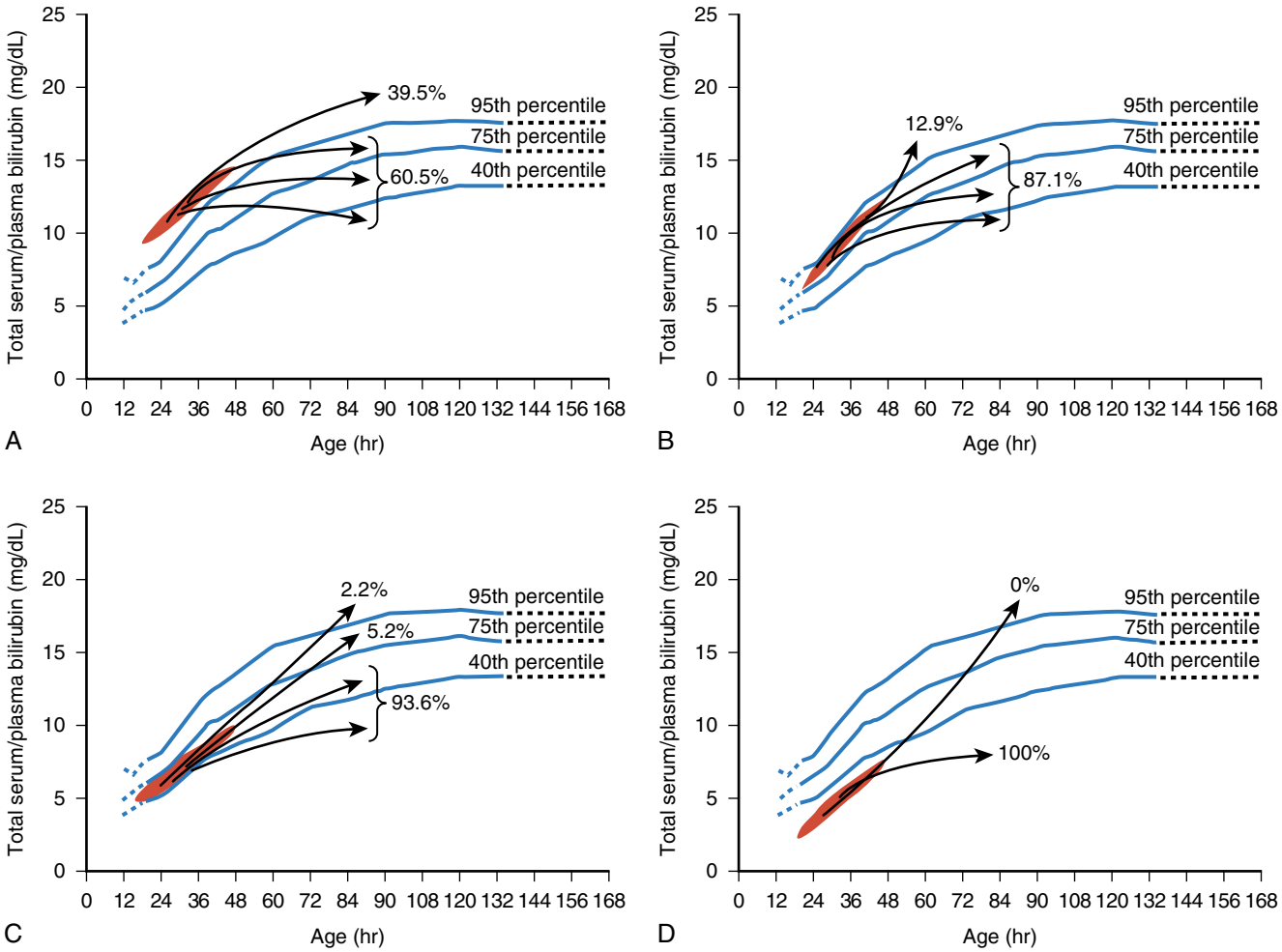
• **Fig. 95.19** Zones of risk for pathologic hyperbilirubinemia based on hour-specific TB levels. (From Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a pre-discharge hour-specific TB level for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103(1):6–14.)

Recently, Bahr et al.[77] constructed a new TB nomogram from 397,395 tests drawn from 407,842 primarily Caucasian and Hispanic newborns, all ≥35 0/7 weeks of gestation. This nomogram is displayed, superimposed on the 1999 Bhutani nomogram, for comparison. Increased values for the 95th percentile shortly after birth may be a reflection of selection bias (Fig. 95.20).

All TB measurements should be plotted on an hour-specific bilirubin nomogram, which takes into account the rapidly occurring changes in TB concentrations. It is clear from a study of the nomogram that an individual TB reading may have different connotations depending on the postnatal hour at which the blood was sampled. The closer the TB reading to the 95th percentile, the greater becomes the risk for subsequent hyperbilirubinemia. Conversely, a TB reading below the 40th percentile is usually associated with a low risk for hyperbilirubinemia. A useful method of assessing whether the rate of rise of TB is greater than normal (0.2 mg/dL/h or greater) is to plot several TB points on the graph and determine whether the trajectory runs in parallel with the graph or at a more rapid rate or "jumping" to a higher percentile track (Fig. 95.21). TcB may provide a



• **Fig. 95.20** New total serum/plasma bilirubin (*TB*) nomogram (*solid lines*) superimposed on the 1999 Bhutani nomogram (*broken lines*), for comparison. (From Bahr TM, Henry E, Christensen RD, et al. A new hour-specific serum bilirubin nomogram for neonates ≥35 weeks of gestation. *J Pediatr*. 2021;236:28.e1–33.e1.)



• **Fig. 95.21** Outcome of newborns as defined by the percentage of newborns that remain or move up to the high-risk zone after their risk assessment with the pre-discharge total serum/plasma bilirubin (*TB*) value (represented by the *red shaded areas*). (A) Outcome for newborns designated in the high-risk zone (*N* = 172). (B) Outcome of newborns in the upper-intermediate-risk zone (*N* = 356). (C) Outcome of newborns in the lower-intermediate-risk zone (*N* = 556). (D) Outcome of newborns in the low-risk zone (*N* = 1756). (From Bhutani VK, Johnson L, Sivieri EM . Predictive ability of a pre-discharge hour-specific TB level for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*. 1999;103(1):6–14.)

useful, quick, and painless method of assessing these bilirubin modalities (see Transcutaneous Bilirubinometry later).

Classification of hyperbilirubinemia as conjugated or unconjugated requires fractionation of serum or plasma bilirubin into direct- and indirect-reacting pigments, respectively. Simple techniques that fail to distinguish direct- from indirect-reacting fractions are not recommended, but they may be useful as less expensive methods for screening and frequent repeat determinations and in emergency situations. The prototype of colorimetric methods is the van den Bergh test, a modification of the Ehrlich diazo reaction. The Jendrassik-Grof method has also been used widely as an automated procedure in many hospital laboratories.

Newer automated methods such as the Ektachem system provide greater precision and the ability to measure the covalently bound δ-bilirubin. Compared with the values of direct bilirubin obtained by high-performance liquid chromatography, the Jendrassik-Grof and other diazo methods exaggerate this fraction to variable degrees. These older, nonspecific diazo reaction methods also fail to measure the δ-bilirubin fraction, underestimating TB by 0.2 to 0.3 mg/dL (3–5 μmol/L) in the normal adult. High-performance liquid chromatography probably provides the most accurate measurement of TB but is impractical for routine clinical laboratory use. The Ektachem method has gained wide acceptance. It uses a dry-film system for measurement of TB and subfractions (indirect, direct, and δ) for general clinical laboratory use. The adult upper limit of normal for TB by this method is 1.3 mg/dL (22 μmol/L); for the conjugated fraction, it is 0.3 mg/dL (5 μmol/L), of which two-thirds is in the form of δ-bilirubin. For newborns younger than 2 weeks, a simpler and less expensive single-film method can be used, omitting measurement of δ-bilirubin, which is not found in this age group.

High levels of direct-reacting bilirubin reflect a different set of pathologic entities that do not respond to the usual interventions for unconjugated hyperbilirubinemia and will be considered later in this chapter. It is useful to consider four groups of newborns when making decisions regarding laboratory evaluation and therapy of unconjugated hyperbilirubinemia: (1) healthy term (more than 37 completed gestational weeks), (2) sick term, (3) healthy premature, and (4) sick premature neonates. Studies to date have dealt almost exclusively with the healthy term newborn. Although unconjugated hyperbilirubinemia is a disease of multiple causes, and neonates should be treated differently based on gestational age and relative state of health, some general comments can be made.

Further laboratory investigation should be considered when TB concentrations are (1) 4 mg/dL (68 μmol/L) or greater in cord blood, (2) increasing at a rate of 0.5 mg/dL (9 μmol/L) or greater per hour over a 4- to 8-hour period, (3) increasing at a rate of 5 mg/dL (86 μmol/L) or greater per day, (4) 13 to 15 mg/dL (222–257 μmol/L) or greater in full-term infants at any time, (5) 10 mg/dL (171 μmol/L) or greater in premature neonates at any time, or (6) when clinical jaundice persists beyond 10 to 14 days of life.

Further diagnostic studies are based on a thorough history and physical examination, which can narrow the differential diagnosis. A careful history from the parents may reveal familial patterns of neonatal hyperbilirubinemia or anemia or ethnic patterns associated with severe neonatal jaundice. Observation of the parents for jaundice and even determination of TB concentrations in them also may be useful in the diagnosis of familial types of hemolytic disease or inherited hepatic dysfunction. Patterns of feeding, the time of onset, and the frequency of breastfeeding also may be important. A careful physical examination with special attention to liver and spleen size, skin appearance, and neurobehavioral status should be performed in the evaluation of a jaundiced neonate.

Initial studies that may be indicated include determination of maternal blood group and Rh type, a screen for antibodies directed against minor erythrocyte antigens, determination of neonatal blood group and Rh type, DAT (formerly known as the Coombs' test), hemoglobin or hematocrit, RBC morphology by peripheral blood smears, and reticulocyte counts. In the presence of significant jaundice with a potential ABO incompatibility (type O mother and type A or B infant), the DAT should be repeated at least once if originally negative, because initial false-negative results have often been noted. Hematologic indices indicative of hemolysis in older children and adults may not be useful in the diagnosis of increased hemolysis in neonates because of overlap in these indices between hemolytic and nonhemolytic conditions. An elevated ETCOc level may be indicative of ongoing hemolysis (see End-Tidal Carbon Monoxide Measurements). Determination of erythrocyte G6PD activity may be useful in cases of unexplained hyperbilirubinemia, an exponential bilirubin trajectory, or hyperbilirubinemia not responding to intense phototherapy. Consideration of the family's ethnic group may be useful in deciding whether to perform a G6PD test. Cord blood should be saved in the event further testing is indicated.

More extensive studies for rarer forms of hemolytic disease and enzyme assays or genetic testing for UGT1A1 activity may be deferred until the chronicity of the disease has been established. Studies for hepatocellular disease (such as hepatitis and obstructive biliary disease), including serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase, and cholesterol, need to be performed only when there is a significant elevation of conjugated bilirubin or liver disease.

Until a laboratory method is proved to accurately assess the risk for developing bilirubin neurotoxicity, the need for treatment and the progression from phototherapy to exchange transfusion must be based on clinical judgment in conjunction with consideration of TB, risk factors, gestational age, and the presence of hemolysis. The method of management chosen for an individual neonate is determined in part by the TB concentration at which therapy is instituted. The 2004 AAP Clinical Practice Guideline formulated an established and uniform policy regarding the methods to be used and criteria for initiation of therapy.[11]

• BOX 95.3 Risk Factors for Development of Severe Hyperbilirubinemia in Infants ≥35 Weeks of Gestation

Major Risk Factors

- Pre-discharge TB or TcB level in the high-risk zone (see Figs. 95.15 and 95.20)
- Jaundice observed in the first 24 hours
- Blood group incompatibility with positive DAT, other known hemolytic disease (e.g., G6PD deficiency)
- Gestational age 35 to 36 weeks
- Previous sibling received phototherapy
- Cephalohematoma or significant bruising
- Exclusive breastfeeding, particularly if nursing poorly and weight loss is excessive
- East Asian race

Minor Risk Factors

- Pre-discharge TB or TcB in the high intermediate-risk zone
- Gestational age 37 to 38 weeks
- Jaundice observed before discharge
- Previous sibling with jaundice
- Macrosomic infant of diabetic mother
- Maternal age ≥25 years
- Male sex

Factors Associated With Decreased Risk of Significant Jaundice[19]

- TB or TcB in the low-risk zone (see Figs. 95.15 and 95.20)
- Gestational age ≥41 weeks
- Exclusive bottle feeding
- African American race
- Discharge from hospital after 72 hours

Refer to the 2022 AAP Clinical Practice Guideline for updated recommendations.[19]
DAT, Direct antiglobulin test; *G6PD*, glucose-6-phosphate dehydrogenase; *TB*, total serum/plasma bilirubin; *TcB*, transcutaneous bilirubin.
[a]Listed in order of decreasing importance.
From the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* 2004;114(1):297–316.

The risk factors for the development of severe hyperbilirubinemia in infants 35 weeks of gestation or more listed in the 2004 AAP Clinical Practice Guideline are shown in Box 95.3 (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]).

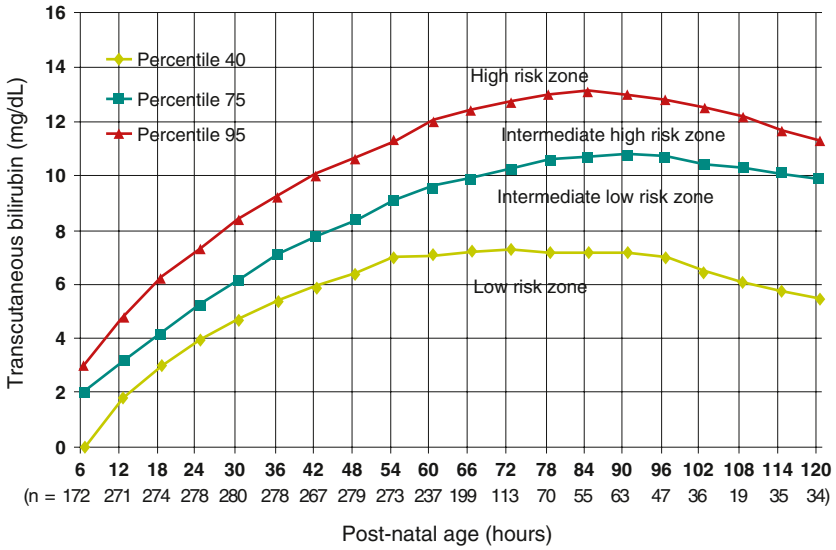
Another problem in the assessment of any particular TB concentration involves variation within and between laboratories. High interlaboratory and interinstrument variations in the measurements of TB were reported. It was found that imprecision for a specific method or a given laboratory was acceptable, but inaccuracy was highly variable and should be taken into account in the interpretation of all TB measurements. Another factor contributing to these variations is the lack of appropriate bilirubin standards and consistent handling of clinical specimens. All of these observations warrant the need for universal standardization of all bilirubin measurements. Lo and co-workers[77a] analyzed specimens of TB from the College of American Pathologists (CAP) Neonatal Bilirubin (NB) and Chemistry (C) surveys using the reference method. They found that the use of different methods in the NB and C surveys, together with the presence of nonhuman protein base and ditaurobilirubin in the survey specimens, accounted for wide variations in accuracy and imprecision between instruments and laboratories. Furthermore, they recommended that survey samples should consist only of human serum enriched with unconjugated bilirubin.

The measurement of unbound or "free" bilirubin concentrations is another potential measure to assess the risk for developing bilirubin neuropathy or encephalopathy. Data are limited, but preliminary studies show that unbound bilirubin levels may be better correlated with bilirubin neurotoxicity than TB.[2,78] In late preterm and term infants with severe jaundice, it has recently been demonstrated that unbound bilirubin is a more sensitive and specific predictor of auditory neuropathy spectrum disorder than TB or bilirubin-to-albumin molar ratio (BAMR).[78]

Transcutaneous Bilirubinometry

Historically, visual inspection has been the most commonly used means of screening newborns for hyperbilirubinemia. Jaundice progresses cephalocaudally. Digital pressure that blanches the skin diminishes the effects of pigmentation and local cutaneous perfusion and allows the detection of jaundice. Proper lighting is important in detecting subtle levels of jaundice. However, visual assessment is subjective, dependent on observer experience and is notoriously inaccurate.

TcB is a method using reflectance photometry or transcutaneous colorimetry as a noninvasive estimate of TB levels. The technique offers an objective measurement of skin color, from which a reading, reflecting the TB, is derived. The two devices most commonly used are the BiliChek (Philips Children's Medical Ventures, Monroeville, PA) and the Konica Minolta/AirShields JM-103 Jaundice Meter (Draeger Medical, Lubeck, Germany), but others are also commercially available. They differ in their ease of use and their propensity to be affected by variations in skin color. A number of studies have shown that these instruments provide fairly accurate estimates of TB in term and near-term newborn infants of varying races and ethnicities, generally providing values within 2 to 3 mg/dL (34–51 μmol/L) of the TB if the TB is less than 15 mg/dL (257 μmol/L). The technology tends to underestimate the actual TB level and should be regarded as a screening mechanism rather than an accurate reflection of the TB. The devices have been evaluated as potential pre-discharge screening tools to identify infants at risk (i.e., TB level at the 95th percentile or higher). As a result of these studies, hour-specific nomograms based on TcB measurements have been established for both term and late preterm infants.[2] It may be more prudent to plot TcB readings on a TcB nomogram than on a serum bilirubin-based nomogram such as that of Bhutani et al.[10c,11] However, additional studies are warranted to establish a strong and reliable correlation between TcB and TB at levels of 15 mg/dL (257 μmol/L) and higher, as well as during and after phototherapy and in premature or low birth weight infants, before its routine use can be advocated. The device has also



• **Fig. 95.22** Hour-specific transcutaneous bilirubin (*TcB*) nomogram constructed from 3303 measurements from 1059 neonates. The percentile values were divided into four groups following the pattern of the Bhutani hour-specific TB nomogram. (From Bromiker R, Goldberg A, Kaplan M. Israel TcB nomogram predicts significant hyperbilirubinemia. *J Perinatol.* 2017;37(12):1315–1318.)

been used successfully in community TcB screening.[79] Clinicians should be aware that there may be variation in results among devices,[80,81] as well as overestimation of results in African American neonates.[82] Akin to the TB-based nomogram, a recently developed TcB nomogram from Israel demonstrated a greater than 100-fold increase in neonates meeting requirements for phototherapy, from 0 in those whose highest TcB reading fell below the 40th percentile through 27/120 (22.5%) for the >95th percentile group (Fig. 95.22).[83]

TcB measurements should be used with caution in preterm newborns. In a meta-analysis of 22 published studies, Nagar et al.[84] found that TcB measurements correlated reasonably well with TB measurements in preterm infants. Results of subjects born at <32 weeks of gestation were similar to those of the entire preterm population. However, in a multisite cohort study of Canadian infants born from 24 to 35 completed weeks of gestation, TcB provided a reasonably accurate assessment of TB in those born between 33 and 35 weeks of gestation but demonstrated better agreement with TB in the age group of 33 to 35 weeks of gestation than among those born prior to 33 weeks of gestation.[85]

End-Tidal Carbon Monoxide Measurements

The breakdown of heme by the rate-limiting enzyme HO produces equimolar quantities of CO and biliverdin, the latter being reduced to bilirubin. Therefore the measurement of CO in the end-tidal breath, corrected for ambient CO to derive ETCOc, can be used as an index of in vivo heme degradation and bilirubin production and hence hemolysis.[3] Portable devices that provide automatic sampling and bedside analysis of ETCOc have yielded results comparable to gas chromatography and have been used to accurately estimate heme catabolism in neonates, children, and adults. A new device is currently available and has been used successfully.[86–88] The measurement of ETCOc reflects whether RBC breakdown is occurring at a physiologic or an accelerated rate and should be elevated in all cases of hemolysis, regardless of etiology. This is important when DAT screening is used to screen for hemolysis due to alloimmunity. A negative DAT will rule out alloimmune causes of hemolysis, but other non-immune-mediated causes of hemolysis may be ongoing but not detected.[23]

In a large multicenter study, a measurement of ETCOc at 30 ± 6 hours of age, alone or in combination with a TB measurement, did not improve the predictive ability of the age-in-hours–specific TB level, although it was as good as a measurement of TB alone for this purpose. The study did find, however, that the use of an ETCOc measurement in combination with the TB level aids in the following: (1) discrimination between infants with increased bilirubin production rates and infants with decreased elimination, (2) identification of infants with increased bilirubin production due to ABO incompatibility or other causes of hemolysis, and (3) identification of infants with impaired conjugation defects who have a normal ETCOc level in the face of rising TB levels. In other words, high producers of CO and bilirubin are most likely undergoing a hemolytic process, whereas infants with high TB levels and normal bilirubin production rates most likely have a defect in bilirubin conjugation. The measurement of ETCOc could provide direct information of the rate of bilirubin production. Bhutani and co-workers[88a] in 2001, using data from this study, constructed an age-in-hours–specific ETCOc nomogram for measurements taken between 4 and 48 hours' postnatal age, revealing that an ETCOc value at the 75th percentile at 30 ± 6 hours of age is 1.7 ppm or less and is considered the threshold of increased bilirubin production. The importance of determining ETCOc levels and their application in identifying infants at risk for developing hyperbilirubinemia associated with hemolysis has been reviewed.

Christensen et al.[87] found that ETCOc reference intervals were higher in healthy neonates during the first postnatal week (5th–95th percentile; reference range, 1.4–1.7 ppm) than after 7 days (when all values were ≤1.0 ppm). In their clinical practice, these authors utilized the cutoff of 2.0 ppm to define hemolysis in neonates during the first week of life.

