Core Concepts: Bilirubin Metabolism

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Core Concepts: Bilirubin Metabolism

Thor Willy Ruud Hansen, MD, PhD*

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Dr Hansen has disclosed no financial relationships relevant to this article. This commentary does not contain a discussion of an unapproved/investigative use of a commercial product/device.

Abstract
Bilirubin is formed in the reticuloendothelial system as the end product of heme catabolism through a series of oxidation-reduction reactions. The predominant bilirubin isomer in humans is IX-alpha (Z,Z), which, because of its lipophilic nature, can cross phospholipid membranes. In fetal life, this characteristic permits passage of bilirubin through the placenta into the maternal organism for excretion. Postpartum, this same characteristic enables passage of bilirubin across the blood-brain barrier, which is why clinicians worry about jaundice in newborns. Bilirubin is transported in serum bound to albumin. When the bilirubin-albumin complex reaches the liver, bilirubin is transferred into the hepatocytes, where it is bound to ligandin. The next step, which occurs inside the hepatocyte, is binding of bilirubin to glucuronic acid (conjugation) through the enzyme uridine diphosphate glucuronyl transferase (UDPGT). Both ligandin and UDPGT have very low concentrations and activities in the fetus, but activity increases greatly after birth. However, during the time required to increase these enzyme activities, bilirubin accumulates. An important factor in this process is increased bilirubin production through the breakdown of fetal erythrocytes. Once conjugated in the liver, bilirubin is excreted into the bile and transported through the gut with food and further broken down, contributing to the color of stool. Deconjugation and reabsorption of bilirubin can occur in the bowel, a process known as enterohepatic circulation. Increased enterohepatic circulation is believed to contribute to prolonged jaundice in some newborns and may be partially responsible for human milk-associated jaundice. Some of the steps in bilirubin metabolism can be influenced by drugs or feeding.

Objectives
After completing this article, readers should be able to:

1. Recognize the different steps in bilirubin metabolism.
2. Describe how bilirubin metabolism changes from fetal to postnatal life.
3. Explain why bilirubin accumulates in the newborn.
4. Describe how the isomers of bilirubin may be relevant to its distribution in the body.
5. Explain why the enterohepatic circulation of bilirubin may influence the course of neonatal jaundice.

Definition
Neonatal jaundice occurs when the concentration of bilirubin in serum (TSB) increases to the point where the accumulation of bilirubin in skin becomes visible to the unaided eye in daylight conditions (or similar-quality artificial light). Such visual detection is possible when the TSB exceeds 5 to 6 mg/dL (85 to 100 mcmol/L) and varies between observers and lighting conditions. A distinction often is made between physiologic and nonphysiologic jaundice. This distinction is useful didactically but often not possible clinically. For simplicity, physiologic jaundice may be said to be present when bilirubin production is increased and excretion capacity is low as part of a normal transitional process. Nonphysiologic jaundice may be said to be present when bilirubin production is exaggerated beyond normal or bilirubin excretion is reduced below normal for the newborn period. The Table summarizes some key concepts of neonatal bilirubin metabolism.

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Bilirubin Metabolism

Production

The life span of red cells in the newborn is about 1.5 to 3 months. (1) Degradation of heme from red cells, myoglobin, cytochromes, and catalases leads to production of bilirubin. (2) Biliverdin is formed as an intermediate step. Carbon monoxide is a byproduct of this process and can be measured in exhaled breath, providing an estimate of bilirubin production. (3) Bilirubin production in the neonate has been estimated to be about 8.5 mg/kg per day, approximately double the rate of 4 mg/kg per day in adults. (4)

The first step in heme catabolism (Figure) involves heme oxygenase, an enzyme that is found in the reticuloendothelial system but also in other tissues. (6)(7) Reduction of biliverdin IX-alpha to bilirubin IX-alpha takes place in the cytosol, catalyzed by biliverdin reductase. (8) Bilirubin IX-alpha in the serum of humans occurs almost exclusively as the (4Z,15Z) isomer. The apparent lipophilic nature of this bilirubin isomer can be explained by the intramolecular hydrogen bonds between the side groups (Z = zusammen - German for “together”). (8) A more detailed review recently was published in NeoReviews. (9)