Bilirubin to Albumin Molar Ratio

The molar ratio of bilirubin (mg/dL) to albumin (g/dL) correlates with unconjugated bilirubin levels in newborns and therefore can be used as a surrogate for unbound bilirubin or residual binding capacity of albumin. The serum albumin concentration is inherently low in preterm infants, and, in addition, albumin levels and the ability of albumin to bind bilirubin vary significantly in sick infants. It has also been shown that binding increases with increasing gestational and postnatal age. The BAMR may be used as an adjunct to measurements of TB in determining the need for exchange transfusion. A serum albumin level <3.0 g/dL (4.4 µmol/L) is suggested as a risk factor for bilirubin neurotoxicity and can be used as an option for lowering the indication for commencing phototherapy.[11] The AAP has suggested the BAMR can be used together with, but not in lieu of, the TB level as an additional factor to be considered in determining the need for exchange transfusion (Table 95.4). However, use of the BAMR in addition to TB for the management of hyperbilirubinemia in a preterm population did not improve long-term neurodevelopmental outcomes when compared with controls in whom TB alone was used.[89] In an Egyptian study of severely hyperbilirubinemic neonates, both TB and BAMR were strong predictors of neurotoxicity, although BAMR did not improve prediction over TB alone.[90] In a study of paired TB and albumin levels in preterm and term neonates, both gestational and postnatal age influenced TB, albumin, and BAMRs. Furthermore, hypoalbuminemia and extreme BAMRs were associated with an increased risk of death.[91]

TABLE 95.4 Bilirubin to Albumin Molar Ratio (BAMR) as a Determinant of the Need for Exchange Transfusion

Risk Category	BAMR at Which Exchange Transfusion Should Be Considered: TB (mg/dL)/Albumin (g/dL)	BAMR at Which Exchange Transfusion Should Be Considered: TB (µmol/L)/Albumin (µmol/L)
Infants ≥38 0/7 wks	8.0	0.94
Infant 35 0/7 to 37 6/7 wks and well or ≥38 0/7 wks if higher risk or isoimmune hemolytic disease or G6PD deficiency	7.2	0.84
Infant 35 0/7 to 37 6/7 wks if higher risk or isoimmune hemolytic disease or G6PD deficiency	6.8	0.80

Refer to the 2022 AAP Clinical Practice Guideline for updated recommendations.[19]
If TB is at or is approaching the exchange level, send blood for immediate type and cross-match. Blood for exchange transfusion is modified whole blood (red blood cells and plasma) cross-matched against the mother and compatible with the infant.
G6PD, Glucose-6-phosphate dehydrogenase; *TB*, total serum/plasma bilirubin.
From the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114(1):297–316.

Newer Methods of Diagnosis

In the past, the etiologies of many cases of nonimmune hemolysis were unidentified and termed idiopathic. Modern methods of diagnosis, including flow cytometry–based eosin-5-maleimide uptake to identify RBC membrane defects and next-generation sequencing (NGS)–based panels to identify mutations responsible for hemolysis, may shed light on specific hemolytic conditions, the etiology of which may have remained unknown.[2] It has been advised to use this methodology only if simpler and less expensive technologies have failed to arrive at a diagnosis. One such technique includes studying RBC morphology, which may lead to diagnoses of spherocytosis, elliptocytosis, bite and blister cells, echinocytes, and schistocytes.[92]

Next-Generation Sequencing

NGS is a technology for genome sequencing at high speed and low cost. Simultaneous sequencing of thousands to millions of DNA molecules with the capability of sequencing multiple individuals simultaneously is possible. An accurate diagnosis is all-important in our understanding of the hyperbilirubinemic disease process, in guiding the clinical management, and in facilitating genetic counseling. Christensen and colleagues[93] reported that in neonates with TB values greater than 30 mg/dL (513 µmol/L), a clinical diagnosis was not identified in 66% of newborns. In contrast, NGS of DNA utilizing a panel of 27 genes known to be associated with neonatal hyperbilirubinemia determined an etiology, including hereditary spherocytosis, (some combined with ABO hemolytic disease), pyruvate kinase deficiency, severe G6PD deficiency, and ABO hemolytic disease, for hyperbilirubinemia in each of 10 neonates studied. In a subsequent study of seven Utah-based neonates with ABE, the same group determined a diagnosis using this genetic technology for all.[94] These reports contrast with the high number of newborns labeled as idiopathic in the US Kernicterus Registry, as well as in an earlier report from Utah. A study is currently underway in which NGS will be used to study the genetic etiology of 100 US cases of ABE.

Therapy for Unconjugated Hyperbilirubinemia

The current mainstay of treatment is phototherapy, which has been proved to be instrumental in containing the rate of rise and lowering the TB. In cases of failure of phototherapy,

or in newborns presenting with extremely high TB concentrations, exchange transfusion will definitively lower the TB to a level that will no longer be of danger to the neonate. IVIG and phenobarbital therapy also have a role in the management of hyperbilirubinemia. The intensity and invasiveness of therapy are determined by the many factors discussed thus far, including the gestational age and relative health of the neonate, the current level of TB, and an estimation of the rate of rise in view of the dynamic nature of bilirubin metabolism in the newborn. An example of a clinical pathway for the management of the newborn infant readmitted for phototherapy or exchange transfusion is given in Box 95.4.

Indications for Therapy

Guidelines for the management and prevention of neonatal hyperbilirubinemia have been formulated in many countries, including the United States, Canada, Norway, South Africa, and Israel, among others. There is some evidence that guidelines complemented by statewide learning collaboratives are instrumental in decreasing the rates of extreme hyperbilirubinemia and exchange transfusion. In California, from 2007 to 2012 (3 years after publication of the 2004 AAP Clinical Practice Guideline) the rates of TB ≥25 mg/dL and exchange transfusion both decreased significantly. A similar report emanated from Canada, where national guidelines were published in 2007. In the period prior to 2007, neonates were 3.5 times more likely to develop a TB ≥25 mg/dL than in the period from 2011 to 2013. This improvement was attributed to introduction of the Canadian Pediatric Society guidelines and improved physician awareness of severe neonatal hyperbilirubinemia.[95] A significant decrease in the number of exchange transfusions was reported from New South Wales, Australia, following publication of guidelines related to this procedure.[96]

These guidelines were derived from consensus and were not evidence based. In surveys from California, both bilirubin-induced neurotoxicity and sensorineural hearing loss occurred at TB levels well above the 2004 AAP recommendations for exchange transfusion[63,97] (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Cerebral palsy consistent with kernicterus was limited to infants with TB levels >5.0 mg/dL (85.5 μmol/L) above exchange transfusion indications and at least two risk factors for neurotoxicity, including prematurity, G6PD deficiency, and hypoxic-ischemia.[98] It has been suggested that, as kernicterus is rare and occurs only at very high TB levels (>35 mg/dL [599 μmol/L]), previously

• BOX 95.4 Examples of Management of the Newborn Infant Readmitted for Phototherapy or Exchange Transfusion

Treatment

- Use intensive phototherapy (Fig. 95.27) and/or exchange transfusion (Fig. 95.28).

Laboratory Tests

- TB and direct bilirubin level
- Blood type (ABO, Rh)
- Direct antiglobulin test (Coombs' test)
- Serum albumin
- CBC with differential and peripheral blood smear for RBC morphology
- Reticulocyte count
- ETCOc (if device available)
- G6PD screen, if indicated by ethnicity or geographic origin or if poor response to phototherapy
- Urinalysis for reducing substances
- If history or presentation suggests sepsis, perform blood culture, urine culture, and CSF examination for protein, glucose, cell count, and culture.

Interventions

- If TB is ≥25 mg/dL (428 μmol/L) or ≥20 mg/dL (342 μmol/L) in a sick infant or infant <38 weeks of gestation, obtain a type and cross-match, and request blood in case exchange transfusion becomes necessary.
- In infants with isoimmune hemolytic disease and a rising TB despite intensive phototherapy or rising to within 2 to 3 mg/dL (34–51 μmol/dL) of exchange level (see Fig. 95.28), administer IVIG (500–1000 mg/kg) over 2 hours and repeat if necessary.
- If infant's weight loss from birth is greater than 12% or there is clinical or biochemical evidence of dehydration, recommend formula or expressed breast milk. If oral intake is in question, give IV fluids.

For Infants Receiving Intensive Phototherapy

- Breastfeed or bottle feed (formula or expressed breast milk) every 2 to 3 hours.
- If TB ≥25 mg/dL (428 μmol/L), repeat TB within 2 to 3 hours.
- If TB = 20 to 25 mg/dL (342–428 μmol/L), repeat TB within 3 to 4 hours.
- If TB <20 mg/dL (342 μmol/L), repeat TB in 4 to 6 hours.
- If TB continues to fall, repeat TB in 8 to 12 hours.
- If TB is not decreasing, or is moving closer to the level for exchange transfusion or if the BAMR exceeds levels shown in Table 95.4, consider exchange transfusion.
- When TB is <13 to 14 mg/dL (222–239 μmol/L), discontinue phototherapy.
- Depending on the cause of the hyperbilirubinemia, it is an option to measure TB 24 hours after discharge to check for rebound hyperbilirubinemia.

Refer to the 2022 AAP Clinical Practice Guideline for updated recommendations.[19]
BAMR, Bilirubin-to-albumin molar ratio; *CBC*, complete blood count; *CSF*, cerebral spinal fluid; *ETCOc*, end-tidal carbon monoxide, corrected for inhaled CO; *G6PD*, glucose-6-phosphate dehydrogenase; *IV*, intravenous; *IVIG*, intravenous immunoglobulin; *RBC*, red blood cell; *TB*, total serum/plasma bilirubin.
From the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* 2004;114(1):297–316.

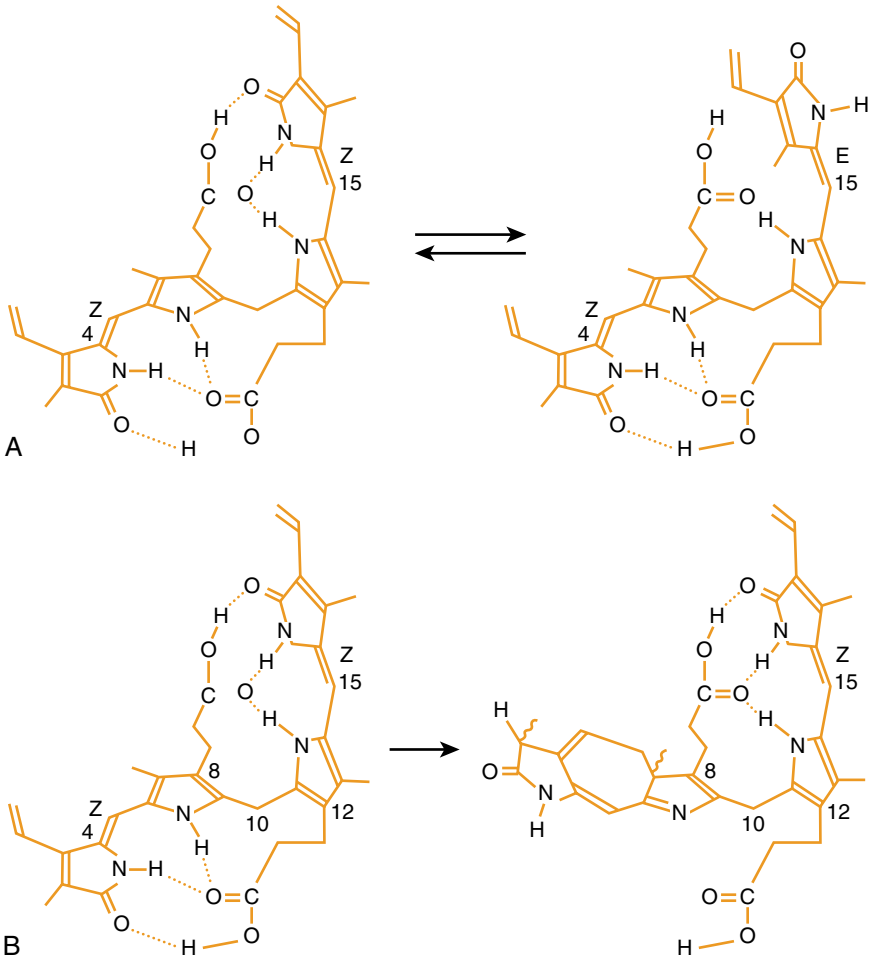
recommended phototherapy and exchange transfusion treatment thresholds may be unnecessarily aggressive.[99] Similarly, Maisels asked that, if 3000 newborns need to receive phototherapy to prevent one exchange transfusion, could phototherapy have been avoided in many of these neonates?[100]

Phototherapy

Phototherapy is the most widely used form of therapy for the treatment and prophylaxis of neonatal unconjugated hyperbilirubinemia. In nearly all neonates, phototherapy reduces or blunts the rise of TB levels (regardless of maturity, the presence or absence of hemolysis, and the degree of skin pigmentation). Extreme hemolysis, however, especially when combined with immature conjugation, may result in phototherapy failure. Given the decades of experience with its use worldwide and the lack of reported serious long-term side effects thus far, short-term phototherapy appears to be safe. The initial report from the Collaborative Study on the Effectiveness and Safety of Phototherapy undertaken under the auspices of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) demonstrated that neonates receiving phototherapy require significantly fewer exchange transfusions. More important, these infants had an incidence of gross kernicterus and subsequent neurobehavioral performance that was lower than that for those who received only exchange transfusions to control hyperbilirubinemia. Furthermore, subsequent follow-up studies revealed no adverse outcome in the infants who received phototherapy in the neonatal period.

Mechanism of Action

Three independent mechanisms have been proposed to explain the action of phototherapy in reducing TB levels in neonates. The first and most important pathway is geometric photoisomerization of the native unconjugated bilirubin IX-α (Fig. 95.23). Unconjugated bilirubin IX-α is normally in the *4Z,15Z* configuration. In this form, the –COOH group of each carboxymethyl side-chain interacts through three hydrogen bonds with the C=O and N-H groups of the pyrrole rings in the opposite half-molecule. As a result, ionization of the two –COOH and two keto groups is

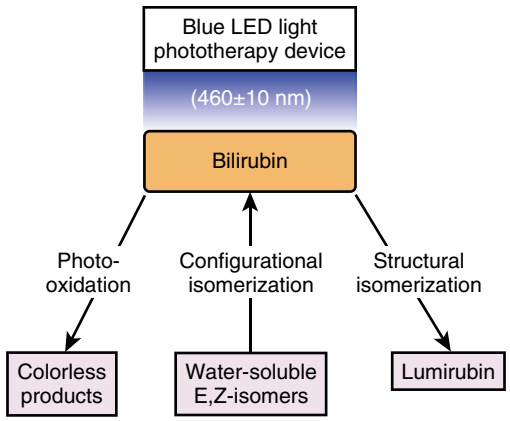


• **Fig. 95.23** Isomerization pathways for bilirubin during phototherapy. (A) Z-E carbon double-bond configurational isomerization of bilirubin. (B) Intramolecular cyclization of bilirubin to form lumirubin. (Modified from McDonagh AF, Lightner DA. "Like a shrivelled blood orange": bilirubin, jaundice, and phototherapy. *Pediatrics*. 1985;75(3):443–455. Used with permission of the American Academy of Pediatrics.)

inhibited, making the molecule nonpolar and water insoluble. When illuminated by wavelengths (peak, 460 ± 10 nm), light is absorbed by bilirubin, and unconjugated bilirubin undergoes *Z* to *E* (configurational) isomerization at either one or both of the bridge double bonds to yield potentially three isomers: *4E,15Z*; *4Z,15E*; and *4E,15E*. The *E* configuration spatially precludes hydrogen bonding of the molecule which therefore remains open or unfolded and free to ionize. Thus the *E* isomer is more polar and soluble than the *Z* isomer. Because the liver can transport only soluble bilirubin into bile, the *E* isomers can be excreted without the need for conjugation.

At least two pairs of geometric photoisomers have been identified in vivo. The first pair, photoisomers IA and IB, is presumably the two possible *E,Z* isomers. The second pair, photobilirubins IIA and IIB, is most likely two rotamers of the *4E,15E* isomer. Both pairs are presumably formed rapidly in the skin, subcutaneous tissue, and capillaries. Being more polar, all of these isomers partition into the plasma, continuously shifting the equilibrium to promote more isomer formation. These isomers are rapidly taken up by the liver and transported into bile, where they are destabilized by bile acids, rapidly reverting to native unconjugated bilirubin IX-α in the bile ducts and intestine. The rotameric isomers remain mostly intact and are the major polar photoproducts found in bile. This photoisomerization pathway may be responsible for more than 80% of the augmented bilirubin elimination during phototherapy. Geometric photoisomerization is not, however, the only isomerization pathway open to photoexcited bilirubin. The proximity of the side-chain double bond at C-3 to the adjacent pyrrole ring allows an intramolecular cyclization, again rendering the bilirubin molecule more polar (see Fig. 95.24). This structural isomer of bilirubin is referred to as *lumirubin*, and it is also excreted into bile without need for hepatic conjugation. Lumirubin, a soluble compound, is the major photoproduct excreted with the bile and urine, and its conversion is the rate-limiting step in the elimination of bilirubin by phototherapy.

The third pathway of phototherapy involves a variety of bilirubin oxidation reactions, resulting from an autosensitized reaction involving singlet oxygen. The products formed by these reactions are multiple but include biliverdin, dipyrroles, and monopyrroles. Many of these products are colorless, nonreactive in the van den Bergh test, and presumably excreted by the liver and kidney without need for conjugation. Compared with the photoisomerization pathway, the oxidation mechanism appears to play a very minor role in photoalteration of unconjugated bilirubin in vivo (Fig. 95.24).



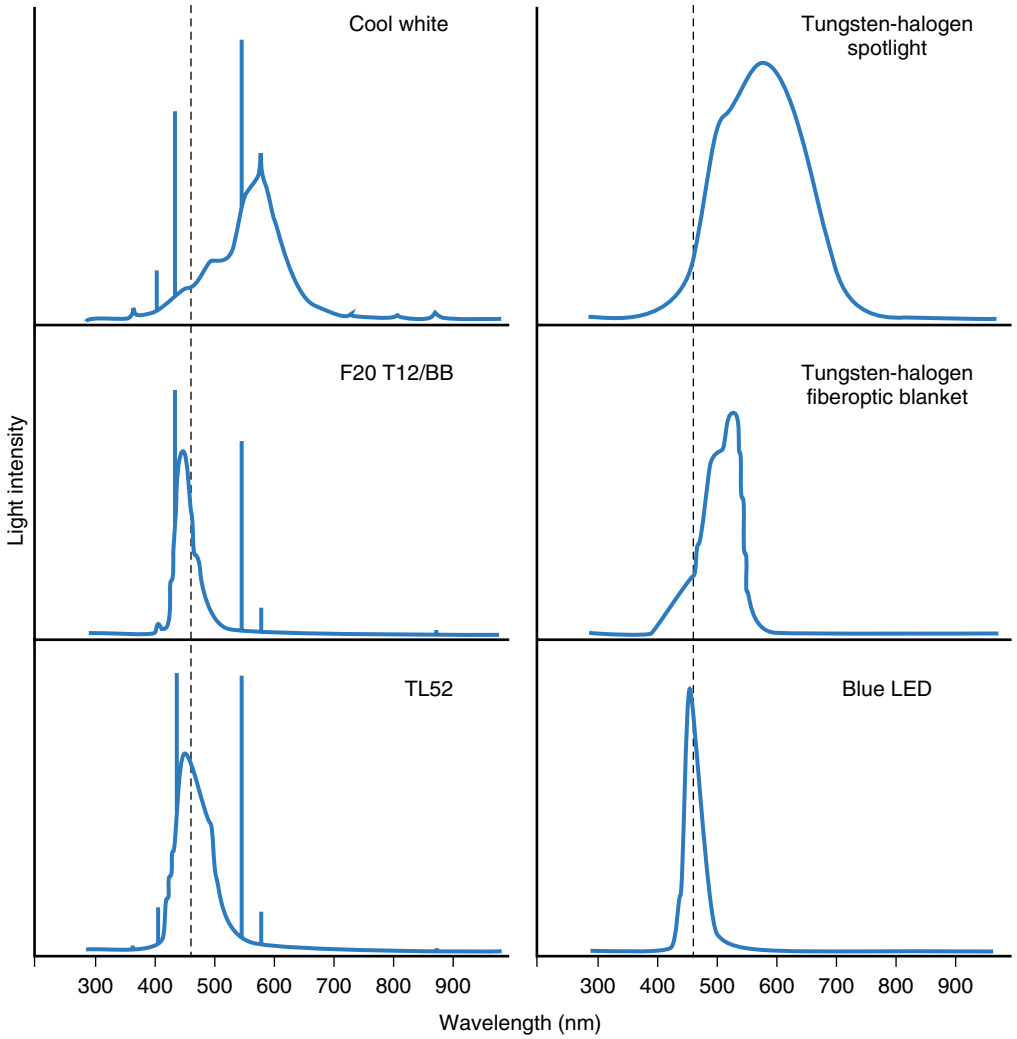
• **Fig. 95.24** The major mechanisms of bilirubin photoalteration. *LED*, Light-emitting diode. (Redrawn from Vreman HJ, Wong RJ, Stevenson DK. Light emitting diodes for phototherapy for the control of jaundice. In Holick M, ed. *Biology of light 2001: Proceedings of a symposium.* Boston: Kluwer Academic; 2002:355–367.)

Technique

Phototherapy is not a standardized practice in the United States at this time, but there exist many different devices capable of delivering phototherapy with varying efficacies. Any physician using one of these devices must be cognizant of the variables influencing the efficacy of phototherapy and ensure that the device is used appropriately. The efficacy of phototherapy units varies widely because of differences in light source and configuration and depends on emission of light in the blue-to-blue-green range that overlaps the in vivo bilirubin absorption spectrum (~460–490 nm), irradiance of at least 30 μW/cm^2/nm, illumination of maximal body surface area, and demonstration of a decrease in TB levels during the first 4 to 6 hours of exposure.[101]

The first variable to consider is the wavelength of light used to induce photoisomerization (Fig. 95.25). Bilirubin absorbs light maximally in the blue range (340–540 nm), with peak absorption for albumin-bound bilirubin at about 460 nm and for unbound bilirubin at about 440 nm. Daylight and cool white lamps have a spectral emission of 380 to 720 nm with a peak of 578 ± 10 nm and are less effective than blue lamps (F20 T12/B), which have a narrower spectral range and peak between 420 and 480 nm. Special blue lamps (F20 T12/BB and TL52 tubes; Philips, Amsterdam, the Netherlands) emit narrower spectra of light with greater irradiance at the main therapeutic spectrum and have been shown to be most effective. The blue hue that is produced by these lamps can interfere with skin color assessment in jaundiced neonates, and it has been reported to produce dizziness and nausea variably in those caring for these patients. These side effects may be readily tempered by the addition of daylight fluorescent lamps to the phototherapy unit. However, incorporation of white fluorescence "dilutes" the blue intensity, thereby dramatically decreasing the effectiveness of phototherapy.

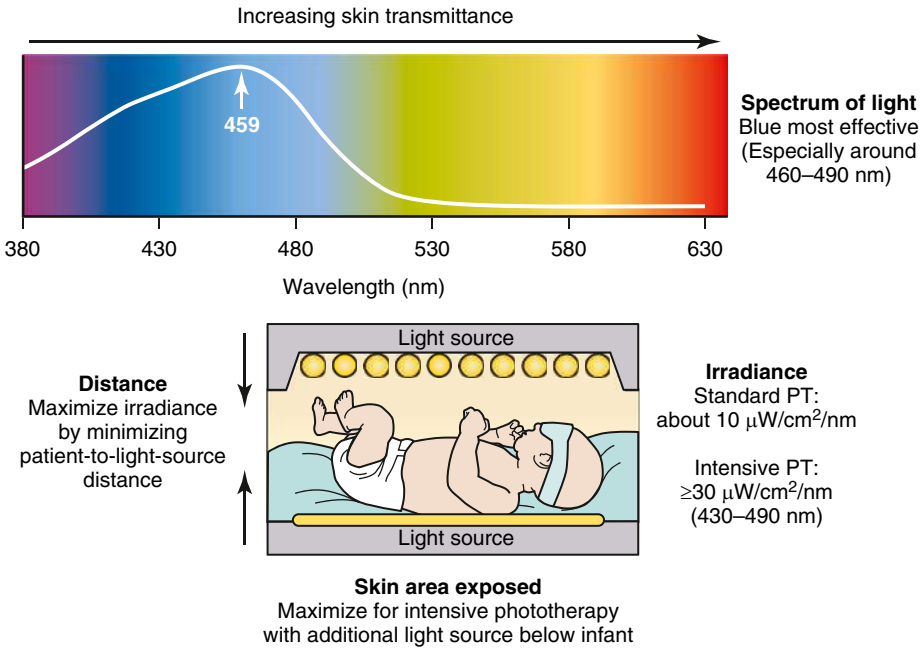
Several studies have shown that phototherapy with blue-green to green light (peak at 525 nm) is as effective as that with blue light and better than white light in reducing bilirubin levels.[102,103] Green light lacks the untoward side effects often associated with intense blue light. However, further study is needed to definitively determine the clinical benefit of green light, because green light phototherapy has not been widely adopted.



• **Fig. 95.25** Emission spectra of phototherapy devices. The intensities are shown on a linear relative scale. The spectra of the three fluorescent lamps (cool white and special blue; F20 T12/BB and TL52), tungsten-halogen (spotlight [Mini Bili-Lite, Olympic Medical, Seattle, WA] and fiberoptic blanket [Biliblanket, Ohmeda, Columbia, OH]), and blue light-emitting diodes (*LEDs*; neoBLUE [Natus Medical, San Carlos, CA]) were measured under identical conditions on an S-2000 spectrophotometer (Ocean Optics, Inc., Dunedin, FL). The dotted line represents the peak absorption of bilirubin at about 460 nm. (From Vreman HJ, Wong RJ, Murdock JR, Stevenson DK. In vitro efficacy of an LED-based phototherapy device [neoBLUE] compared to traditional light sources. *Pediatr Res*. 2003;53:400A [#2895].)

The second variable that influences the efficacy of phototherapy is energy output or irradiance as measured in units of microwatts per square centimeter per nanometer (μW/cm^2/nm). Effective phototherapy must provide irradiance well above the levels that have been determined to be minimally effective in producing bilirubin degradation while not exceeding levels beyond which no significant increases in response are evident. This also helps avoid potential side effects such as elevation in body temperature. A standard phototherapy unit operating under optimal conditions would provide clinically significant, but minimally effective, levels of phototherapy (about 6–12 μW/cm^2/nm). In intensive phototherapy, irradiance is increased to 30 μW/cm^2/nm or greater.[11] Standard phototherapy lamps are normally positioned within 40 cm from the infant, but, should more intensive phototherapy be required, the lamps may be placed within 15 to 20 cm of the patient, provided blue fluorescent or light-emitting diode (LED) lamps are used rather than heat-producing halogen lamps with a potential danger of causing thermal injury. Conversely, increasing the distance from the lamp to the skin surface of the neonate results in a theoretical diminution of light energy by a factor equal to the square of the increase in the distance (Fig. 95.26).[2]

Skin pigmentation does not reduce the effectiveness of phototherapy. With use, fluorescent lamp energy output declines to a degree that varies from one type of lamp to another. A meter for monitoring lamp energy output should be used to ensure optimal treatment. Although commercially available photometers have been found to vary in their absolute measurement of irradiance because of differences in meter sensitivity (i.e., peak and range) and the emission



• **Fig. 95.26** Important factors in the efficacy of phototherapy (*PT*). The absorbance spectrum of bilirubin bound to human serum albumin (*white line*) is shown superimposed on the spectrum of visible light. Clearly, blue light is most effective for phototherapy, but, because the transmittance of skin increases with increasing wavelength, the best wavelengths to use are probably in the range of 460 to 490 nm. Term and near-term infants should be treated in a bassinet, not an incubator, to allow the light source to be brought to within 10 to 15 cm of the infant (except when halogen or tungsten lights are used), increasing irradiance and efficacy. For intensive phototherapy, an auxiliary light source (fiberoptic pad, blue light-emitting diode mattress, or special blue fluorescent tubes) can be placed below the infant or bassinet. If the infant is in an incubator, the light rays should be perpendicular to the surface of the incubator in order to minimize loss of efficacy due to reflectance. (From Maisels MJ, McDonagh AF. Phototherapy for neonatal jaundice. *N Engl J Med*. 2008;358(9):920–928.)

characteristics of their light sources, they are still useful in determining relative lamp decay. Lamps that have lost more than 20% of the acceptable output should be replaced.

All lamps should be housed behind Plexiglas to reduce the danger to the patient should a lamp explode. Also, it has been shown that Plexiglas provides protection from any harmful ultraviolet irradiation arising from the available light sources. Patients may be cared for on an open warmer or in a crib. Use of a closed incubator may increase the distance of the light source from the infant, thereby attenuating the irradiance delivered to the patient.

The third variable affecting the efficacy of phototherapy is the body surface area of the neonate exposed to light. Ideally, neonates should be naked when under phototherapy; however, use of diapers, folded back to cover as little of the infant's surface as practically possible, may improve on the obvious disadvantages of leaving the infant unclothed. Positioning of several phototherapy units around the newborn or placing the infant on a phototherapy mattress in addition to the overhead lights, may increase exposure. A white sheet draped around the periphery of the bed may also act to reflect light onto relatively under-exposed areas, thereby increasing the overall light irradiance.

Other conventionally used devices incorporate tungsten-halogen lamps as their light source for use as spotlights. Another technique involves transmission of light through a fiberoptic bundle to a pad or blanket around the infant. The advantage of the latter technology is that the source of light and therefore heat will be at a distance from the infant. In infants with severe hyperbilirubinemia, the previously mentioned techniques can be used in combination to increase light intensity (irradiance) and body surface area exposure. Too frequently, currently used doses of phototherapy are well below the optimal therapeutic range. The AAP recommends the use of intensive phototherapy, especially for infants readmitted for hyperbilirubinemia or if the threshold for exchange transfusion is approaching. Intensive phototherapy implies the use of high levels of irradiance in the therapeutic range (usually ≥30 μW/cm²/nm) delivered to as much of the infant's surface area as possible[11] (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]).

Use of arrays of blue LEDs, which can deliver high-intensity, narrow-band light in the absorption spectrum of bilirubin, has nearly replaced fluorescent tube–based devices. This technology has been shown to be an effective and safe alternative to other modes of phototherapy. LED light sources are now available in many configurations, including mattresses, side panels, blankets, and overhead lights. Advantages of the LED technology include the ability to increase the light intensity to a level higher than that of many conventional technologies without significantly warming the

baby, although hypothermia has been observed in naked, term neonates.[104] The lamps remain effective without the need for replacement.[105]

Clinical studies comparing intermittent to continuous phototherapy have yielded conflicting results. Several studies failed to show effectiveness of the intermittent therapy. These results may have resulted from prolonged light-on and light-off cycles—for example, 6- to 12-hour on-off schedules. Photoisomerization of bilirubin occurs primarily in skin layers, and the restoration of the bilirubin pool in the skin takes about 1 to 3 hours. Thus a prolonged on-off schedule may not be as effective as continuous therapy, but an on-off cycle of less than 1 hour is apparently as effective as continuous treatment. Phototherapy lights should be shut off and eye patches removed during feeding and family visiting for up to 1 hour, because this does not significantly reduce phototherapy effectiveness.

Some reports have demonstrated that home phototherapy may be an effective and safe alternative to prolonged hospitalization for healthy full-term neonates with jaundice. Clear advantages of home-centered phototherapy include (1) reduced cost, (2) avoidance of parent-infant separation, and (3) parental satisfaction. However, complications of home phototherapy that might result from inadequate nursing supervision include corneal abrasion, eye patch misuse, excessive weight loss, temperature derangement, and ineffective bilirubin reduction. Whether there is any valid indication for phototherapy for those neonates who could be safely managed at home is questionable. Those with valid indications are generally too sick, too small, or too close to the exchange transfusion level to be safely treated at home. The AAP Committee on Fetus and Newborn has not endorsed home phototherapy, but it has issued a strict guideline for its use.[11,101] Because the devices available for home phototherapy may not provide the same degree of irradiance or surface-area exposure as those available in hospital, the AAP Subcommittee on Hyperbilirubinemia recommends that home phototherapy should be used only in infants whose bilirubin levels are in the "optimal phototherapy" range (see Indications).[11] It is not appropriate for infants with higher bilirubin concentrations or if the TB is approaching the exchange transfusion level. As with hospitalized infants, the TB must be monitored regularly.

Indications

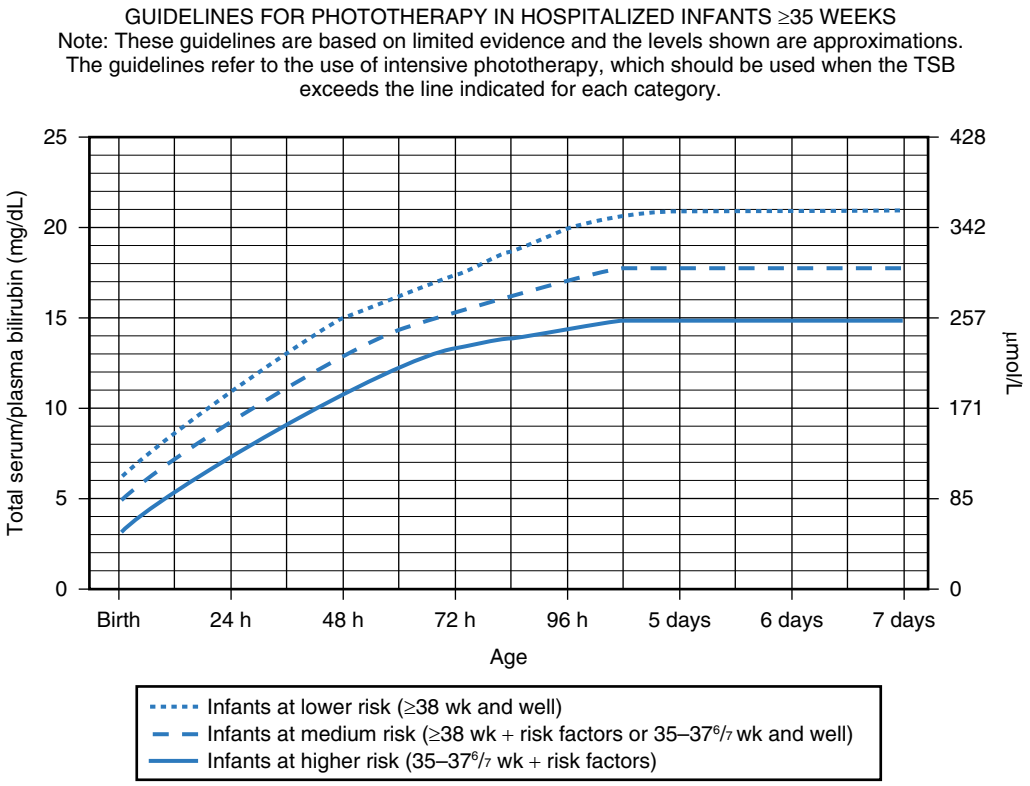
Although the guideline is not "evidence-based," the AAP in 2004 issued a comprehensive guideline for the commencement of phototherapy in infants greater than 35 weeks of gestation (Fig. 95.27)[11] (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). This guideline takes into account the presence of risk factors as well as late prematurity and also is adapted to the dynamic changes in TB levels during the first days of life. Thus there is no single TB level at which phototherapy should be commenced, but each newborn must be assessed individually, taking into consideration postnatal age, gestational age, and other risk factors. The TB level at which phototherapy may be discontinued must also be decided in view of these factors. It may be useful to continue plotting the TB concentrations on the hour-specific bilirubin nomogram during phototherapy and discontinue phototherapy when the TB has decreased below the 75th percentile for hours of age, or even closer to the 40th percentile in infants with lower gestational age or in the presence of risk factors. Chang et al.[106] recently proposed a formula by which rebound hyperbilirubinemia can be predicted by an infant's gestational age, age at initiation of phototherapy, and relative TB at phototherapy termination: Score = 15 (if gestational age <38 weeks) – 7 × (age in days at phototherapy initiation) – 4 × (AAP phototherapy threshold – TB at phototherapy termination) + 50. With a prediction score of ≤20, phototherapy can be discontinued with <4% probability of rebound. Follow-up for rebound, not necessarily requiring continued hospitalization, should be performed in cases of newborns younger than 37 weeks of gestation, those with positive DAT, and those treated at or before 72 hours' postnatal age. Phototherapy should be used during transport of hyperbilirubinemic newborns to centers for exchange transfusion.[107]

Wickremasinghe et al.[108] studied the effectiveness of subthreshold phototherapy (administration of phototherapy at TB levels below 2004 AAP indications) during birth hospitalization in preventing readmissions for phototherapy (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). They found a decreased rate of readmission for phototherapy among those in whom phototherapy had been started below the threshold, but many newborns had to be unnecessarily treated for each readmission prevented.[108]

The 2004 AAP Clinical Practice Guideline does not extend to premature infants younger than 35 weeks of gestation.[11] Previously, guidelines suggested by Maisels and Watchko[108a] were used. These suggest a wide range of indications for phototherapy and exchange transfusion, offering options based on gestational age and birth weight. Guidelines published by the National Institute for Health and Clinical Excellence (NICE) include graphs incorporating indications for phototherapy and exchange transfusion for each week of gestational age, starting at 23 weeks. TB values can be plotted directly on the graphs (available at http://www.nice.org.uk/CG98). A revised guideline (albeit one that is not evidence based) for the management of hyperbilirubinemia in preterm infants was formulated in the United States by neonatologists who were involved in the preparations of the 2004 AAP Clinical Practice Guideline,[11] the 2009 clarification,[109] or both (Table 95.5).[110] Additional guidelines for use in premature infants have been published from Norway, Holland, and South Africa.[111]

Aggressive Versus Conservative Phototherapy in Premature Infants

In an NICHD study,[111a] 1974 premature infants were randomized to aggressive (earlier timing) or conservative phototherapy. Two subgroups of infants, birth weights 501 to 750 g and 751 to 1000 g, were randomized at 12 to



- Use total bilirubin (TB). Do not subtract direct reacting or conjugated bilirubin.
- Risk factors = isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis, or albumin <3.0 g/dL (if measured).
- For well infants 35–37⁶/₇ wk, can adjust TB levels for intervention around the medium risk line. It is an option to intervene at lower TB levels for infants closer to 35 wk and at higher TB levels for those closer to 37⁶/₇ wk.
- It is an option to provide conventional phototherapy in hospital or at home at TB levels 2–3 mg/dL (35–50 μmol/L) below those shown, but home phototherapy should not be used in any infant with risk factors.

• **Fig. 95.27** The 2004 AAP Clinical Practice Guideline for phototherapy in hospitalized infants ≥35 weeks of gestation. Infants are designated as higher risk because of the potential negative effects of the conditions listed on albumin binding of bilirubin, the blood-brain barrier, and the susceptibility of the brain cells to damage by bilirubin. (Adapted from the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114(1):297–316.)

TABLE 95.5 Suggested Guidelines for Initiating Phototherapy or Exchange Transfusion in Premature Infants

	TB, Phototherapy		TB, Exchange Transfusion	
Gestational Age (wk)	mg/dL	μmol/L	mg/dL	μmol/L
<28 0/7	5–6	86–103	11–14	188–239
28 0/7–29 6/7	6–8	103–137	12–14	205–239
30 0/7–31 6/7	8–10	137–171	13–16	222–274
32 0/7–33 6/7	10–12	171–205	15–18	257–308
34 0/7–34 6/7	12–14	205–239	17–19	291–325

TB, Total serum/plasma bilirubin.
Adapted from Maisels MJ, Watchko JF, Bhutani VK, Stevenson DK. An approach to the management of hyperbilirubinemia in the preterm infant less than 35 weeks' gestation. *J Perinatol*. 2012;32(9):660–664.

36 hours to aggressive phototherapy (immediately after enrollment) or conservative phototherapy (started at 8.0 mg/dL [137 μmol/L] in the 501 to 750 g birth weight subgroup and 10.0 mg/dL [171 μmol/L] in the 751 to 1000 g subgroup). Aggressive phototherapy significantly reduced the mean peak TB, thereby confirming the efficacy of phototherapy in decreasing TB in the 1000 g or less birth weight group. In only seven infants were the criteria for exchange transfusion met. There was no difference (52% vs. 55%) in the rate of the primary outcome (a composite of death or neurodevelopmental impairment [NDI], including blindness, severe hearing loss, moderate or severe cerebral palsy, or a Mental or Psychomotor Developmental Index [Bayley] <70). However, aggressive phototherapy was instrumental in reducing the rate of NDI when analyzed alone (26% vs. 30%). This apparently was offset, however, by a 5% increase in mortality (39% vs. 34%) in the very smallest infants (501–750 g birth weight) that was attributable to phototherapy. Although aggressive phototherapy may be appropriate for relatively larger babies, these results emphasize the potential for phototherapy-related adverse effects. Aggressive phototherapy should be administered with caution in those less than 750 g birth weight. Suggested possible strategies for delivering safer but effective phototherapy to this premature very low birth weight group include reduced initial irradiance with a subsequent increase should the TB increase[112] and the use of narrow-spectrum LEDs. Cycled phototherapy was recently addressed by Arnold et al.[113] to determine whether TB levels could be controlled while reducing phototherapy exposure to potentially avoid increased mortality with continuous phototherapy among extremely low birth weight newborns. Cycled phototherapy (≥15 min/h titrated to an infant's TB level) compared with continuous phototherapy substantially decreased mean phototherapy hours (34 vs. 72) with a minimally BAER mean wave V latency (7.42 vs. 7.32 ms) and, most importantly, fewer deaths (10.2% vs. 13.4%). The authors suggested the need for a large, randomized trial to assess whether cycled phototherapy would increase survival and survival without impairment in small, preterm infants.

Complications

Several potential complications may occur with the use of phototherapy. The effect of high-intensity light exposure on the eyes of human neonates is uncertain, but animal studies indicate that retinal degeneration may occur after several days of continuous exposure. It is essential therefore that the eyes of all newborns exposed to phototherapy be covered with sufficient layers of opaque material to guard against the possibility of damage. The use of fiberoptic phototherapy does not eliminate the need to cover an infant's eyes.

Phototherapy may produce an increase in body and environmental temperature. Fluid balance, especially in the premature neonate, is a key management issue in patients being treated with phototherapy. There is increased insensible and intestinal water loss during phototherapy, which must be compensated for by an increase of about 25% above the estimated fluid need without phototherapy. In addition, stools may be slightly looser and more frequent. Fiberoptic phototherapy appears to result in lower insensible water losses and therefore slightly less need for increased maintenance fluids (see Chapter 96). Blue LED-based devices, however, do not release a significant amount of heat, and their use results in less insensible water loss. Term infants treated with blue LED light in open cribs should have their body temperatures monitored to detect excessive body cooling. In infants who are exclusively breastfed and infant intake is sufficient, exclusive breastfeeding or supplementation with expressed breast milk should be continued while the infant receives phototherapy. Supplementation with infant formula may decrease the TB level but may also interfere with the establishment and continuation of breastfeeding.[114] However, although not universally agreed upon, formula supplementation may diminish TB levels and avoid the need for readmission for phototherapy, an intervention that interferes with parent/infant bonding, interrupts breastfeeding, and is disturbing for the parents.[100]

A well-recognized side effect of phototherapy is the *bronze baby syndrome*. In this disorder, the serum, urine, and skin become brown-black (bronze) several hours or more after a neonate is placed under the phototherapy lamps. All reported neonates with this syndrome have recovered without apparent sequela, except one term neonate who died and was found to have kernicterus on autopsy. In nearly all patients, conjugated hyperbilirubinemia and retention of bile acids have been noted either before light exposure or after the syndrome has developed. This syndrome has been reproduced in Gunn rats when their smaller bile ducts become obstructed by precipitated bile pigment during phototherapy. Photobilirubin II is shown to be degraded to brown pigments in vitro and when administered to Gunn rats in vivo. It seems likely that the bronze color of the plasma and urine in bronze baby syndrome results from retention of bile pigment photoproducts when biliary excretion is impaired by concomitant cholestasis. A study in two infants with this syndrome showed an increase in coproporphyrin in the blood, and photo-irradiation of this substance produced copper coproporphyrin degradation products similar to those found in neonates with this syndrome. It is generally recommended that phototherapy not be used in neonates with significant conjugated hyperbilirubinemia or other evidence of cholestasis. However, because there have been case reports of newborns who developed kernicterus in the presence of predominantly direct hyperbilirubinemia, the role of conjugated bilirubin in the pathophysiology of bilirubin encephalopathy is not clear, and affected infants should be assessed for the need for phototherapy on an individual basis.

Congenital erythropoietic porphyria is another syndrome in which phototherapy is contraindicated because it may lead to death. This rare disorder is characterized by hemolysis, splenomegaly, and pink to red urine that fluoresces orange under ultraviolet light. Exposure to visible light of moderate to high intensity and of wavelengths between

400 and 500 nm (blue to blue-green) produces severe bullous lesions on the exposed skin and accelerated hemolysis. Mixed hyperbilirubinemia with a significant direct-reacting fraction may also be seen in this disease.