Table. Mechanisms of Neonatal Hyperbilirubinemia

<table>
<thead>
<tr>
<th>Physiologic Jaundice in the Newborn</th>
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<tbody>
<tr>
<td>• Catabolism of heme</td>
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<tr>
<td>– From breakdown of fetal erythrocytes</td>
</tr>
<tr>
<td>– From myoglobin, cytochromes, catalase</td>
</tr>
<tr>
<td>• Decreased uptake into and excretion from liver cells</td>
</tr>
<tr>
<td>– Low neonatal concentration of ligandin, the intracellular binding protein</td>
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<tr>
<td>– Low neonatal activity of uridine diphosphate glucuronyl transferase (UDPGT)</td>
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<table>
<thead>
<tr>
<th>Nonphysiologic Jaundice</th>
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</thead>
<tbody>
<tr>
<td>• Increased heme catabolism</td>
</tr>
<tr>
<td>– Congenital hemolytic anemias (eg, glucose-6-phosphate dehydrogenase deficiency, spherocytosis)</td>
</tr>
<tr>
<td>– Immunologically mediated hemolysis (eg, Rhesus and ABO incompatibility)</td>
</tr>
<tr>
<td>• Decreased bilirubin conjugation and excretion</td>
</tr>
<tr>
<td>– Genetic defects in UDPGT (eg, Crigler-Najjar, Arias type 2, Gilbert)</td>
</tr>
<tr>
<td>– Hepatic and biliary disease (eg, neonatal hepatitis, intra- and extrahepatic biliary atresia)</td>
</tr>
<tr>
<td>• Increased enterohepatic circulation of bilirubin</td>
</tr>
<tr>
<td>– Decreased bowel passage (eg, intestinal atresias, necrotizing enterocolitis, fasting, inadequate nutrition [“breast feeding jaundice”])</td>
</tr>
<tr>
<td>– Increased deconjugation of bilirubin in the bowel (eg, breast milk jaundice)</td>
</tr>
</tbody>
</table>

Figure. Schematic illustration of bilirubin metabolism in fetal and neonatal life. Bilirubin is formed in the reticuloendothelial system from heme through reactions catabolized by heme oxygenase and biliverdin reductase. Bilirubin is transported in serum bound to albumin, but a minor fraction is unbound (“free”) and can cross the blood–brain barrier or (in the fetus) the placental barrier. Bilirubin is transported into the cell and bound to ligandin. UDPGT catabolizes the conversion of bilirubin to a water-soluble form through binding to one or two molecules of glucuronic acid, forming bilirubin conjugates. Excreted in the bile, these conjugates subsequently are transformed to urobilinoids through bacterial action. Conjugated bilirubin also may undergo deconjugation, and the unconjugated bilirubin can be reabsorbed into the circulation (enterohepatic circulation). B=bilirubin, Fe=iron, CO=carbon monoxide, UDPGT=uridine diphosphate glucuronyl transferase. Modified from Hansen. (5)
**Transport and Protein Binding**

Unconjugated bilirubin is transported in plasma bound to albumin, with a high binding affinity at the primary binding site. In addition, a secondary binding site has a lower affinity. Because bilirubin is tightly bound to albumin, concentrations of free (“unbound”) bilirubin are in the low nanomolar range, even in babies who exhibit significant jaundice. (11) If the molar concentration of bilirubin exceeds that of albumin, the primary binding site is saturated, and free bilirubin concentrations increase. (12) The binding of bilirubin to albumin increases with postnatal age (13) but is reduced in sick infants (14) and in the presence of exogenous or endogenous binding competitors such as certain drugs. (15) Bilirubin also can bind to other proteins (e.g., alpha-fetoprotein and ligandin), lipoproteins, and erythrocytes.