Other theoretical dangers of phototherapy include electrical shock or fire from poorly grounded or defective equipment and unproven potential long-term effects on endocrine and sexual maturation and DNA repair mechanisms in skin epithelial cells. Phototherapy may cause DNA damage, but there is no evidence for long-term effects.[115] The risk of genotoxicity appears to be transient. However, the possibility of potential harm supports the need for clinical caution in adhering to therapeutic guidelines and avoiding excess phototherapeutic intensity and or duration. Irradiance levels must be monitored clinically, the goal being to offer the lowest possible level of phototherapy to control TB levels.[116]

In the main follow-up studies, except for the above-mentioned NICHD study, phototherapy-treated premature and full-term neonates have failed to demonstrate any increase in morbidity or mortality ascribed to the appropriate use of phototherapy. Recent studies have excluded phototherapy as an implication in the etiology of autism and diabetes mellitus,[117] although there may be a slight increase in the risk of infantile cancer.[118]

No scientific evidence exists indicating that hydration directly lowers TB levels. However, dehydration is not known to be beneficial to patients in this context, so all neonates should receive appropriate replacement and maintenance fluids. Conjugated bilirubin is water soluble and eliminated from the body in urine, bile, and stool. Inasmuch as appropriate hydration maintains adequate urine output, bile flow, and stool excretion, fluid administration indirectly assists in the removal of unconjugated bilirubin. Ideally, this fluid is given enterally to stimulate gastrointestinal tract motility. The use of a milk-protein formula may be considered in infants not responding to phototherapy to inhibit enterohepatic reabsorption of bilirubin, thereby lowering TB levels.

Pharmacologic Therapy

Phenobarbital

In experimental animals, UGT1A1 activity can be increased or induced with administration of phenobarbital, ethanol, chloroquine, antihistamines, heroin, and chlorophenothane (also known as DDT). These substances are not specific for any one enzyme but stimulate many hepatic membrane-bound enzyme systems and hepatic protein synthesis in general. Because of the known and potential toxicity of these agents, only phenobarbital has been used with regularity in humans.

After the demonstration that phenobarbital administration to a child with Crigler-Najjar syndrome type II disease reduced TB levels, phenobarbital administration to pregnant mothers and their offspring was shown to reduce peak TB levels caused by physiologic jaundice by about 50%. Studies in newborn rhesus monkeys have demonstrated that the major effect of this therapy is to increase hepatic UGT1A1 activity and the conjugation of bilirubin. It also may enhance hepatic uptake of bilirubin in the newborn. The administration of phenobarbital to newborns at the time jaundice is first observed or even immediately after delivery is much less effective than its administration to the mother during pregnancy for at least 2 weeks before delivery. The drug is much less effective in premature neonates. As a prophylactic measure, it would be necessary therefore to administer phenobarbital to large numbers of pregnant women for prolonged periods during pregnancy, because the time of delivery could not be predicted with certainty. Even then, the premature neonates most susceptible to the toxic effects of hyperbilirubinemia would receive little or no beneficial effect.

Phenobarbital is potentially addictive, may lead to excessive sedation of the newborn, and has other potent metabolic effects in addition to those on bilirubin metabolism. For these reasons, its use has not achieved wide application but has been reserved largely for specific high-risk populations. For example, in the pre-phototherapy era, in unexplained severe hyperbilirubinemia of newborns from the Greek coastal islands, the frequency of kernicterus was significantly reduced by general administration of phenobarbital to pregnant women during the last trimester. A dosage of 60 mg/d is sufficient for maternal administration and 5 mg/kg/d for neonatal treatment. Similar effects have been observed in full-term Korean newborns. Phenobarbital is also useful in the differentiation of Crigler-Najjar syndrome types I and II. Combining phenobarbital treatment with phototherapy has no advantage, the effect being no greater than that of phototherapy alone.

Metalloporphyrins

The pharmacologic basis for using this class of compounds to control TB levels is the targeted blockade of bilirubin production through the competitive inhibition of HO, the rate-limiting enzyme in the bilirubin production pathway.[119] Originally proposed by Maines in 1981[119a] for use in modulating bilirubin production, these compounds have been extensively studied. The first metalloporphyrin to be evaluated for use in preventing neonatal unconjugated hyperbilirubinemia was tin protoporphyrin (SnPP). Administration of this compound was shown to decrease bilirubin concentrations in newborn rats, rhesus monkeys, and adult rats with hemolytic anemia, and it was the first synthetic heme analog used for the purpose of inhibiting HO in human neonates. Although highly efficacious, the photoreactivity of this metalloporphyrin made it a less desirable drug. In newborn rhesus monkeys, administration of SnPP produced skin ulcerations, whereas in human neonates who also received phototherapy some mild erythema of the skin was observed. Human trials with tin mesoporphyrin (SnMP) in preterm neonates have shown a dose-dependent reduction in peak TB levels irrespective of gestational age and a reduction in the need for phototherapy compared with controls.[2,119] Mild transient erythema in patients requiring phototherapy was the only side effect noted. In human studies, it has been shown that a single IM dose of

6 μM SnMP/kg body weight eliminates the need for phototherapy during the postnatal period. In a study of SnMP, the drug was administered IM to newborns whose pre-discharge TcB reading was >75th percentile. In treated infants, compared with controls, phototherapy time was halved, the natural TB trajectory was reversed, and the TB at 3 to 5 days was sixfold lower.[120] In a recent study, SnMP administered in conjunction with phototherapy significantly lowered the TB by 48 hours. Other than transient erythema in some patients, there were no serious or life-threatening complications.[121] A long-term follow-up of subjects enrolled in this study to determine safety is ongoing. The efficacy of SnMP has been well described by several investigators in patients with Crigler-Najjar syndrome,[2,59] but SnMP does contain a foreign metal, it induces the HO-1 promoter,[119] and it can inhibit other enzymes such as nitric oxide synthase and soluble guanylyl cyclase (sGC). An alternative compound, zinc protoporphyrin (ZnPP), has been proposed, but it has a much lower inhibitory potency and is not well absorbed after oral administration. Nevertheless, it is a naturally occurring metalloporphyrin possessing both in vitro and in vivo inhibition of both HO-1 and HO-2 isozymes in studies using neonatal rodents and nonhuman primates. Moreover, ZnPP is minimally photoreactive in vivo.

Other Nonmetalloporphyrin Inhibitors

Some nonmetalloporphyrin inhibitors of HO-1 have been identified. Originally designed for use in transplantation survival studies, peptide inhibitors have been reported not only to be immunosuppressive in vitro and in vivo but also to inhibit in vitro total HO enzyme activity dose-dependently. However, in mouse studies, it has been found that administration of peptides may upregulate HO-1 mRNA and protein in the liver, spleen, and kidney. These findings have precluded human studies investigating the efficacy of peptides for the treatment of hyperbilirubinemia.

Imidazole dioxolanes, inhibitors of cholesterol production, have also been found to inhibit in vitro[1] and in vivo HO activity, despite being structurally different from metalloporphyrins. It has been demonstrated that these compounds are highly selective for inhibiting the inducible HO-1, but, like metalloporphyrins, some imidazole dioxolanes may affect other important enzymes, such as nitric oxide synthase (NOS) and sGC.[1]

Miscellaneous Agents

As already discussed, reabsorption of unconjugated bilirubin may contribute to a significant portion of hepatic bilirubin load in the newborn period. Frequent milk feeding (cow or human) may slow the rise of TB levels and enhance the bilirubin-reducing effect of phototherapy. Oral administration of nonabsorbable substances that bind bilirubin in the intestinal lumen and presumably reduce enteric absorption of bilirubin may reduce peak TB levels in physiologic jaundice. Orlistat has been used to increase fecal fat excretion, thereby enhancing bilirubin elimination and decreasing the serum unconjugated bilirubin concentrations in Crigler-Najjar syndrome. Feeding breastfed newborns β-glucuronidase inhibitors (L-aspartic acid or enzymatically hydrolyzed casein) during the first week reduced jaundice without affecting breastfeeding deleteriously.[2] Activated charcoal has been used but is effective only when administered during the first 12 hours of life.[1] Agar has also been shown to be effective. Further study of this type of therapy is needed before recommendations can be made regarding clinical applications. These pharmacologic agents may be no more effective than frequent milk feeding (every 2 hours).

Intravenous Immunoglobulin

High-dose IVIG (500–1000 mg/kg) administered over 2 to 4 hours has been shown in several small studies to reduce TB levels and the need for exchange transfusion in fetuses and neonates with Rh or ABO immune hemolytic disease. COHb studies performed 24 hours after IVIG infusion in DAT-positive, ABO-heterospecific neonates have demonstrated that, in those infants who responded to IVIG infusion with a decrease in TB, hemolysis was inhibited compared with the pre-administration status. Exchange transfusion was avoided in responding newborns, but not in those in whom there was no response. Lack of response was attributed to higher rates of hemolysis, in which case use of a higher dose of IVIG was suggested. Although the efficacy of IVIG therapy remains controversial,[112] its administration should be considered in neonates with DAT-positive immune hyperbilirubinemia who are not responding to intense phototherapy and whose TB level is approaching exchange transfusion indications. This dose can be repeated after 12 hours if necessary. Recommendations for IVIG have been included in the revised 2004 AAP Clinical Practice Guideline (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Although the use of IVIG may be effective in preventing exchange transfusion in ABO-heterospecific neonates, it may not be as valuable in hemolytic disease of the newborn because of Rh isoimmunization, although its administration may delay the increase in TB, thereby allowing for stabilization of the infant.[122]

Exchange Transfusion

Exchange transfusion (see Chapter 84), first described by Diamond and associates,[122a,122b] is the standard mode of therapy for immediate treatment of hyperbilirubinemia to prevent kernicterus and for correction of anemia in erythroblastosis fetalis. Its use has been reduced as a result of the use of RhoGAM to prevent Rh isoimmune disease, the application of phototherapy, and, more recently, the administration of IVIG in cases of isoimmunity. In a recent multicenter study of exchange transfusions performed between 1997 and 2016, including preterm neonates ≥23 weeks of gestation, as well as late term and term newborns, the incidence of exchange transfusion among the 1¼ million newborns surveyed decreased from a peak of 0.3% in 1997 to a nadir of 0.05% in 2016.[123] However, in infants with severe hyperbilirubinemia caused by isoimmunity or other hemolytic conditions, especially G6PD deficiency, exchange transfusion may be the only effective method of adequately reducing TB levels.

Objectives

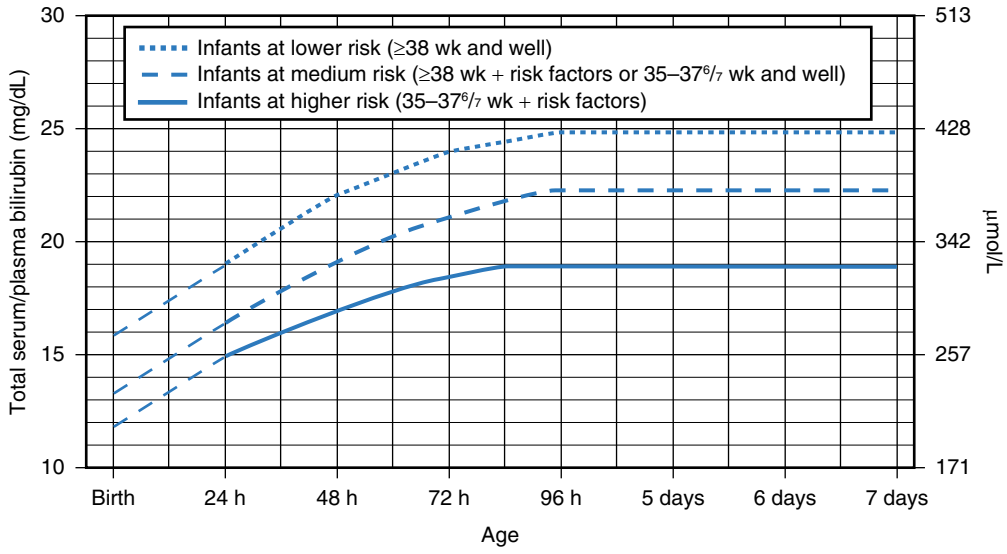
With this technique, the equivalent of two neonatal blood volumes (160 mL/kg of body weight) is replaced in aliquots not to exceed 10% of the total blood volume. This results in the replacement of about 85% of the circulating RBCs. Bilirubin concentrations are usually reduced by 50%. Although the procedure is relatively safe when performed by experienced practitioners in term neonates, it nevertheless carries a risk for both mortality (0.1%–0.5% in term neonates) and morbidity, in addition to being time consuming and expensive. The procedure usually takes 1 to 2 hours. Slower exchanges should theoretically increase the quantity of bilirubin removed by permitting equilibration of pigment from tissue, but the differences are too small to justify the increased risk of prolonging the duration of the procedure. The indications for exchange transfusion need to be individualized, taking into account gestational age and severity of illness (Fig. 95.28). During acute hospitalization, exchange is recommended if TB rises to the indicated levels despite intensive phototherapy. For readmitted infants, if TB is above the exchange transfusion threshold level, intensive phototherapy may be considered, provided the infant does not display clinical signs of ABE. Serial TBs should be performed every 2 to 3 hours, and, if TB remains at or above levels indicated, exchange is recommended after 6 hours of intensive phototherapy.[11]

Indications

In the past, it was regularly recommended that TB, even in healthy term infants, be kept below 20 mg/dL (342 μmol/L) during the first 28 days of life. This recommendation has been questioned, however, and a growing consensus has developed that levels as high as 25 mg/dL (428 μmol/L) are acceptable for otherwise healthy, full-term, asymptomatic infants with no obvious hemolytic condition. When the exchange level is considered, conjugated bilirubin should not be subtracted from the total. Despite the inability of direct-reacting bilirubin to enter the CNS, it is possible that

GUIDELINES FOR EXCHANGE TRANSFUSION IN INFANTS ≥35 WEEKS

Note: These guidelines are based on limited evidence and the levels shown are approximations. During birth hospitalization, exchange transfusion is recommended if TB rises to these levels despite intensive phototherapy. For readmitted infants, if TB is above exchange level, repeat TB every 2–3 hours and consider exchange if TB remains above levels indicated after intensive phototherapy for 6 hours.



- The dashed lines for the first 24 hours indicate uncertainty due to a wide range of clinical circumstances and a range of responses to phototherapy.
- Immediate exchange transfusion is recommended if infant shows signs of ABE (hypertonia, arching, retrocollis, opisthotonos, fever, high-pitched cry) or if TB is 25 mg/dL, (85 μmol/L) above these lines.
- Risk factors—isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis.
- Measure serum albumin and calculate bilirubin/albumin (B/A) ratio.
- Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin.
- If infant is well and 35–37⁶/₇ wk (median risk), can individualize TB levels for exchange based on actual gestational age.

• **Fig. 95.28** The 2004 AAP Clinical Practice Guideline for exchange transfusion in hospitalized infants ≥35 weeks of gestation. Note that these suggested levels represent a consensus of most of the committee but are based on limited evidence, and the levels shown are approximations. (Adapted from the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;114(1):297–316.)

direct-reacting bilirubin can partially displace unconjugated bilirubin from albumin-binding sites to increase the risk for kernicterus.

In the face of rapidly rising TB levels, as may be seen in Rh erythroblastosis or other types of hemolytic disease, the decision to perform an exchange transfusion should anticipate the rate of rise (from previous TB levels, hemoglobin concentrations, and reticulocyte counts). Such prediction permits blood for the exchange to be ordered so that the exchange transfusion is underway by the time the critical level is reached.

The indications for exchange transfusion are based on the infant's TB levels in combination with postnatal age, gestational age, and other risk factors, such as isoimmune hemolytic disease, G6PD deficiency, asphyxia, lethargy, temperature instability, sepsis, acidosis, and BAMR. In 2004, the AAP published a comprehensive guideline for initiating exchange transfusion for term and near-term neonates[11] (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Indications for low birth weight neonates or those with lower gestational ages than those specified in the AAP guideline have also been published (see Phototherapy, discussed previously).

In the severely affected erythroblastotic neonate, clinical judgment rather than laboratory data should be used to decide whether the neonate requires immediate exchange transfusion after delivery. In this situation, a partial exchange transfusion using packed RBCs, coupled with reduction in blood volume if venous pressure is elevated, and with measures to ensure adequate ventilation and correction of acidosis and shock, will often be lifesaving. In most exchange transfusions, fresh whole or reconstituted citrate-phosphate-dextrose anticoagulated blood should be used. If blood older than 5 days must be used, the pH should be checked and sodium bicarbonate added to correct the pH to 7.1. Full correction to pH 7.4 may result in later excessive rebound alkalosis as the citrate is metabolized. Repeat exchange transfusion may sometimes be required. In a developing country, ABE and TB >30 mg/dL were predictive of the need for repeat exchange transfusion.[124]

Technique

Although there are numerous different combinations of blood components that can be used safely and effectively, there is no single combination or component that is superior. RBCs reconstituted with 5% albumin or fresh frozen plasma are most frequently used. Mixtures containing a citrate anticoagulant may cause hypocalcemia, but the administration of calcium during the exchange is seldom practiced. Transfused blood should not contain RBC antigens to which the mother has antibodies. Irradiation of blood is recommended for all exchange transfusions, especially if the infant had undergone an intrauterine transfusion, but may be omitted in clinical emergencies. Because the transfused blood is frequently deficient in platelets, the platelet count should be monitored after exchange transfusion, and platelet transfusion should be considered in infants who are severely thrombocytopenic or who have a bleeding tendency.

The administration of salt-poor albumin (1 g/kg) to neonates 1 to 2 hours before exchange transfusion to increase the efficiency of bilirubin removal by shifting more tissue-bound bilirubin into the circulation has been advocated and shown to increase the bilirubin removed by 40%. As the total amount of bilirubin removed during an exchange transfusion is only a small portion of the total-body pool of bilirubin, this increase may not significantly alter subsequent bilirubin concentrations or the need for additional exchange transfusions. In addition, theoretically, the transient increase in TB concentration after albumin administration could increase, rather than reduce, the risk for kernicterus if there are local phenomena at the brain level that enhance entry of bilirubin into neurons. Finally, constituents of some albumin solutions may act to displace bilirubin from its binding sites, potentially increasing the percentage of free bilirubin present in the plasma. Thus pretreatment with albumin before exchange transfusion is not routinely recommended.

Complications

The AAP recommends that exchange transfusions be performed only by trained personnel in a neonatal intensive care unit with full monitoring and resuscitation capabilities[11] (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Exchange transfusion is an invasive procedure, and complications may be related to the blood transfusion itself, catheter complications, and the procedure. The potential complications of exchange transfusion are listed in Box 95.5. Severe hemolysis caused by the use of incompatible RBCs may be life threatening. With modern-day screening for infection, there is only a slight chance of transmission of viral or bacterial infection. Hyperkalemia may result if the transfused blood has been stored for a long period. Rebound hypoglycemia may occur if the glucose load during exchange transfusion is large. Graft-versus-host disease is rare but may occur in premature infants or those who have had in utero transfusions. Umbilical

BOX 95.5 Potential Complications of Exchange Transfusion

- Thrombocytopenia, particularly with repeat transfusions
- Portal vein thrombosis or other thromboembolic complications
- Umbilical or portal vein perforation
- Acute necrotizing enterocolitis
- Arrhythmia, cardiac arrest
- Hypocalcemia, hypomagnesemia, hypoglycemia
- Respiratory and metabolic acidosis, rebound metabolic alkalosis
- Graft-versus-host disease
- Human immunodeficiency virus, hepatitis B and C infections
- All other potential complications of blood transfusions

venous catheterization may result in air embolism, hemorrhage, or infection.

Currently, serious complications of exchange transfusion are uncommon. Mortality rates are very low in healthy, term neonates but are increased for sick or extremely premature infants. In a study of 106 neonates who underwent 140 exchange transfusions between 1980 and 1995, the overall mortality was 2% but increased to 8% in the subset of infants who were ill. In a smaller series, no serious adverse effects or death occurred among 22 term neonates who had 26 exchange transfusions between 1990 and 1998. In another review of 55 neonates who underwent 66 exchange transfusions between 1992 and 2002, 74% had some form of adverse event, with the most common being thrombocytopenia, hypocalcemia, and metabolic acidosis. In a study of exchange transfusion in neonates with hemolytic disease of the newborn, the procedure was associated with an increased risk of sepsis, leukocytopenia, thrombocytopenia, hypocalcemia, and hypernatremia, but not mortality, when compared with a similarly affected group who did not undergo exchange transfusion.[125] Adverse events related to exchange transfusion, including thrombocytopenia, metabolic acidosis, hypocalcemia, seizures, and death were more frequent in preterm infants: 32 weeks or less, 87%; 33 to 36 weeks, 78%; and 37 weeks or more, 67%. More recently, in a multicenter US-based study, a high death rate within 7 days of exchange transfusion was noted in preterm infants, especially those ≤29 weeks of gestation (17%; 24/14). This may not be surprising if co-existing morbidities of prematurity are taken into consideration. Of concern, however, is the reported death rate following exchange transfusion in late preterm and term neonates: 3/311 neonates born at 35 to 37 weeks of gestation and 5/457 of those ≥37 weeks of gestation died (1%).[123]

If performed under intensive care facilities and with appropriate expertise, the advantages of performing an exchange transfusion clearly outweigh the potential risk, albeit small, of serious complications. Preparations for emergency situations should be made before initiation of the procedure.

Individualization of Therapeutic Guidelines

The TB level is one of the major criteria to be evaluated when considering the initiation or escalation of treatment for unconjugated hyperbilirubinemia. Although it is believed that it is the unconjugated fraction that presents the danger of kernicterus, the exact ratio of unconjugated and conjugated bilirubin is difficult to assess, because its quantitation exhibits great variability among laboratories. In view of this, the conjugated bilirubin level should not be subtracted from the TB level unless it constitutes more than 50% of the total. As was stated previously, many variables other than the TB level influence the susceptibility of a particular patient to the sequelae of unconjugated hyperbilirubinemia; these include genotype, gestational age, chronologic age, and the presence of hemolytic or other disease states. Therefore it is useful to consider four groups of patients at risk for kernicterus and modify treatment based on the category: the healthy term (>37 weeks' estimated gestational age), the sick term, the healthy premature, and the sick premature neonate.

In 1994, the AAP Provisional Committee for Quality Improvement and Subcommittee on Hyperbilirubinemia published recommendations for the management of unconjugated hyperbilirubinemia in healthy term neonates.[126] The recommendations were revised in 2004[11] and clarified in 2009,[109] and they are detailed in Figs. 95.19, 95.27, and 95.28. Whereas the 1994 AAP Practice Parameter did not offer suggestions for the management of newborns with hemolytic conditions, the 2004 AAP Clinical Practice Guideline takes into account both term and late preterm infants, with and without risk factors (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Risk factors to be considered for the purpose of deciding whether to institute phototherapy or exchange transfusion include isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, and acidosis. Jaundice manifesting in the first 24 hours of life is emphasized as an important risk factor, and recommendations for infants with early jaundice are included in the therapeutic guidelines. Therefore a more conservative approach should be taken to initiating therapy for hyperbilirubinemia (Table 95.5). Similarly, TB levels rising at a rate greater than 0.5 mg/dL/h (9 μmol/L/h) or "jumping tracks" on the bilirubin nomogram indicate a state of active hemolysis; such patients should be considered as falling into the "sick," or risk factor, category. It is exceedingly important in these cases to institute and revise therapies on the basis not only of the current level of bilirubin but also of an estimate of the anticipated peak. Thus early in the patient's course, phototherapy or exchange transfusion may be performed at a relatively lower TB level than for a similar level occurring at a later time (see Box 95.4).

Prediction of Hyperbilirubinemia and Post-Discharge Follow-Up

An examination of the hour-specific bilirubin nomogram will reveal that the TB continues to rise in a steady fashion throughout the first days of life, arriving at its peak at about 4 to 5 days. An infant who is discharged home at or around 48 hours of age will only have started to increase the TB, which will peak at home. Great responsibility is therefore placed on the parents to detect deepening jaundice and to approach the appropriate facilities should hyperbilirubinemia develop. Many cases of kernicterus have developed at home in newborns previously thought to be well and discharged from the newborn nursery as healthy. To assist in discharge planning and in an attempt to detect at least some of the infants with developing hyperbilirubinemia, the AAP in 2004 issued recommendations for post-discharge follow-up[11] (Table 95.6) (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]).

TABLE 95.6 Post-Discharge Follow-Up

Infant Discharged	Should Be Seen by
Before age 24 h	72 h
Between age 24 and 47.9 h	96 h
Between age 48 and 72 h	120 h

Refer to the 2022 AAP Clinical Practice Guideline for updated recommendations.[19]
From the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*. 2004;11(1):297–316.

It is recommended that every newborn should be seen by a pediatrician within 2 to 3 days of discharge, even those who were not jaundiced at the time of discharge. Clinical judgment should be used when scheduling follow-up, and infants with risk factors for hyperbilirubinemia may be seen earlier than recommended in Table 95.6, as judged clinically necessary.

In the 2009 update with clarifications[109] to the 2004 AAP Clinical Practice Guideline,[11] a pre-discharge measurement of TB or TcB is recommended, as well as determining the risk for hyperbilirubinemia based on an infant's age in hours and the TB measurement, which may require readmission to the hospital for treatment (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Moreover, all infants should be screened for risk factors prior to discharge when planning for the post-discharge visit. Pre-discharge TB percentile risk assessment in combination with gestational age may be the most sensitive index of subsequent hyperbilirubinemia requiring phototherapy or readmission,[127] whereas complete absence of clinical jaundice is highly predictive of infants who will not develop significant hyperbilirubinemia. The incidence of hazardous hyperbilirubinemia, defined as TB ≥30 mg/dL (513 µmol/L), decreased in at least three US health systems following adoption of universal pre-discharge bilirubin screening with closer post-discharge follow-up.[63]

Recently, Kuzniewizc et al.[128] evaluated a predictive model incorporating the difference between the last TB prior to discharge and the 2004 AAP phototherapy threshold (D-TB) to predict newborns who would subsequently, post-discharge, meet AAP phototherapy thresholds (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). Their objective was to use a single graph, rather than a combination of the Bhutani nomogram for prediction and the 2004 AAP graphs for phototherapy indications. Factors such as infant age in hours, gestational age, and DAT positivity were integrated by using the 2004 AAP phototherapy guideline (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations[19]). The closer the pre-discharge TB was to phototherapy indications, the greater became the risk of meeting phototherapy requirements. The predicted probability of exceeding the phototherapy threshold after discharge ranged from 56% for a pre-discharge Δ-TB 0 to 1 mg/dL (17.1 µmol/L) below the threshold to 0.008% for Δ-TB >7 mg/dL (120 µmol/L) below the threshold.

Few studies have evaluated the rate of compliance with the post-discharge follow-up recommendations proposed in the 2004 AAP Clinical Practice Guideline (refer to the 2022 AAP Clinical Practice Guideline for updated recommendations).[19] Profit et al.[129] reported that, of 698 newborns discharged in Houston, Texas, in 2006, only 37% were seen before 6 postnatal days, and as many as 37% were seen after as long as 10 days. O'Donnell et al.[130] found that of 3282 newborns discharged from a single New York City birthing center, 44% were not seen within the preset time frame, and Shakib et al.[131] reported that, of over 50,000 newborns discharged within 48 hours of birth in Utah, only 15% had had a well child visit within 72 hours of discharge. In a study from Israel, the early community surveillance rate for jaundice was low, with only 32.9% of 659 infants surveyed having approached a health service facility within 3 days of discharge.[132] In contrast, there was near universal compliance among those who received a specific instruction, including time and place, for a post-discharge bilirubin blood test. These findings emphasize the need for arranging appointments prior to discharge to ensure post-discharge follow-up.

Although the AAP does not recommend home deliveries, guidelines for those who are born at home emphasize the need to screen these newborns for hyperbilirubinemia between 24 and 48 hours to determine the risk of developing severe hyperbilirubinemia.[133]

Hyperbilirubinemia in Resource-Limited Countries

Severe hyperbilirubinemia, frequently with clinical evidence of ABE, is one of the most frequent causes for admission in countries with limited resources. Management of hyperbilirubinemia is compromised by long distances to travel to obtain medical care, lack of adequate phototherapy devices, and erratic electricity supply. Exchange transfusion is frequently performed under inadequate circumstances. To cite only a few examples, in Nigeria severe hyperbilirubinemia accounted for one in five neonatal admissions and has been associated with case fatality and neurodevelopmental sequelae, including cerebral palsy and auditory impairments. G6PD deficiency, prematurity, low birth weight, infection, and ABO incompatibility were prevalent among the etiologies of hyperbilirubinemia.[134] In Myanmar, half of all neonatal hospital admissions are for hyperbilirubinemia with a high rate of exchange transfusion.[135] In an attempt to circumvent the inability to provide adequate phototherapy, Slusher et al.[136] devised a method by which filtered sunlight can serve as a surrogate for electric-powered phototherapy by using window-tinting films to filter the broad spectrum of sunlight, allowing transmission of only safe and efficacious

blue light. In a randomized, controlled noninferiority trial in which filtered sunlight was compared with conventional phototherapy for the treatment of hyperbilirubinemia in term and late-preterm neonates in Nigeria, filtered sunlight was not inferior to conventional phototherapy for the treatment of neonatal hyperbilirubinemia. Because the need for exchange transfusion may be high, and blood banking and other supportive services lacking, it has been proposed that infants at high risk of kernicterus should be given priority over those at moderate risk. Factors to be taken into consideration include the presence of intermediate or advanced ABE, neurotoxicity risk factors, and TB levels.[137]

Visual inspection in the assessment of neonatal jaundice in developing countries in which TcB or TB testing may not be readily available was recently re-addressed. In a multinational study including six centers, physicians and primary health care workers evaluated 5250 infants. High sensitivity for detecting neonates with TB >20.0 mg/dL (340 µmol/L) was found for "any jaundice of the distal extremities (palms or soles) OR deep jaundice of the trunk or head." For the TB threshold >15.0 mg/dL (260 µmol/L), "any jaundice of the distal extremities OR deep jaundice of the trunk or head" had the highest sensitivity across sites. The authors concluded that, in settings where TB cannot be measured, neonates with any jaundice on the distal extremities should be referred to a hospital for evaluation and management.[138]

Globally, and especially in resource-limited countries, neonatal jaundice is a major contributor to mortality in the immediate postnatal period. A recent estimate assessed the 2016 global neonatal jaundice-associated deaths during the first 6 days as 1309.3 deaths per 100,000 live births. The burden was highest in countries with low-middle or low sociodemographic indices, especially in Sub-Saharan Africa and South Asia. Industrialized countries were not exempted as neonatal jaundice was the ninth leading cause in Western Europe and 13th in North America.[82]

2022 Revised Clinical Practice Guideline for the Management of Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation

In August 2022, the AAP published an update[19] to replace the 2004 Clinical Practice Guideline with an accompanying technical report.[139] Also incorporated in the new guideline are some recommendations included in the 2009 update with clarifications,[109] although the latter document was not published under the auspices of the AAP. Major features of the new guideline include a cautious raising of therapeutic thresholds, incorporation of risk factors for hyperbilirubinemia neurotoxicity in addition to those for hyperbilirubinemia, and refining treatment thresholds by gestational age to a larger extent than previously. It also includes a comprehensive approach to the prevention, risk assessment, monitoring, and management of hyperbilirubinemia. Highlights of the guideline are summarized below but should not be regarded as comprehensive, and the complete AAP guideline should be consulted for clinical decision making. Some tables and figures from the 2004 Clinical Practice Guideline, clearly labeled as such, have been left intact for the readers' reference and comparison.

Bilirubin Nomograms

The current guideline differs from the previous set with regard to the use of a bilirubin nomogram. A major recommendation of the 2004 Clinical Practice Guideline was to plot any total bilirubin value, serum or transcutaneously derived, on an hour-specific bilirubin nomogram to assess the risk of developing clinically significant hyperbilirubinemia. Current recommendations include plotting a bilirubin value on a graph, also hour-specific, to determine the difference between the bilirubin value and phototherapy indications at the postnatal age at which the bilirubin test was sampled. This approach will determine the interval between discharge and follow-up and the need for additional bilirubin testing. The principle is that the closer the bilirubin value is to the phototherapy indication, the greater becomes the risk for subsequent development of hyperbilirubinemia. The reasons stated for not including the 1999 Bhutani nomogram in the current guideline are that it did not take gestational age and neurotoxicity risk factors into account and was created from a study population that excluded DAT-positive infants. However, as pediatricians have become accustomed to assessing TB values on the nomogram, and as the authors have found it useful for comparing TB values and assessing the rate of rise of TB graphically, we have left the nomogram intact in the current version of this chapter for reference.

Risk Factors

It is important to identify risk factors for hyperbilirubinemia, as these newborns require closer monitoring. There is no longer a distinction between major and minor risk factors as was the case in the 2004 Clinical Practice Guideline. Importantly, factors exacerbating the risk for neurotoxicity, which were first listed in the 2009 update with clarifications,[109] are now incorporated.

Risk factors for hyperbilirubinemia now include lower gestational age, jaundice in the first 24 hours after birth, pre-discharge TcB or TB close to the phototherapy threshold, hemolysis from any cause based on rate of rise of bilirubin, phototherapy before discharge, parent or sibling who required phototherapy or exchange transfusion, family or genetic history suggestive of inherited RBC disorders, exclusive breastfeeding with suboptimal intake, scalp hematoma or significant bruising, Down syndrome, and the macrosomic infant of a diabetic mother. A rapid rate of bilirubin rise (as assessed by TB or TcB), an indicator of possible hemolysis, has been stratified into >0.3 mg/dL/hour in the first 24 hours or >0.2 mg/dL/hour thereafter.

Hyperbilirubinemia neurotoxicity risk factors are important to identify, as they lower the threshold for treatment

with phototherapy and the level at which care should be escalated. Hyperbilirubinemia neurotoxicity risk factors include <38 weeks of gestation, with risk increasing with the degree of prematurity, serum albumin <3.0 g/dL (4.4 μmol/L), presence of isoimmune hemolytic disease (positive DAT), G6PD deficiency or other hemolytic conditions, sepsis, and significant clinical instability in the previous 24 hours.

G6PD deficiency receives special emphasis both as a risk factor for hyperbilirubinemia as well as hyperbilirubinemia neurotoxicity. The guideline recognizes this condition as one of the most important causes of hazardous hyperbilirubinemia leading to kernicterus. Knowledge of family ancestry will be useful in identifying *some but not all* (authors' emphasis and clarification), newborns with G6PD deficiency. The guideline acknowledges that G6PD-deficient newborns are more likely to receive phototherapy before hospital discharge, have a greater risk of readmission and retreatment, and can develop an unanticipated, sudden, and extreme increase in TB. G6PD enzyme activity should be measured in any infant with hyperbilirubinemia of unknown cause when TB increases despite intensive phototherapy, rises suddenly or increases after an initial decline, or when escalation of care is required (see below).

Phototherapy

Based on evidence that bilirubin neurotoxicity does not occur until TB levels are well above the 2004 exchange transfusion thresholds, the AAP raised phototherapy treatment thresholds by a narrow range. Therapeutic graphs are now guided by a combination of postnatal age in hours, gestational age, hyperbilirubinemia risk factors, and hyperbilirubinemia neurotoxicity factors. In this chapter, the new graphs for phototherapy and exchange transfusion (Figs. 95.29 to 95.33) have replaced those of the 2004 Clinical Practice Guideline. Intensive phototherapy, with an irradiance of at least 30 μW/cm^2/nm at a wavelength around 475 nm should be provided. Sunlight is not recommended as a therapeutic option. Although filtered sunlight has been used in resource-constrained communities, the AAP emphasizes that the guideline is not developed for such settings. It is an option to initiate phototherapy at a subthreshold level to reduce the risk of readmission if the absolute TB level or rate of TB rise suggests a high likelihood of exceeding the threshold after discharge. Phototherapy may be discontinued when the TB has decreased by at least 2 mg/dL (34 μmol/L) below the hour-specific threshold at the time of initiation of phototherapy. Follow-up post-phototherapy is recommended as follows: all infants who exceeded phototherapy thresholds during the birth hospitalization should have a repeat TB no later than the day after phototherapy discontinuation, and earlier in those who received phototherapy earlier than 48 hours, had a positive DAT, and had a suspected or known hemolytic disease.

Escalation of Care

Escalation of care refers to the intensification of care with elevated or rapidly increasing TB concentrations to prevent the need for exchange transfusion. The escalation of care threshold is defined as 2 mg/dL (34 μmol/L) below the exchange transfusion threshold. Infants should be managed in or transferred to an intensive care unit with capabilities for performing exchange transfusion. Blood should be set up for an exchange transfusion, the infant hydrated, intensive phototherapy given, and TB tested at least every 2 hours until lowered.

Post-Discharge Follow-Up for Newborns Who Did Not Receive Phototherapy

Follow-up is now based on the difference between the predischarge bilirubin value and phototherapy indications at the time of sampling. The smaller the difference, the shorter the time to repeat bilirubin testing or the doctor's visit. A graded table, based on phototherapy threshold minus TcB or TB measurement, is provided to guide the pediatrician with regard to repeat bilirubin testing or clinical follow-up. Early discharge (<12 hours) requires a bilirubin test between 24 and 48 hours of age.

Routine Assessment of Jaundice

All infants should be visually assessed for jaundice at least every 12 hours following delivery until discharge. Jaundice appearing at <24 hours will most likely be due to a hemolytic process, and TcB or TB should be measured as soon as possible. TcB or TB should be measured universally between 24 and 48 hours, or before discharge should that occur earlier, and assessed in relation to phototherapy indications at the time of testing.

Transcutaneous Bilirubinometry

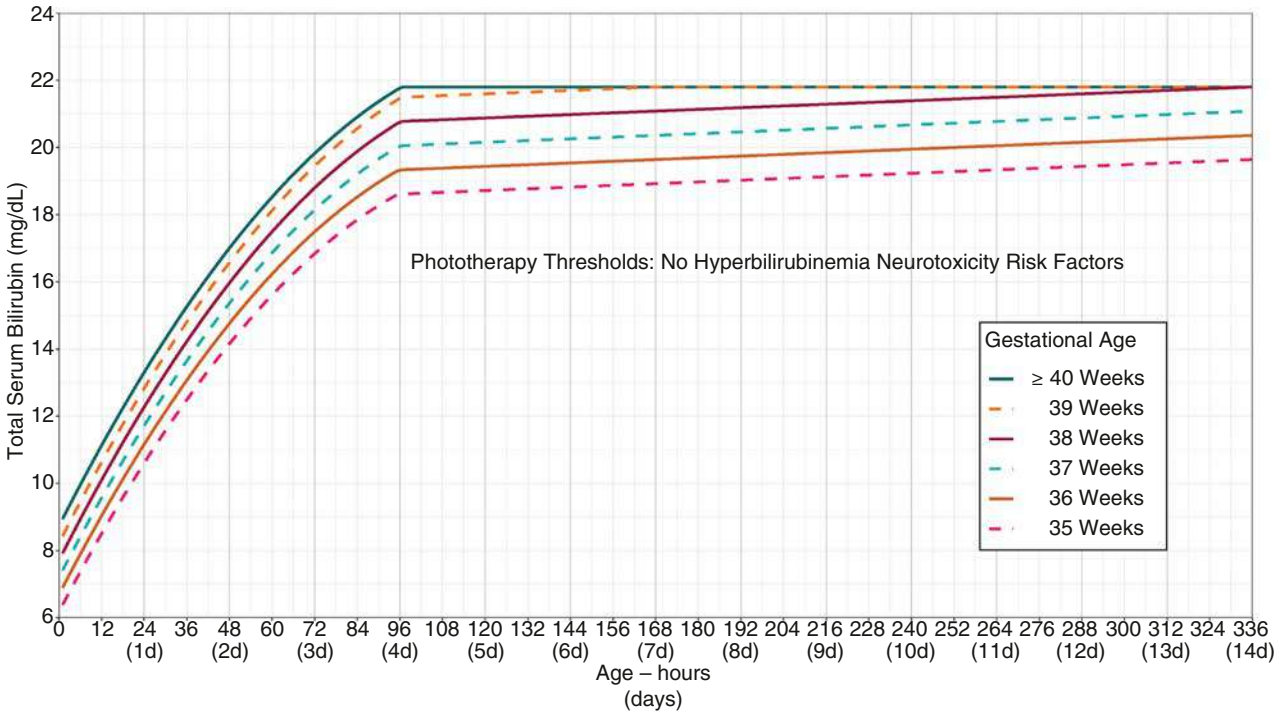
TcB measurements are reliable when used as a screening test to identify those infants who require a TB measurement. There is good correlation between TB and TcB at TB concentrations of <15 mg/dL (257 μmol/L). TB should be measured if the TcB exceeds or is within 3 mg/dL (51 μmol/L) of the phototherapy treatment threshold, or if the TcB value is ≥15 mg/dL (257 μmol/L).

Use of the Guideline Outside of the United States

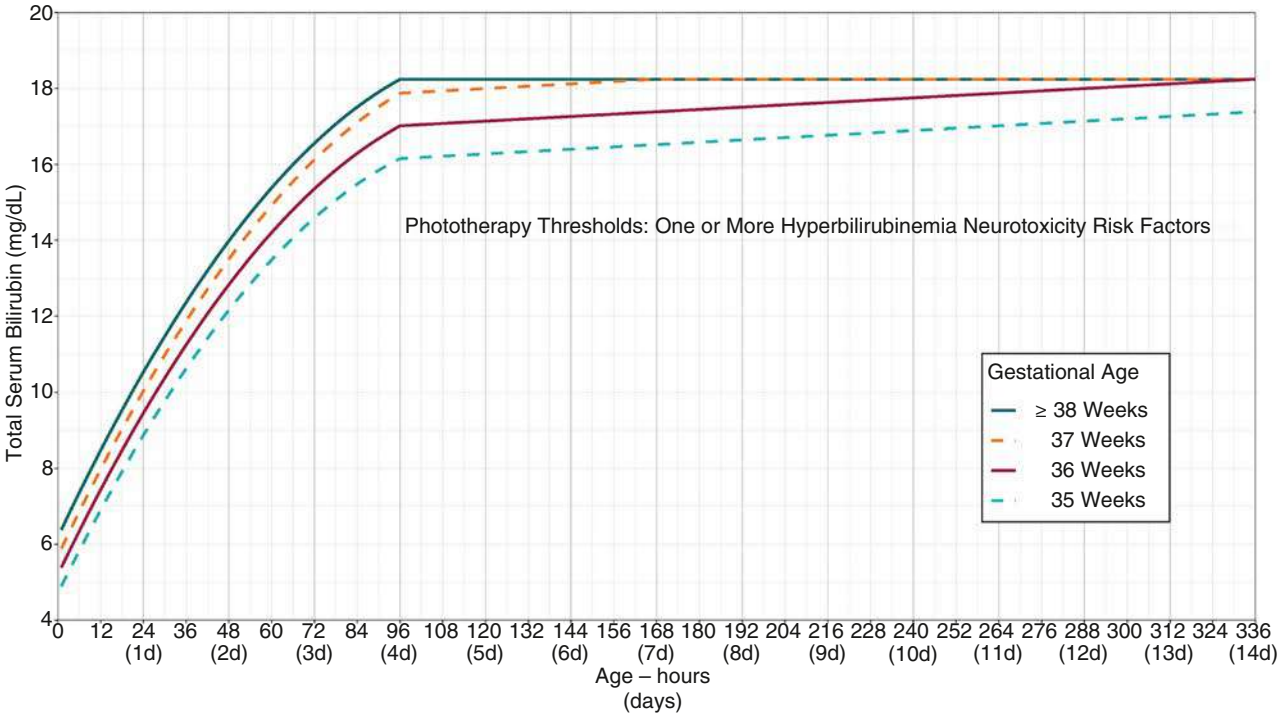
The AAP emphasizes that this guideline was not devised for resource-constrained settings, for low- and middle-income countries, or even high-resource countries where early discharge from the mother-baby unit is less common. The AAP recommends caution and incorporation of local expertise in adapting this guideline for use outside the United States.

Conjugated Hyperbilirubinemia

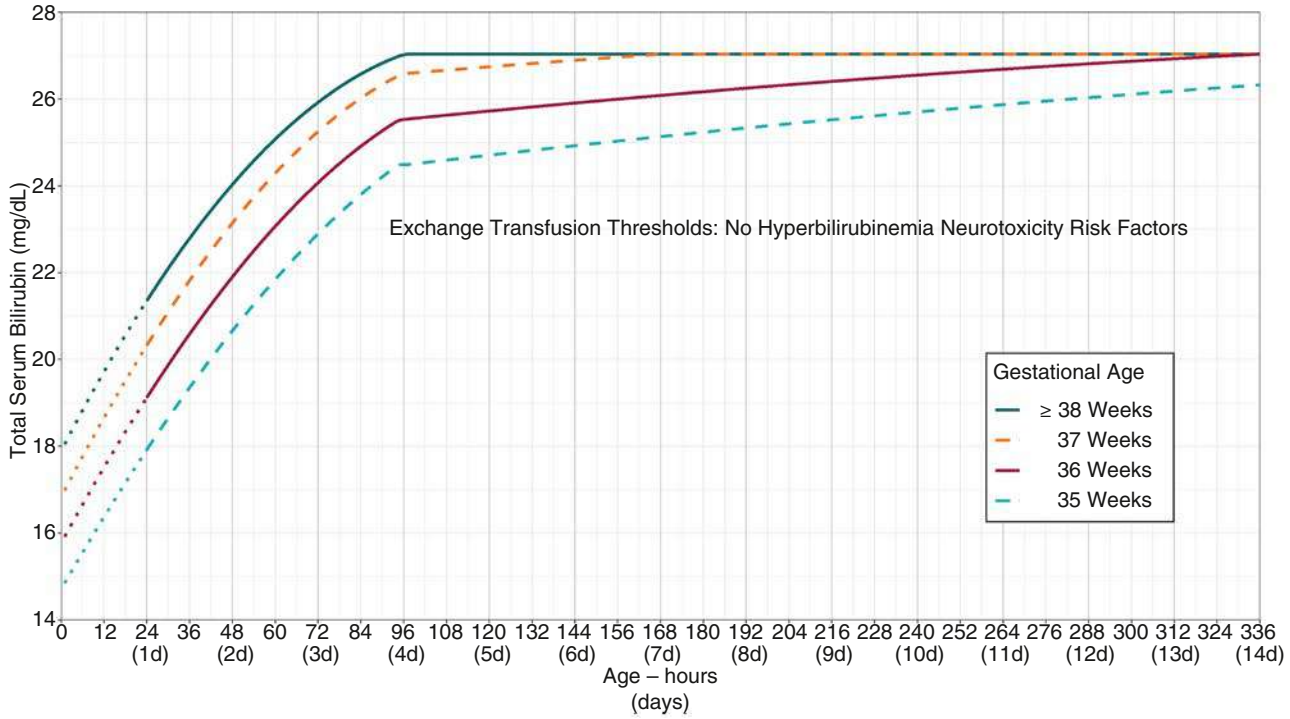
Neonatal jaundice associated with a rise in conjugated bilirubin is indicative of a defect or insufficiency in bile secretion, biliary flow, or both and is always pathologic. It is



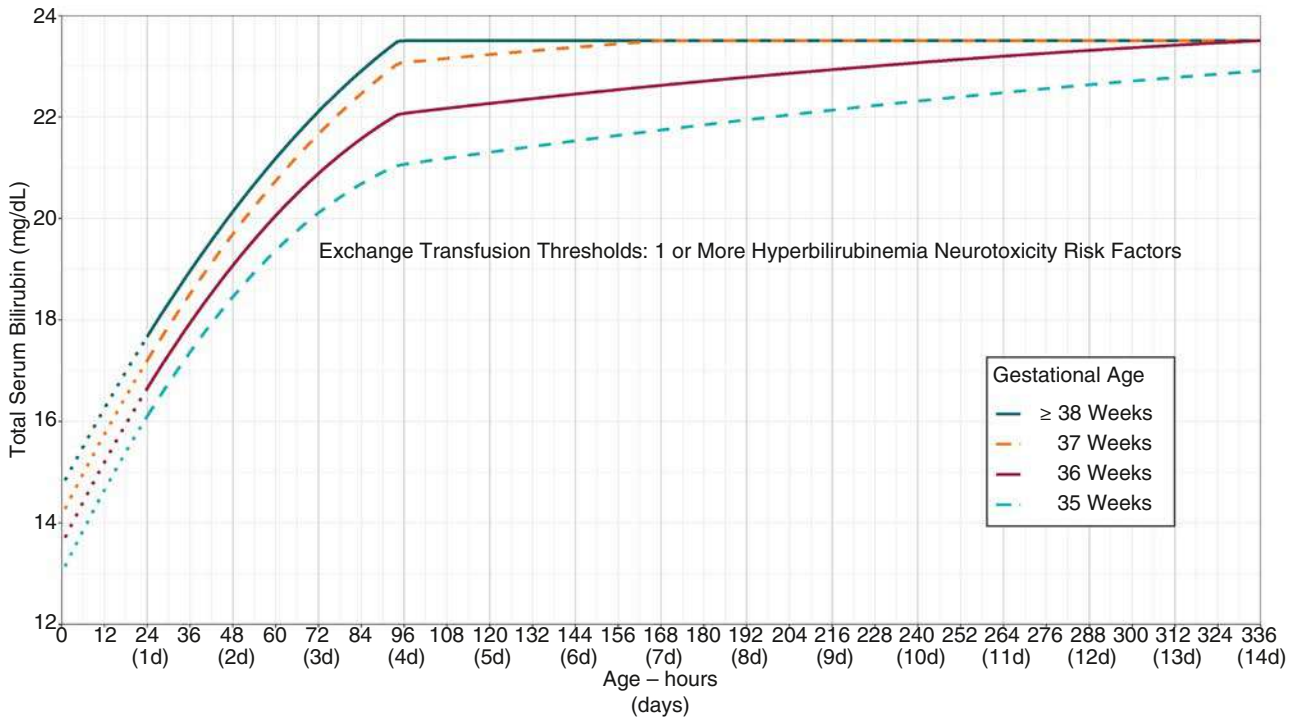
• **Fig. 95.29** The 2022 AAP phototherapy thresholds by gestational age and age in hours for infants with no recognized hyperbilirubinemia neurotoxicity risk factors other than gestational age. (From Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation *Pediatrics*. 2022;150(3):e20220588859.)



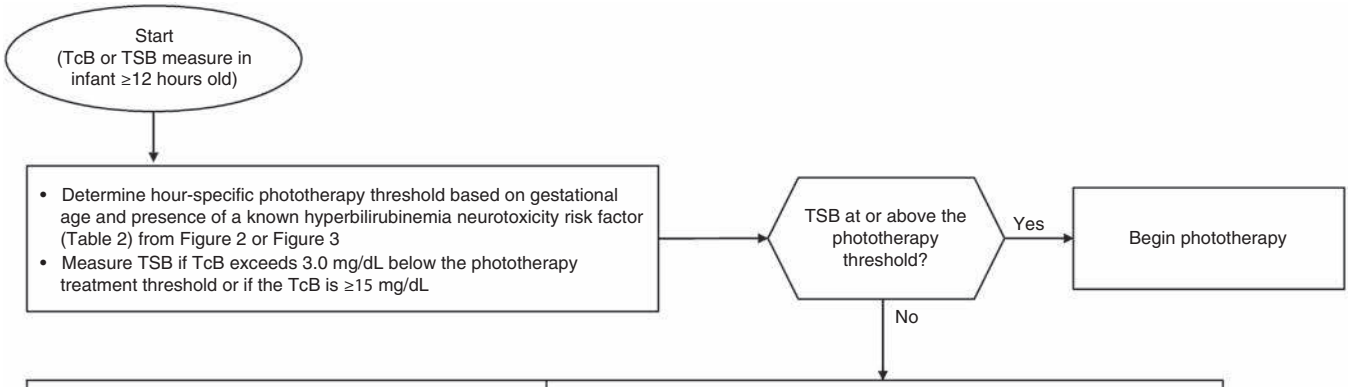
• **Fig. 95.30** The 2022 AAP phototherapy thresholds by gestational age and age in hours for infants with any recognized hyperbilirubinemia neurotoxicity risk factors other than gestational age. (From Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation *Pediatrics*. 2022;150(3):e20220588859.)



• **Fig. 95.31** The 2022 AAP exchange transfusion thresholds by gestational age for infants with no recognized hyperbilirubinemia neurotoxicity risk factors other than gestational age. (From Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation *Pediatrics*. 2022;150(3):e20220588859. Used with permission of the American Academy of Pediatrics.)



• **Fig. 95.32** The 2022 AAP exchange transfusion thresholds by gestational age for infants with any recognized hyperbilirubinemia neurotoxicity risk factors other than gestational age. (From Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation *Pediatrics*. 2022;150(3):e20220588859.)



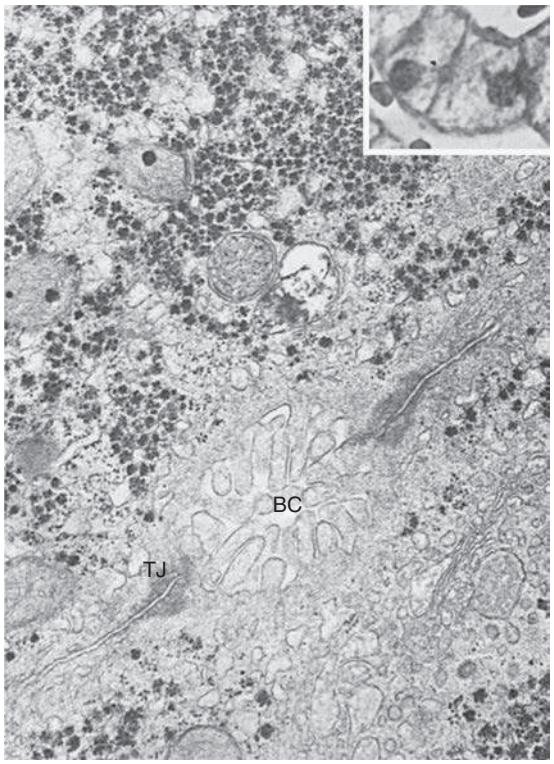
Phototherapy threshold minus TcB or TSB		Discharge Recommendations
0.1-1.9 mg/dL	Age <24 hours	Delay discharge, consider phototherapy, measure TSB in 4 to 8 hours
	Age≥24 hours	Measure TSB in 4 to 24 hours[a] Options: • Delay discharge and consider phototherapy • Discharge with home phototherapy if all considerations in the guideline are met • Discharge without phototherapy but with close follow-up
2.0-3.4 mg/dL	Regardless of age or discharge time	TSB or TcB in 4 to 24 hours[a]
3.5-5.4 mg/dL	Regardless of age or discharge time	TSB or TcB in 1-2 days
5.5-6.9 mg/dL	Discharging <72 hours	Follow-up within 2 days; TcB or TSB according to clinical judgment[b]
	Discharging ≥72 hours	Clinical judgment[b]
≥7.0 mg/dL	Discharging <72 hours	Follow-up within 3 days; TcB or TSB according to clinical judgment[b]
	Discharging ≥72 hours	Clinical judgment[b]

• **Fig. 95.33** Flow diagram for infants during the birth hospitalization to determine post-discharge follow-up for infants who have not received phototherapy. *TcB*, Transcutaneous bilirubin; *TSB*, total serum bilirubin. (From Kemper AR, Newman TB, Slaughter JL, et al. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation *Pediatrics*. 2022;150(3):e20220588859.)

commonly accompanied by a rise in serum levels of other constituents of bile, such as bile salts and phospholipids. The designation *cholestasis*, meaning reduction in bile flow, is used to describe this group of disorders. The rise of conjugated serum bilirubin may be the result of primary defects in the hepatocellular production, transport or excretion of bile, or secondary to impairment of biliary flow due to abnormalities in bile duct function or structure. Sequelae are specific to the many diverse diseases producing this clinical entity; therefore treatment, when possible, is directed at the underlying disease.

The hepatocellular phase of bile secretion involves the transport of conjugated bilirubin across the hepatic cell membrane at the biliary pole. The lateral cell membrane at this site is folded to form microvilli and becomes part of the canalicular space, surrounded by two or more adjacent hepatocytes. Microvilli and the underlying cytoplasm contain microfilaments visible by electron microscopy. These structures consist of the contractile protein actin, which is necessary for normal canalicular contraction and microvillous motility, important elements in the generation of intrahepatic bile flow. At the border of the bile canaliculus, hepatocytes are joined in a "tight junction," which under normal circumstances forms an efficient barrier preventing the contents of the bile canaliculus from entering the perisinusoidal space of Disse or the vascular compartments (Fig. 95.34). The bile canaliculus is an integral part of hepatocytes. It follows that any hepatocellular injury may result in impairment of the cellular phase of bile excretion and breakdown of the tight junctions, leading to the clinical and laboratory findings of cholestasis.

Hyperbilirubinemia resulting from hepatocellular injury may be associated with other abnormalities that reflect impairment of other hepatocellular functions. These abnormalities include hypoglycemia, fluid retention, toxin and medication metabolism, and bleeding. However, injury may selectively impair bile secretion at the biliary pole of hepatocytes, resulting in an isolated laboratory finding of conjugated hyperbilirubinemia. A liver biopsy taken in the early stages of one of the diseases caused by a hepatocellular defect in bile secretion would characteristically show bile pigment granules in hepatocytes and canaliculi, referred to as *canalicular cholestasis*. Bile pigment granules are not seen in either hepatocytes or canaliculi of normal liver parenchyma. In fact, the canalicular lumen is not visualized in routine sections of normal liver parenchyma, because it is partially obliterated by microvilli identifiable only by electron microscopy. In cholestasis, there is usually blistering, blunting, or destruction of these microvilli, transforming the bile canaliculus into a widened, round space containing bile (the bile plug). On occasion, liver biopsies from patients with conjugated hyperbilirubinemia fail to show any abnormalities when examined with the light microscope.

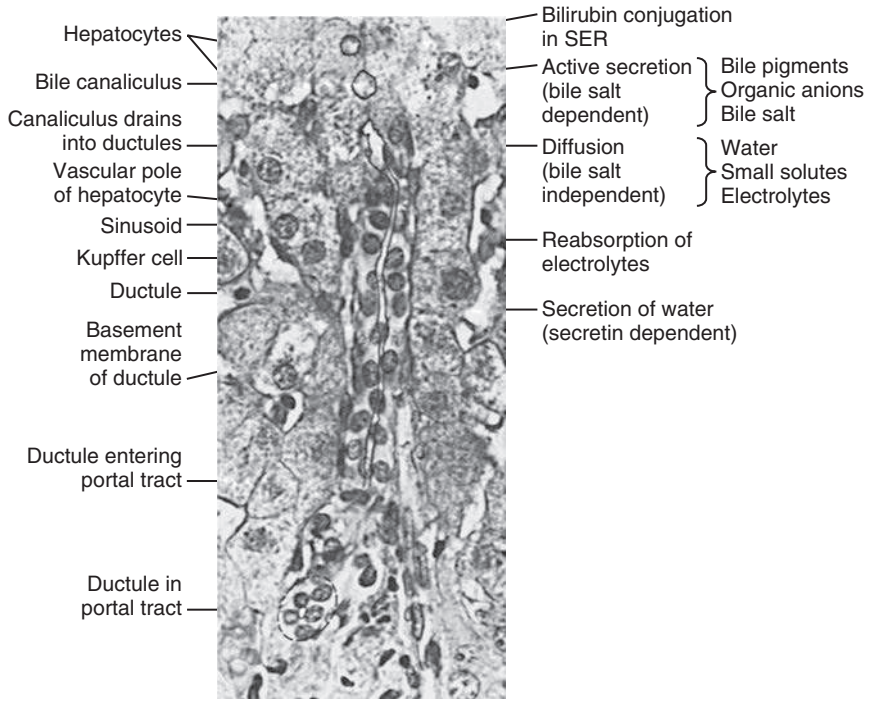


• **Fig. 95.34** Electron micrograph of two adjacent normal hepatocytes. Note the microvillar surface of the bile canaliculus (*BC*). Tight junctions (*TJ*) border the canaliculus. *Insert:* A high-power view of two adjacent hepatocytes as seen in the light microscope. In such preparations, bile canaliculi appear as poorly defined condensations of the cell membrane. Epon-embedded, 6000×. (Courtesy L. Biempica, Albert Einstein College of Medicine, Bronx, NY.)

The ductal phase of bile excretion includes those events that take place in the biliary system distal to the bile canaliculus (Fig. 95.35). The intrahepatic biliary system is comprised of the bile ductules (the initial portion of which is frequently referred to as the *canals of Hering*); the interlobular (portal) bile ducts, recognized by their constant association with a vein and an arteriole; and the right and left hepatic ducts, which in some individuals may partially extend beyond the liver capsule at the liver hilum. The extrahepatic component includes the common hepatic duct, the cystic duct, the gallbladder, and the common bile duct.

A constant and characteristic tissue response to complete mechanical obstruction of a major bile duct is dilation and proliferation of proximal portions of the intrahepatic biliary system, including the ductules and canals of Hering, structures that are outside the confines of the portal tracts. A constant accompaniment to bile duct proliferation is an increase in surrounding connective tissue, which eventually leads to fibrous bridging between adjacent portal tracts, causing biliary cirrhosis. Although bile secretory defects may initially be purely hepatocellular or ductal, any long-standing abnormality in the flow of bile leads to some degree of hepatocellular damage.

Box 95.6 lists diseases that may manifest as conjugated hyperbilirubinemia in the neonatal period. This list is divided into those disorders caused by a defect in the hepatocellular phase of bile secretion and those caused by ductal disturbances. In most of these disorders, direct-reacting bilirubin accounts for 50% to 90% of the TB level. A small amount of indirect-reacting bilirubin is always present, reflecting mild hemolysis, defective uptake and excretion, or hydrolysis of conjugated bilirubin. Early in the onset of conjugated hyperbilirubinemia in



• **Fig. 95.35** Microscopic section of normal liver depicting transition between the bile canaliculus and bile ductule entering the portal tract. Anatomic structures are identified to the left of the illustration, and the corresponding physiologic events are listed on the right. *SER*, Smooth endoplasmic reticulum.

• BOX 95.6 Diseases That May Manifest as Conjugated Hyperbilirubinemia in the Neonatal Period

Neonatal Idiopathic Hepatitis (Giant Cell Hepatitis)

Infection
- Viral
  - Cytomegalovirus
  - Rubella
  - Hepatitis B
  - Enteroviruses, including coxsackie and echoviruses
  - Herpes simplex
  - Varicella-zoster
  - Adenovirus
  - HIV
- Bacterial
  - Sepsis or urinary tract infection (e.g., *Escherichia coli*)
  - Syphilis
  - Tubercle bacillus
  - *Listeria* organisms
- Protozoal
  - Toxoplasma organisms

Toxic
- Maternal drugs
- Medications
- Intestinal failure associated liver disease

Metabolic Disorders
- α_1-Antitrypsin deficiency
- Disorders of carbohydrate metabolism
  - Galactosemia
  - Hereditary fructose intolerance (fructosemia)
  - Glycogen storage disease type IV
- Disorders of lipid storage
  - Niemann-Pick disease
  - Gaucher disease
  - Wolman disease (lysosomal acid lipase [LAL] deficiency)
  - Fatty acid oxidation defects
  - Short- and long-chain acyl-CoA dehydrogenase deficiency (SCAD, LCAD)
- Disorder of amino acid metabolism
  - Tyrosinemia
- Urea cycle defect
  - Citrin deficiency
- Mitochondrial disorders
  - Mitochondrial DNA depletion syndromes (e.g., *DGUOK*, *POLG*, *MPV17*/Navajo neurohepatopathy)
  - Growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death (GRACILE) syndrome
- Disorders of bile acid synthesis
  - Primary disorders of bile acid synthesis (types 1 to 6)
  - Secondary
    - Cerebrohepatorenal syndrome (Zellweger syndrome)
    - Smith-Lemli-Opitz syndrome
- Congenital disorders of glycosylation

Endocrine
- Hypopituitarism
- Hypothyroid

Immune-Mediated
- Gestational alloimmune liver disease (GALD)
- Hemophagocytic lymphohistiocytosis (HLH)
- Neonatal lupus

Hypoxic/Ischemic/Vascular
- Shock/hypoperfusion
- Congenital heart disease

Excess Production Exceeding Capacity of Bilirubin Excretion
- Erythroblastosis fetalis (severe forms)

Impaired Bile Acid Transport
- Progressive familial intrahepatic cholestasis (PFIC) types 1 to 5
- Dubin-Johnson syndrome
- Abnormal villin expression in the bile canaliculi
- NISCH syndrome (neonatal ichthyosis and sclerosing cholangitis)

Other Genetic Disorders
- Arthrogryposis renal dysfunction cholestasis (ARC) syndrome
- Hereditary cholestasis with lymphedema (Aagenaes) syndrome

Impaired Bile Flow

Extrahepatic Obstruction/Malformation
- Biliary atresia
  - Isolated
  - Trisomy 18
  - Biliary atresia splenic malformations (polysplenia-heterotaxia syndrome)
- Biliary cysts
- Spontaneous bile duct perforation
- Inspissated bile/bile plug, biliary sludge, choledocholithiasis
- Tumors/malignancy (e.g., hepatoblastoma, neonatal leukemia)

Impaired Intrahepatic Duct Formation or Obstruction
- Alagille syndrome (arteriohepatic dysplasia)
- Non-syndromatic paucity of bile ducts
- Cystic fibrosis
- Neonatal sclerosing cholangitis

the neonate, the direct-reacting portion may account for only 10% to 25% of TB. As hepatic conjugation and uptake of bilirubin mature, the indirect-reacting portion decreases and the direct portion increases.

Causes of Conjugated Hyperbilirubinemia

Conjugated hyperbilirubinemia results from interference with the hepatic excretion of conjugated bilirubin into bile. Idiopathic neonatal hepatitis and biliary atresia together account for the greatest proportion of cases of neonatal conjugated hyperbilirubinemia, about 50% of all cases.[140] Therefore these entities will be discussed first.

Idiopathic neonatal hepatitis is defined as prolonged conjugated hyperbilirubinemia without the apparent stigmata of a generalized viral illness, the evidence of identifiable infectious agents, or an etiologically specific metabolic abnormality. On liver biopsy, this group is characterized by

extensive transformation of hepatocytes into multinucleated giant cells, and it is therefore sometimes referred to as *neonatal giant cell hepatitis*. Giant cell transformation of hepatocytes does not reflect any specific etiology. It is caused by rupture of lateral cell membranes of adjacent hepatocytes, with consequent reduction in the number of bile canaliculi and retention of conjugated bilirubin. It is seen in various inherited metabolic disorders and some infections. Necrosis of hepatocytes and inflammation are usually present, although special stains (e.g., reticulin using silver impregnation) may be necessary to demonstrate loss of hepatocytes. Necrosis and inflammation may be transient, with giant hepatocytes persisting for many months or even years.

Biliary atresia is defined as a condition in which there is luminal obliteration or apparent absence of segments of parts or all of the extrahepatic biliary system. Although confirming the etiology of neonatal cholestasis may be difficult in the early stages, an early accurate diagnosis is essential for the choice of proper clinical management (see Treatment of Biliary Atresia). The prognosis in idiopathic neonatal hepatitis is uncertain and cannot be predicted based on clinical or laboratory findings. Furthermore, the prognosis continues to change as new conditions are identified and removed from this category.

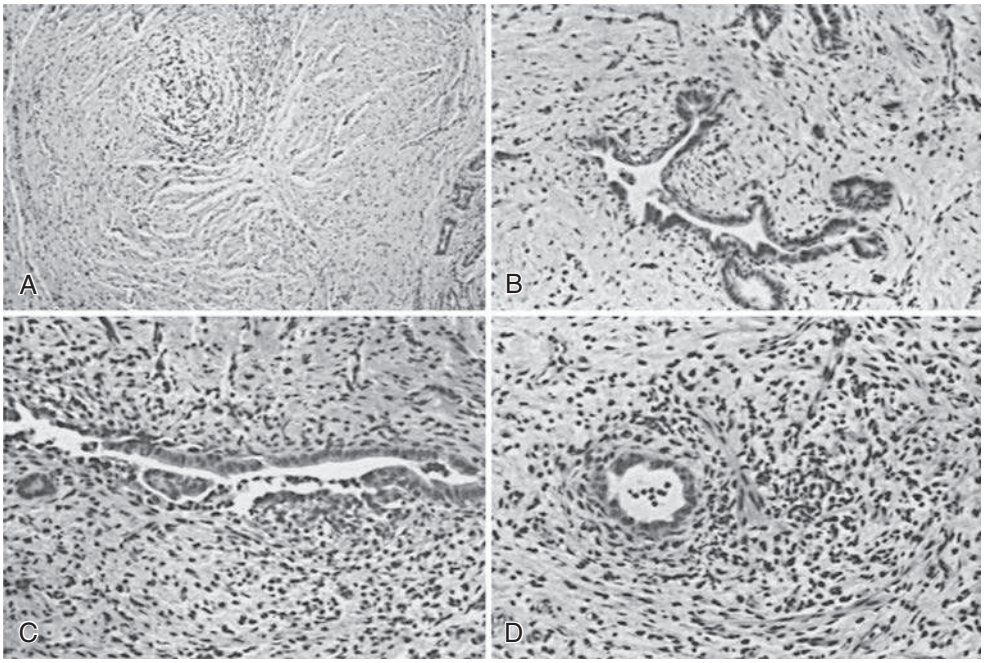
The causes of idiopathic neonatal hepatitis and biliary atresia remain undetermined. The long-held view that biliary atresia represents a simple congenital developmental anomaly with failure of canalization is now thought untenable. In most cases, biliary atresia and neonatal hepatitis occur as isolated abnormalities, and both are considered to represent acquired conditions that may be initiated by the same or similar noxious factors. In support of the acquired nature of most cases of biliary atresia is the absence of reported cases in stillborn fetuses and the relatively rare association with other malformations. Similarly, clinical evidence of total obstruction to the flow of bile (such as acholic stools or colorless meconium) is not detected in the early stages of jaundice. The onset of acholic stools is frequently delayed until 2 weeks of life or later, and liver biopsy findings may be non-specific in the first weeks with overlapping features of neonatal hepatitis. Microscopic changes observed in the extrahepatic biliary system, or its fibrous remnant removed during the hepatoportoenterostomy, strongly suggest a sequence of changes that include acute cholangitis, necrosis, inflammation, attempted regeneration, and obliterative fibrosis (Fig. 95.36).

Injury to the structures involved in bile secretion (hepatocytes and biliary epithelium) may occur either in utero or in the perinatal period, but the consequences of such injury and therefore clinically manifested disease are delayed until sometime after birth. Clinical manifestations and eventual outcome may depend on the severity and persistence of lesions in a specific location. Thus primary injury to hepatocytes may result in clinical manifestations of neonatal hepatitis, whereas injury to major bile ducts and gallbladder may result in biliary atresia. Viral illness including reovirus, rotavirus, and cytomegalovirus, and toxins have been investigated for possible causes of biliary atresia but have failed to definitively demonstrate a link.

Although genetics may play a role in biliary atresia splenic malformations (BASMs), studies have failed to identify a genetic etiology in the majority of biliary atresia patients without other malformations. The cohort of patients classified as having idiopathic neonatal hepatitis constitute a heterogeneous group that undoubtedly includes various, as yet undefined, hereditary metabolic disorders. A metabolic disorder may explain the recurrent incidence of this disease in some families and poorer prognosis compared with sporadic cases. With advances in identification of genetic and metabolic disorders, this diagnosis continues to decline over time.

Most patients with idiopathic neonatal hepatitis or biliary atresia represent isolated cases without familial incidence or associated anomalies. Neonatal hepatitis has a familial incidence of 10% to 15%, whereas no familial cases of histologically proven extrahepatic biliary atresia have been observed. Rare occurrences of neonatal hepatitis and biliary atresia in two siblings have been reported. Both neonatal hepatitis and biliary atresia occur more frequently in patients with trisomy 18 than in the general population. Biliary atresia has been observed in association with laterality defects and is often referred to as BASM or polysplenia-heterotaxia syndrome in 10% to 15% of cases. This syndrome is characterized by situs inversus of abdominal organs, intestinal malrotation, asplenia or multiple spleens, centrally placed liver, and a variety of cardiac, pulmonary, and vascular malformations. The inferior vena cava is frequently absent.

Early clinical manifestations of both idiopathic neonatal hepatitis and biliary atresia may be limited to jaundice. In a small proportion of patients, especially among those who later develop neonatal hepatitis, jaundice may be apparent at birth, documented by increased concentrations of conjugated bilirubin in cord blood. In biliary atresia, direct-reacting bilirubin levels have been demonstrated to be mildly elevated within the first days of life and higher than in healthy infants, but it may be overlooked when direct bilirubin levels remain <20% of TB levels.[141] Jaundice usually becomes apparent between the second and sixth weeks of life. The dark yellow staining of diapers from the presence of bilirubin in the urine or scleral icterus often prompts the parents to seek medical advice. Jaundice may be first noted by the physician during a well-baby visit. Hepatomegaly may be present in both neonatal hepatitis and biliary atresia. Obstruction to the flow of bile is reflected by acholic stools and may be observed in both neonatal hepatitis and biliary atresia. It is always transient and incomplete in neonatal hepatitis, but its duration is variable and may extend beyond the crucial period during which an accurate diagnosis must be established if surgical correction is needed. Routine clinical and laboratory findings usually do not distinguish between extrahepatic biliary atresia and neonatal hepatitis. A routine series of diagnostic laboratory tests is suggested, however, to establish the severity of hepatic involvement and to screen for possible causes (Box 95.7). The failure of routine tests to distinguish between neonatal



• **Fig. 95.36** Microscopic preparations of fibrous remnant of extrahepatic biliary system resected during Kasai procedure for biliary atresia. (A) Most distal portion of specimen, showing complete obliteration of lumen by fibrous tissue. (B–D) More proximal segments, illustrating a spectrum of changes that includes necrosis of lining epithelium, acute and chronic inflammation, mural fibrosis with distortion of lumen, and great variation in size of ductlike structures. All micrographs are 60× magnification.

• BOX 95.7 Laboratory Tests Recommended for Evaluation of Neonatal Conjugated Hyperbilirubinemia

Determine Degree of Elevation of Liver Enzymes and Assess Liver Synthetic Function

- Total and fractionated serum/plasma bilirubin
- SGOT (AST), SGPT (ALT), alkaline phosphatase, and γ-glutamyl transpeptidase
- Prothrombin time/INR
- Serum albumin
- Glucose

Evaluate for Conditions Requiring Timely Therapy

- Tests for infectious disease
  - Complete blood count
  - Viral: PCRs from nose, pharynx, blood, stool, urine, and cerebrospinal fluid as indicated
  - Bacterial: UA/urine culture ± blood culture ± CSF cultures
- Testing for metabolic disease
  - Newborn screen (e.g., galactosemia, hypothyroidism, cystic fibrosis, tyrosinemia, citrullinemia)
  - Lactate ± ammonia
  - Urine reducing substances or erythrocyte galactose-1-phosphate uridyltransferase (galactosemia)
  - Urine succinylacetone (tyrosinemia)
  - α_1-Antitrypsin level and phenotype
- Testing for endocrine disorders
  - TSH/T4
- Testing for anatomic or vascular abnormalities
  - Abdominal ultrasound with Doppler

Additional Diagnostic Studies in Conjunction With Pediatric Gastroenterologist/Hepatologist (As Indicated)

- Cholestasis panel (e.g., PFIC, Alagille, metabolic disorders)
- Liver biopsy (routine histology ± electron microscopy ± immunohistochemistry ± viral studies ± enzyme analysis)
- Cholangiography (open, percutaneous, endoscopic, MRCP)
- Radiography (e.g., spine images for Alagille, chest x-ray for lung and heart disease, MRI brain for panhypopituitarism)
- Ophthalmologic examination (e.g., posterior embryotoxin for Alagille, chorioretinitis for congenital infection, cataracts)
- MMP7 (biliary atresia)
- Cardiac evaluation
- Sweat chloride analysis (cystic fibrosis)
- Plasma amino acids
- Urine organic acids
- Acylcarnitine profile
- Cortisol
- α-Fetoprotein
- Ferritin
- Serum and urine bile acid concentrations
- Cholesterol
- Ammonia

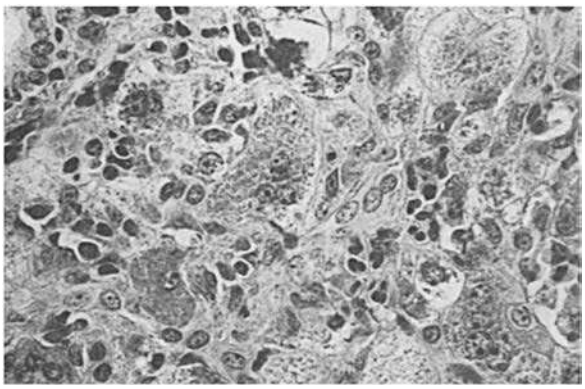
ALT, Alanine transferase; *AST*, aspartate aminotransferase; *CSF*, cerebral spinal fluid; *FTA-ABS*, fluorescent treponemal antibody absorption (test); *INR*, international normalized ratio; *MMP7*, matrix metalloproteinase-7; *MRCP*, magnetic resonance cholangiopancreatography; *MRI*, magnetic resonance imaging; *PCR*, polymerase chain reaction; *PFIC*, progressive familial intrahepatic cholestasis; *SGOT*, serum glutamic-oxaloacetic transaminase; *SGPT*, serum glutamic-pyruvic transferase; *TSH*, thyroid-stimulating hormone; *UA*, urinalysis.

hepatitis and biliary atresia has led to a continued search for other distinguishing biochemical characteristics with recent advances. Yang et al.[142] reported on the high sensitivity and specificity of serum matrix metalloproteinase-7 (MMP-7) to differentiate biliary atresia from other neonatal cholestasis, and it may be a reliable biomarker for biliary atresia. Subsequent studies have supported the utility of MMP7 not only as a marker for biliary atresia, but also for liver fibrosis and disease progression.

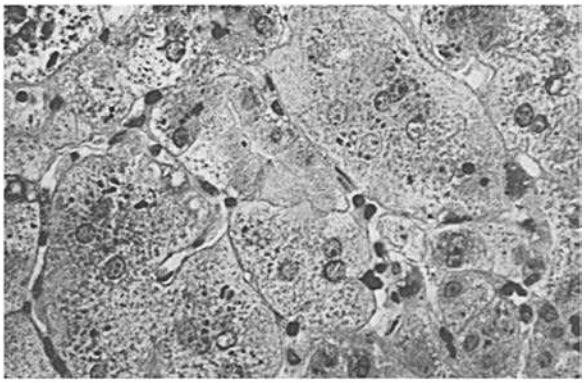
A longstanding method for the evaluation of patency of extrahepatic bile ducts is the use of hepatobiliary scintigraphy (HBS), with technetium-99m acetanilidoiminodiacetic acid (IDA) or IDA derivatives, such as paraisopropyl iminodiacetic acid or di-isopropyl iminodiacetic acid. These compounds are efficiently extracted by hepatocytes and are excreted with bile into the intestines. When complete obstruction exists, no activity is detected in the intestines. Pretreatment with phenobarbital for 3 to 7 days before testing promotes excretion of isotope in neonates with severe intrahepatic cholestasis and thus reduces the chance for a mistaken diagnosis of extrahepatic obstruction. Phenobarbital is given orally at the dose of 5 mg/kg daily. Patients are given nothing by mouth for 1 hour before and 2 hours after injection of the radiotracer to avoid gallbladder contraction and dilution of radiotracer excreted into the intestines. Given the limited specificity of scintigraphy and availability of MMP-7 as a serum biomarker, along with concerns for scintigraphy leading to delays in diagnosis of biliary atresia, HBS is becoming less commonly used in routine evaluation of cholestatic infants.[143]

Ultrasonography has also been applied in the evaluation of neonatal cholestasis to identify anatomic and vascular abnormalities. Although the absence of a gallbladder is suggestive of biliary atresia, reports of a normal gallbladder may be seen in biliary atresia and is therefore not adequate to exclude biliary atresia.

Histopathologic examination of the liver remains an integral part of the evaluation of patient with persistent conjugated hyperbilirubinemia. The timing depends on the clinical status, age of the patient, and results of other pertinent laboratory data. A percutaneous liver biopsy or intraoperative wedge biopsy can yield adequate tissue for microscopic and, if desired, electron microscopic and virologic studies. In most patients (90%–95%), liver biopsy establishes or confirms the correct diagnosis, sparing the neonate with neonatal hepatitis an unnecessary surgical procedure. Before the biopsy, the prothrombin time and platelet count must be ascertained and assessed to determine the safety of performing the procedure and the need for correction. A prolonged prothrombin time may be corrected in some patients by administration of vitamin K. When there is concern for impaired synthetic function or increased risk of bleeding, a thromboelastogram (TEG) or rotational thromboelastometry (ROTEM) may be used to further assess coagulopathy. In some situations in which a biopsy is urgently required, fresh frozen plasma, cryoprecipitate, and/or platelets may be administered during the percutaneous biopsy. Postoperatively, neonates must be observed closely for vital signs or clinical changes that indicate significant bleeding, bile peritonitis, or sepsis, which are rare but significant complications of the procedure.

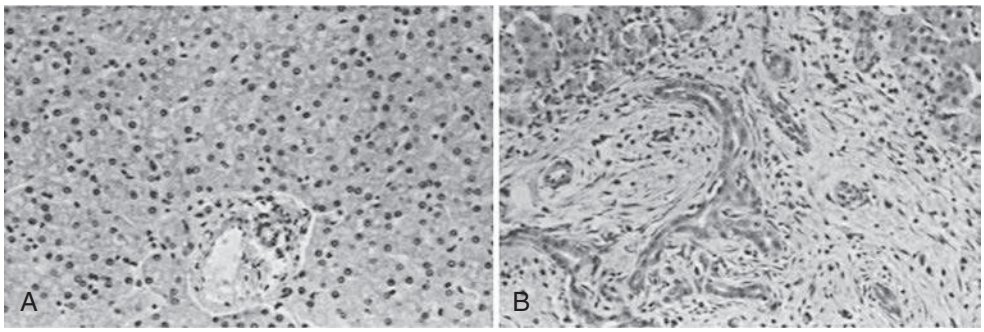


• **Fig. 95.37** Neonatal hepatitis. Note the marked cellular irregularity obliterating the normal orderly plate arrangement. Intracanalicular bile is present. Small cells in sinusoids are Kupffer cells and elements of extramedullary hematopoiesis. Paraffin embedding and hematoxylin and eosin staining (60× magnification).



• **Fig. 95.38** High-power view of transformed giant hepatocytes. Most of the intracytoplasmic granules represent bile pigment. Paraffin embedding and hematoxylin and eosin staining (200× magnification).

The liver biopsy in neonatal hepatitis is characterized by marked irregularity in the size of hepatocytes and, in some cases, by numerous giant hepatocytes (Fig. 95.37 and Fig. 95.38). Giant cells may contain from 4 to 100 nuclei. The cytoplasm of these giant cells is usually foamy and contains bile pigment. Bile canaliculi appear to be reduced in number and proportion to the number of giant hepatocytes. Necrosis and inflammation are frequently detected. Kupffer cells are swollen and contain bile pigment, lipofuscin, iron, and phagocytosed debris of destroyed hepatocytes. Although these findings may also be present in biliary atresia, it is the relative absence of bile duct proliferation that distinguishes neonatal hepatitis from biliary atresia. In some cases of neonatal hepatitis, there is evidence of inflammatory injury to portal bile ducts, with epithelial reduplication interpreted as regenerative activity. The aforementioned changes are usually seen in early stages, soon after onset of jaundice. Biopsies taken after

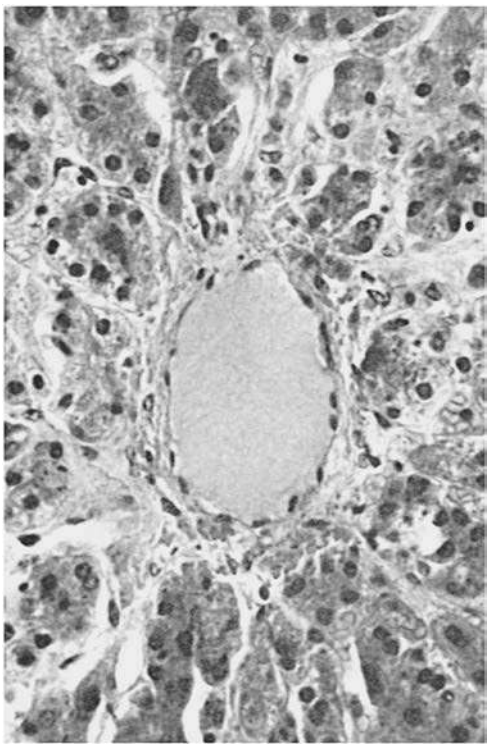


• **Fig. 95.39** (A) Portal tract of normal neonate, containing a large, thin-walled vein and single cross section of a bile duct. (B) Portal tract from neonate with biliary atresia. Note marked enlargement of tract from fibrosis that surrounds multiple elongated bile ducts. Both micrographs are 60× magnification.

3 months of age may show little necrosis and inflammation and, instead, demonstrate fibrosis or cirrhosis. Giant cell transformation of hepatocytes may persist for months or years. However, although the positive predictive value of liver histology for a diagnosis of biliary atresia is 90.7%, the negative predictive value is only 60.7%.[144] Thus the intraoperative or percutaneous cholangiogram remains another important tool in establishing this diagnosis.

It has been shown in patients and in animal experiments that, soon after obstruction of the common bile duct, ducts and ductules in portal tracts and in periportal zones begin to proliferate. This phenomenon involves most, if not all, portal tracts and is present even at the periphery of the liver, far removed from the site of obstruction. Therefore in biliary atresia, changes suggesting blockage in the major (mostly extrahepatic) bile ducts are seen. At least three portal tracts should be available for examination. In biliary atresia, all tracts will show some degree of proliferation. In early stages, ductular proliferation may be present without increased fibrosis. The ducts have a varicose appearance and contain focal bile plugs. Later, ducts and ductules frequently appear distorted (Fig. 95.39) because of a discrepancy between the rate of proliferation of biliary epithelium and that of the surrounding fibrous tissue. An associated inflammatory exudate is occasionally seen around proliferated ductules, but a true cholangitis with epithelial necrosis and intramural inflammation is rarely encountered except in association with surgical complications. Other changes within portal tracts include dilated lymphatics and, occasionally, tortuous and thick-walled arterioles. In later stages of biliary atresia, ductular epithelium may disappear, having been replaced by collagen fibers, and this may lead to a secondary paucity of portal bile ducts.

Hepatocytes in biliary atresia show intracellular and canalicular cholestasis, with the canalicular component predominating (Fig. 95.40). In about one-third of all patients with biliary atresia, there is giant cell transformation of hepatocytes. In most cases, this transformation is primarily centrolobular (around terminal branches of the hepatic vein) and is not associated with necrosis and inflammation. Extramedullary hematopoiesis may also be present.



• **Fig. 95.40** Extrahepatic biliary atresia. Central vein surrounded by hepatocytes. Intracanalicular bile plugs are present. In addition, hepatocytes contain intracytoplasmic bile pigment granules. Paraffin embedding and hematoxylin and eosin staining.

Treatment of Neonatal Hepatitis

Clinical management of neonatal hepatitis consists of supportive measures, because no specific therapy is known. Many neonates have a transient but significant reduction in bile flow, often requiring replacement of fat-soluble vitamins, particularly vitamins D and K. Subclinical rickets is common in these neonates and may contribute to the increase in serum concentrations of alkaline phosphatase. Persistence of acholic or very pale stools without significant lowering of direct-reacting serum bilirubin concentrations after 1 month should be viewed as an indication for repeat clinical study, including possible liver biopsy or intraoperative cholangiogram. This practice permits detection of patients whose extrahepatic bile ducts sclerose after

an initial phase of hepatitis or those whose earlier diagnosis was unclear and who are therefore suitable candidates for exploratory laparotomy and corrective surgery. On rare occasions, patients with neonatal hepatitis and complete obstruction, as evidenced by acholic stools, may recover rapidly after operative cholangiography that shows normal extrahepatic ducts. This phenomenon is probably the result of flushing out of inspissated bile in the extrahepatic biliary system, with consequent relief of obstruction. This situation may be seen in cystic fibrosis or severe dehydration. Fluctuations in stool color should alert the clinician to the possible existence of a biliary cyst, which can be diagnosed with imaging studies, such as ultrasound or MRI, and treated surgically. There are no early reliable criteria on which the prognosis of a particular patient can be based.

Treatment of Biliary Atresia

When the clinical evaluation indicates complete biliary obstruction or proves inconclusive, the patient should undergo an exploratory laparotomy. On entry of the abdomen and after initial scrutiny of the biliary system, an operative cholangiogram should be performed to confirm and characterize the extrahepatic lesion and define its extent. The classification proposed by the Japanese Society of Pediatric Surgeons divides extrahepatic biliary atresia into three types based on gross observations during laparotomy:

- Type I—Atresia of the common bile duct with patent proximal ducts
- Type II—Atresia of the common hepatic duct with patent proximal ducts
- Type III—Atresia of the right and left hepatic ducts at the porta hepatis

Operative examination of these neonates should be performed only by surgeons prepared to proceed with corrective procedures if necessary. Reoperation after exploratory laparotomy increases the technical difficulties and delays the institution of corrective measures. Reconstitution of normal biliary drainage by direct anastomosis of grossly identifiable segments of patent bile ducts to the gastrointestinal tract is possible in only a very small proportion of patients with biliary atresia (estimated at 5%–10%). These include the rare cases of choledochal cyst and occlusion of a short segment of the common bile duct by a valve, a membrane, or fibrosis. In most patients with biliary atresia, there are no grossly visible ducts proximal to the atretic segment. In the past, all of these patients were considered inoperable. Untreated patients, although jaundiced, often appear clinically well in the first few months of life, but they deteriorate rapidly after cirrhosis develops, with clinical manifestations of portal hypertension, ascites, hypersplenism, infection, hyperammonemia, and hemorrhage.

In 1968, Kasai and associates[144a] described for the first time in American literature an operative procedure in which the periphery of the transected fibrous tissue of the porta hepatis, devoid of grossly identifiable ducts, was anastomosed to a Roux-en-Y loop of small intestine (portoenterostomy). Kasai's portoenterostomy or variations of this procedure are currently performed in most medical centers.

The immediate goal for surgical correction is the reestablishment of bile drainage, which is now achieved most effectively when neonates undergo their operation before 45 to 60 days of life. However, even in those with successful biliary drainage, fibrosis increases with time and eventually progresses to cirrhosis. It is currently unclear whether progressive liver fibrosis represents a continuation of the same type of injury responsible for the initial obliterative process in extrahepatic ducts, if it is the result of ascending cholangitis complicating surgery, or if it results from toxicity from retained bile acids. In addition to the age at the time of surgery, other factors that influence the outcome include the size and patency of microscopic ducts in the transected porta hepatis and the preservation of intact epithelial lining. Growth failure and TB greater than 2 mg/dL at 3 months after hepatoportoenterostomy are associated with the need for transplantation or death by 24 months of age.

Orthotopic liver transplantation is the definitive therapy for biliary atresia, with 60% to 80% of individuals with biliary atresia requiring transplantation by the age of 20 years. Survival statistics have steadily improved since 1981, when cyclosporin A and steroid therapy and subsequently FK 506 (tacrolimus) were introduced. Other advancements have lessened the mortality and morbidity of transplantation, resulting in high long-term graft and patient survival.

Known Infectious Causes

In a small proportion of neonatal cholestasis, a specific infectious agent may be identified either by direct isolation and culture or by serologic tests that detect specific antibodies. In addition, microbial antigens may be identified in liver biopsy using monoclonal antibodies and immunocytochemical staining methods. Among infectious agents reported in association with neonatal hepatitis are organisms such as *Treponema pallidum* and *Listeria* species and viruses such as rubella and Coxsackie virus, the herpes group of viruses (herpes simplex, varicella-zoster, cytomegalovirus [CMV]), and adenovirus. The protozoan *Toxoplasma gondii* also has been implicated. Fetal infection may take place in utero either by transplacental spread or by an ascending infection of the amniotic fluid, usually after rupture of membranes. In some cases, infection may occur during delivery by aspiration or swallowing of vaginal contents. Clinically, patients in this group may appear sick and fail to thrive and also may have evidence of CNS and other organ involvement. In many patients, there are stigmata of generalized infection. Laboratory findings are similar to those seen in idiopathic neonatal hepatitis, but stools are not acholic, and therefore biliary atresia usually is not suspected. Congenital infection with human immunodeficiency virus (HIV) is a rare cause of cholestasis in the neonatal period.

Liver biopsy may be helpful in the diagnosis of infectious agents, especially in infections with the herpes viruses (e.g., CMV). These DNA viruses replicate in the nucleus, resulting in intranuclear inclusion bodies that can be seen with the light microscope. Cytomegalic inclusion disease is characterized by marked enlargement of hepatocytes, biliary epithelium, and Kupffer cells caused by intranuclear as well as intracytoplasmic inclusions. In some cases, however, the virus has been isolated from patients in whom liver biopsy showed giant cell transformation of hepatocytes with no evidence of inclusions, leaving it unclear if the virus is the definitive cause of the liver injury.

Hepatitis B surface antigen (HBsAg) may be transmitted from mother to infant, probably by aspiration of vaginal contents, including blood, during delivery. With few exceptions, infants born to HBsAg-positive mothers show no antigenemia in cord blood or in the first month of life. Repeated serologic tests indicate that HBsAg appears in the serum of these infants between 5 and 7 weeks of life and reaches a peak at 10 weeks. Antigenemia in the newborn may be associated with liver injury, and both may persist for many months or possibly years. It is necessary therefore to closely observe all infants of HBsAg-positive mothers for many years for clinical and laboratory evidence of chronic liver disease. Severe and even fulminant neonatal hepatitis associated with HBsAg has been described in infants of chronic carriers and after neonatal transfusions. Prophylaxis with concurrent administration of hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine is effective in preventing neonatal infection in greater than 90% of exposed newborns. HBIG (0.5 mL IM) should be given within 12 hours of birth. In addition, hepatitis B vaccine should be given IM concurrently within 12 hours of birth at a different anatomic site and repeated at 1 to 2 and 6 months of age (for preterm infants who weigh less than 2000 g at birth, a total of four doses of vaccine should be given according to the immunization schedule for preterm infants). Testing for infection (with HBsAg) and immunity (anti-HBs or HBsAb) is now recommended at age 9 to 12 months or 1 to 2 months after final dose. If the infant is not immune (anti-HBs <10 mIU/mL), revaccination is recommended. Concurrent use of HBIG and vaccine does not appear to interfere with vaccine efficacy. A high hepatitis B virus DNA level in HBsAg-positive mothers is the most significant risk factor for vertical transmission despite appropriate vaccination. Antiviral therapy in the third trimester is recommended for pregnant women with serum hepatitis B virus DNA >200,000 IU/mL to reduce the risk of perinatal transmission.[145] Breastfeeding is not contraindicated for mothers with chronic hepatitis B infection.

Hepatitis C virus (HCV), a single-stranded RNA virus in the flavivirus family, has been found to be the etiologic agent in most cases previously referred to as non-A, non-B hepatitis. Transmission occurs through both the percutaneous and the non-percutaneous route. The incubation period varies from 2 weeks to 6 months. The signs of acute disease include malaise, fever, elevation in hepatic transaminases, and jaundice. Fulminant hepatitis and acute hepatic failure are rare. Although about three-fourths of acute infections are asymptomatic, about one-half of affected patients will develop chronic hepatitis, with 20% of these patients progressing to cirrhosis. Progression of the disease is slow, with an average of 10 years to chronic hepatitis, 20 years to cirrhosis, and 30 years to hepatocellular carcinoma. Although infected infants manifest biochemical features of hepatocellular injury, other manifestations of the disease are relatively mild throughout childhood.

The overall risk for vertical transmission of HCV has been shown to be as high as 10%. Women who are infected with both HCV and HIV and those with HCV viremia are at the greatest risk for transmitting HCV to their offspring. The persistence of maternal antibody in the infant is variable but may be as long as 8 to 12 months. Diagnosis in the neonate and infant is made by measuring serial anti-HCV (IgG) titers (using enzyme immunoassays followed by recombinant immunoblot assays detecting antibody against HCV core antigen or other nonstructural proteins) or by directly detecting the presence of HCV ribonucleic acid through the reverse-transcriptase polymerase chain reaction. No proven therapy is currently available to neonates infected with HCV. However, HCV therapies with new direct-acting antiviral agents have been found to be safe and efficacious in children age 3 years and greater. Therefore screening of infants born to HCV-positive mothers is recommended using an antibody-based test at or after 18 months of age. If positive, repeat at 3 years of age to confirm chronic infection and potential for treatment. HCV has been detected in the breast milk of HCV-infected mothers. Although theoretically possible, transmission of HCV through breastfeeding has not been documented in HCV-positive, HIV-negative mothers. Thus breastfeeding is not contraindicated in HCV-positive women.

Sepsis

Microorganisms and their biologic products may have direct toxic effects on the cells and structures responsible for the hepatocellular and ductal phases of conjugated bilirubin excretion. This may be complicated by sepsis-induced hemolysis, further adding to the bilirubin load. Postmortem examination of neonates with severe sepsis has shown centrilobular cholestasis, focal hepatocellular necrosis, and giant cell transformation in some patients. In others, no hepatic lesions can be demonstrated by light microscopy. Severe urinary tract infection, particularly with coliform bacilli, is associated with this syndrome. In this case, generalized septicemia is not an essential feature, and cholestasis may be caused by massive endotoxin release. Antibiotic treatment is followed by prompt relief of hyperbilirubinemia.

Metabolic Disease

Numerous defects in carbohydrate, protein, and lipid metabolism occur with conjugated hyperbilirubinemia.

Galactosemia, caused by deficient activity of galactose-1-phosphate uridyltransferase (GALT) is inherited as an autosomal recessive disease with an incidence of about 1 in 50,000. It results in the accumulation of galactose-1-phosphate in the liver, producing hepatomegaly and conjugated hyperbilirubinemia. Other associated findings in galactosemia include hypoglycemia, emesis, failure to thrive, sepsis (particularly with *Escherichia coli*), cataracts, and ascites. Treatment is focused on dietary avoidance of galactose, along with addressing complications of liver disease/systemic illness. Developmental delay and cirrhosis occur if dietary treatment is not instituted early.

The acute form of tyrosinemia is also an autosomal recessive disease and is characterized by elevations in plasma tyrosine and methionine accompanied by hepatic and renal dysfunction, emesis, and failure to thrive. Dietary tyrosine restriction along with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) has been shown to improve liver and renotubular function and can prevent cirrhosis and early death. In survivors, the risk of hepatocellular carcinoma is high.

Niemann-Pick disease (deposition of sphingomyelin and cholesterol) and Gaucher disease (deposition of glucosylceramide), both autosomal recessive diseases, have also been reported to be associated with conjugated hyperbilirubinemia (see Chapter 94). A variety of mitochondrial disorders and fatty acid oxidation defects can present in the neonatal period; conjugated hyperbilirubinemia is often associated with hypoglycemia, lactic acidosis, hypotonia, and failure to thrive. The diagnosis of a mitochondrial disorders can be confirmed with genetic testing (e.g., for *DGUOK*, *MPV17*, *POLG*) or assessment of respiratory chain enzyme levels in tissue.

Intestinal Failure–Associated Liver Disease

Intestinal failure–associated liver disease (IFALD) is defined as "hepatobiliary dysfunction as a consequence of medical and surgical management strategies for intestinal failure, which can variably progress to end-stage liver disease, or can be stabilized or reversed with promotion of intestinal adaptation."[146] The pathogenesis of IFALD is multifactorial, including immaturity of bile secretion from the infant liver, toxic effects from intravenous lipids, damage from sepsis, disruption of the enterohepatic circulation, and intestinal and biliary stasis. Risk factors include prolonged use (2 weeks or greater) of parenteral nutrition, prematurity, composition and duration of parenteral nutrition, recurrent infection, small intestinal bacterial overgrowth, anatomic considerations, and tolerance of enteral nutrition. Liver biopsy shows evidence of hepatocellular injury with swelling of hepatocytes, necrosis, cholestasis, and occasional giant cell transformation.

Mechanical Obstruction

Inspissated Bile (Bile Plug) Syndrome

Obstruction of a major bile duct by thick bile or mucus is known as the *bile plug syndrome* or the *inspissated* (Latin *inspissatus*, thickened) *bile syndrome*. Although it may be seen in cases of cystic fibrosis, most often the cause is obscure. The obstruction usually resolves gradually, with or without phenobarbital therapy. Severe cases may require irrigation of the biliary tree or direct surgical extraction of the plug.

Choledocholithiasis

Choledocholithiasis is most commonly seen in neonates with a history of severe intrauterine hemolysis. The excessive bilirubin load results in the formation of gallstones, which have the potential to block the secretion of conjugated bilirubin. Gallstones may also be seen in patients receiving total parenteral nutrition. The diagnosis is suspected because of the presence of conjugated hyperbilirubinemia, bilirubinuria, acholic stools, and a palpable gallbladder. It is confirmed with ultrasound. Spontaneous resolution is common, but cholecystectomy may be necessary in cases of cholangitis or progressive elevation of conjugated bilirubin levels.

Cystic Diseases

Cyst formation in the biliary system may result in obstruction of bile flow and produce conjugated hyperbilirubinemia. Congenital hepatic fibrosis is an autosomal recessive disease marked by hamartomatous and fibrotic changes of the interlobular bile ducts. Most cases are associated with cysts of the renal collecting tubules, and the prognosis depends greatly on the degree of renal impairment. *Caroli disease* is the name given to cystic dilation of the major intrahepatic ducts. Cholangitis is a chronic problem, and the outcome is variable. Cysts found along the extrahepatic biliary tree (common hepatic duct, common bile duct, gallbladder) are known as *biliary cysts*. These are more commonly seen in females and may lead to portal hypertension, cirrhosis, and cholangiocarcinoma. Complete surgical excision is needed.

Masses

Obstruction of the extrahepatic biliary ducts may also rarely occur with tumors such as primary hepatoblastoma and metastatic neuroblastoma, nonmalignant tumors such as hepatic hemangiomas, enlarged periductal lymph nodes, and distended loops of bowel. Treatment is directed at the underlying disorder.

Known Genetic Causes of Conjugated Hyperbilirubinemia

Several metabolic disorders result in hepatocellular injury in the neonatal period and give rise to a clinical pathologic syndrome that may resemble neonatal hepatitis or biliary atresia. α_1-Antitrypsin deficiency in the homozygous state (PiZZ) may be manifested by neonatal liver injury. It is estimated that only 10% to 20% of all individuals with this abnormality will have liver disease. Most PiZZ individuals never develop clinical evidence of liver disease, but they may

develop pulmonary emphysema as adults. Patients with α_1-antitrypsin deficiency may show all the signs and symptoms of neonatal hepatitis or biliary atresia, including acholic stools. Liver biopsy also may show changes consistent with either one of the aforementioned conditions. Although in older children periportal hepatocytes frequently contain intracytoplasmic inclusions that give a positive reaction with periodic acid-Schiff stain and resist diastase digestion, these are rarely seen in the neonatal period. Immunocytochemical staining may be helpful in demonstrating granules of α_1-antitrypsin, present in hepatocytes of patients with deficient states but not in normal phenotypes. Phenotyping of the Pi system should be carried out in all suspected cases. In many infants, neonatal cholestasis may regress before the age of 6 months and reappear later in childhood or adolescence when the patient develops cirrhosis and portal hypertension. Studies suggest that the liver disease is a result of toxic gain-of-function mutations that cause the α_1-antitrypsin protein to fold aberrantly and be retained in the endoplasmic reticulum of hepatocytes rather than be secreted into the blood.

Alagille syndrome (arteriohepatic dysplasia) is an autosomal dominant disease with clinical variability characterized by a paucity of intrahepatic bile ducts in the presence of patent extrahepatic ducts. Other findings include unusual facies; vertebral anomalies; cardiac anomalies, including peripheral pulmonary stenosis; renal and vascular anomalies; posterior embryotoxon (incomplete iridocorneal separation); and retarded mental, physical, and sexual development. Mutations in Jagged1 (*JAG1*; chromosome 20p12) have been identified in about 70% of patients studied with Alagille syndrome. Jagged1 is a cell surface ligand for the Notch receptor. The interaction between Jagged1 and Notch is critical for proper cell differentiation during early development. The majority of Alagille syndrome cases (~97%) are caused by haploinsufficiency of the *JAG1* gene. A small percentage (<1%) is caused by mutations in *NOTCH2.*

Early clinical manifestations and laboratory findings are identical to those observed in patients with extrahepatic biliary atresia. The diagnosis is based on clinical features. Liver biopsy typically shows a paucity of the intrahepatic bile ducts, but it is no longer considered mandatory to make a diagnosis of Alagille syndrome, and the presence of cholestasis is acceptable to fulfill this criterion. Later in the first year of life, however, serum cholesterol concentrations rise well beyond those observed in other forms of infantile liver disease. Levels higher than 1000 mg/dL may be seen as early as the third month of life. Cutaneous xanthomas are prominent in the later stages of untreated disease, usually after 1 year of age. It is important to recognize this condition before exploratory surgery, because the patency of the very narrow and collapsed extrahepatic ducts may be extremely difficult to demonstrate. Not only is portoenterostomy unsuccessful in establishing bile flow in this condition, but it also actually accelerates the progression of liver disease.

A nonsyndromic form of intrahepatic bile duct paucity has also been described. A familial form of cholestasis and paucity of intrahepatic bile ducts is associated with development of lymphedema of the lower extremities around the time of puberty. Although initially described cases in this group were from families of Norwegian extraction, similar cases have been reported from England, France, and Sweden.

Progressive familial intrahepatic cholestasis (PFIC) results from defects in specific transporter proteins that are responsible for traffic of bile components from hepatocytes into the bile canaliculus. In addition to cholestasis, the group of PFIC conditions can present with diarrhea and growth failure. PFIC-1, previously known as Byler disease, is caused by mutations in the gene coding for the canalicular surface protein FIC1. PFIC-2 is caused by defects in the gene that codes for the bile salt export pump (BSEP) resulting in toxic retention of bile salts within hepatocytes. PFIC-3 is caused by mutations in the gene encoding multidrug resistant protein 3 (MDR3), resulting in lack of phosphatidylcholine in bile and predisposing to cholangitis. PFIC-3, in contrast to PFIC-1 and PFIC-2, is therefore associated with elevations of γ-glutamyl transpeptidase levels. Two new subtypes of cholestatic liver disease have been reported, including PFIC-4 caused by a mutation in the *TJP2* gene,[147] which results in a disruption of tight-junction structure, and PFIC-5, which is caused by a mutation in the *NR1H4* gene, which encodes a bile acid–activated nuclear hormone (farnesoid X) receptor that regulates bile acid metabolism.[148]

Disorders of bile acid synthesis are caused by defects in the enzymes needed to produce bile acids from cholesterol. Progressive liver injury occurs with inadequate production of bile acids to facilitate normal bile flow along with increased production of abnormal hepatotoxic bile acid intermediaries. Clinical manifestations vary but can include direct hyperbilirubinemia with progressive liver failure in the neonatal period.

Zellweger (cerebrohepatorenal) syndrome is a rare autosomal recessive disease marked by the absence of hepatic and renal peroxisomes. Because peroxisomes have many vital anabolic and catabolic functions within the cell, their absence results in profound cellular dysfunction. In addition to conjugated hyperbilirubinemia, affected patients manifest characteristic facies (high forehead, flat occiput, large fontanelle, shallow orbital ridges, micrognathia), feeding difficulties, hypotonia, seizures, and mental retardation. Death usually occurs early in infancy.

Management

Clinical management of conditions with prolonged cholestasis includes supportive measures. Infants with chronic cholestasis are at risk for failure to thrive. Malabsorption of fat and fat-soluble vitamins results from poor solubilization of dietary fat in mixed micelles due

to reduced intestinal bile flow. In addition, excess catabolism predisposes infants with chronic liver disease to poor weight gain. Fat malabsorption and steatorrhea can be managed by providing a fat source enriched for medium chain triglycerides, which do not require bile salts for intestinal absorption. Fat-soluble vitamins should be supplemented and serum vitamin concentrations should be monitored. The hydrophilic bile acid ursodeoxycholic acid (UDCA; ursodiol, Actigall) has been used in managing cholestatic disorders. Proposed anticholestatic mechanisms of UDCA action include stimulation of bile flow and displacement of more toxic bile acids from hepatocytes into the systemic circulation via Ca^{257+} and protein kinase C-α-dependent mechanisms and/or activation of p38 (mitogen-activated protein kinase [MAPK]) and extracellular signal-regulated kinases (ERKs) to affect transporter molecules (e.g., BSEP and the conjugate export pump multidrug resistance protein 2 [MRP2]). UDCA can be helpful for severe pruritus associated with cholestasis, with the oral antibiotic rifampin often added for refractory pruritus.

Numerous strategies exist to reduce the impact of IFALD, including advancement of enteral feeding as possible, promotion of biliary flow with UDCA, prevention of infection, treating bacterial overgrowth, and adjusting the timing and composition of parenteral nutrition (including cycling of parenteral nutrition, prevention of overfeeding, and change of lipid emulsions).[146] A parenteral fish oil emulsion (Omegaven; Fresenius Kabi, Bad Homberg, Germany) or combination lipid emulsion such as SMOF (Fresenius Kabi), which is composed of soybean oil, fish oil, olive oil, and medium-chain triglycerides, in contrast to the traditional soy-based products, reduces the risk of progressive IFALD. Studies suggest that the pro-inflammatory ω-6-polyunsaturated fatty acids in plant oil–based lipid emulsions (Intralipid, Liposyn III) and the presence of phytosterols contribute to the development of hepatotoxicity. In contrast, fish oil–based lipid emulsions are rich in anti-inflammatory ω-3 fatty acids, which are hepatoprotective and contain no phytosterols. In the United States, Omegaven and SMOF are now allowed for more extensive use.[149] It is important to note that there may be a risk of bleeding in patients receiving parenteral fish oil emulsions.[150] Monitoring for essential fatty acid deficiency remains important when utilizing lipid minimization or alternative lipid emulsions.

Acknowledgments

The authors gratefully acknowledge Ke-You Zhang, MD, and William Berquist, MD, for their helpful review, comments, and suggestions for sections of this chapter.

Key Points

- Neonatal hyperbilirubinemia is usually transient and harmless, but when severe and untreated it can lead to chronic choreoathetotic cerebral palsy (kernicterus) and death or bilirubin-induced neurologic disorders, including severe neurologic hearing impairment (auditory neuropathy/dyssynchrony).
- Although bilirubin neurotoxicity has been limited to a large extent in industrialized countries with functional medical systems, it is still rampant in developing countries and is a major cause of mortality and morbidity globally.
- The etiology of hyperbilirubinemia results from an imbalance between the bilirubin production and elimination; hemolysis and prematurity appear to increase the risk of bilirubin neurotoxicity.
- The serum/plasma total bilirubin (TB), when primarily indirect or unconjugated, is used for clinical assessment and therapeutic decision making. Although unbound bilirubin may offer better prognostic value, its measurement is not available universally in the clinical setting.
- The mainstay of treatment is intensive phototherapy, with the option of exchange transfusion in those infants not responding to that treatment. Therapies using metalloporphyrins have potential in the future.
- The rise of conjugated TB may be the result of primary defects in the hepatocellular transport or excretion of bile or secondary to abnormalities in bile duct function or structure.
- The two most common causes of conjugated hyperbilirubinemia are idiopathic neonatal hepatitis, a noninfectious, nonmetabolic hepatocellular injury, and biliary atresia, a progressive obliteration or absence of extrahepatic bile ducts.
- Abdominal imaging with ultrasound and/or percutaneous liver biopsy for histopathology are key steps to differentiating the many potential causes of neonatal cholestasis, but early genetic testing[151] and newly identified biomarkers are playing an increasingly prominent role.
- Hepatoportoenterostomy to re-establish biliary drainage is an imperative early surgical intervention for biliary atresia.
- Newer fish oil lipid emulsions and combination lipid emulsion products compared to traditional soy-based products may reverse the progression of intestinal failure–associated liver disease.

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