**Uptake, Conjugation, and Excretion**

When the bilirubin-albumin complex comes into contact with hepatocytes, bilirubin is transported into the cell. This process may be carrier-mediated. (16) Inside the liver cells, about 60% of bilirubin is found in the cytosol and about 25% in microsomes. (17) Ligandin, a glutathione-S-transferase, is responsible for binding bilirubin inside the cells. (18) Uptake of bilirubin into hepatocytes increases with increasing concentrations of ligandin. (16) Ligandin concentrations in the liver are low at birth but appear to reach adult values within 1 to 2 weeks of age. (19) The concentration of ligandin in hepatocytes can appear to reach adult values within 1 to 2 weeks of age. (12) The binding of bilirubin to albumin increases with postnatal age (13) but is reduced in sick infants (14) and in the presence of exogenous or endogenous binding competitors such as certain drugs. (15) Bilirubin also can bind to other proteins (e.g., alpha-fetoprotein and ligandin), lipoproteins, and erythrocytes.

Glucuronyl transferase (UDP glucuronate beta-glucuronosyl transferase EC 2.4.1.17) catalyzes the binding of glucuronic acid to bilirubin. This converts the essentially water-insoluble unconjugated bilirubin to a more polar form that can be excreted in the bile. Bilirubin monoglucuronide is formed initially in the endoplasmic reticulum of microsomes; (20) formation of the diglucuronide probably occurs at the cell membrane. (21) Bilirubin-UDP-glucuronyl transferase in humans is a tetramer. (22) The monomer can convert unconjugated bilirubin to monoglucuronide, but the tetramer is needed for formation of the diglucuronide. (21)

Excretion of conjugated bilirubin into bile occurs against a concentration gradient, making it a process that requires energy. Clearance of bilirubin from the hepatocyte is a saturable process, and after the first postnatal week appears to be the rate-limiting step in bilirubin excretion. The excretory transport maximum can be increased by drugs that stimulate bile flow, such as barbiturates. (20)

Glucuronyl transferase activity increases 100-fold after birth, reaching adult values by 1 to 2 months of age. (23) In the first days after birth, conjugated bilirubin constitutes less than 2% of total bile pigment in serum. (24) Diconjugates make up about 20% of the total conjugated fraction in babies, less than half of the estimated 50%+ in adults. (24) Glucuronyl transferase is inducible. Thus, administering phenobarbital to pregnant women increases conjugation in the neonate. (25) Phenobarbital has been used both before and after birth to prevent or treat neonatal jaundice. (26) Dexamethasone and clofibrate also increase bilirubin conjugation.

Heme can be excreted directly in bile, but this is accompanied by the loss of iron. Heme oxygenase can be inhibited through the use of metal meso- and protoporphyrins, (27) and this is a therapy for neonatal jaundice that is undergoing testing. (28) Biliverdin also can be excreted in bile. The biologic puzzle of why humans do not excrete nontoxic biliverdin but process this one step further to potentially toxic bilirubin may have a partial answer in the discovery that bilirubin is a free radical quencher. (29) Also, bilirubin can diffuse through the placental membranes from fetus to mother, whereas biliverdin would have needed a transporter.

Alternate pathways for bilirubin disposition through the liver also may exist. Thus, there is evidence that hemin, biliverdin, and bilirubin can induce such pathways by activating the aryl hydrocarbon receptor that, in turn, activates hepatic CYP1A1 gene expression. (30) Whether such pathways play any role in human infants currently is not known.

When bilirubin enters the bowel, deconjugation may occur in the small intestine, mediated by beta-glucuronidases in the brush border. Unconjugated bilirubin then can be reabsorbed into the circulation. This cycle of conjugation, excretion, deconjugation, and reabsorption is called enterohepatic circulation. Enterohepatic circulation may be significant in the newborn, in part due to limited nutrient intake in the first postnatal days, which prolongs intestinal transit time. However, it also is believed that factors in human milk in some mother-infant dyads, which have not been unequivocally identified, may contribute to increased enterohepatic circulation in so-called human milk-associated jaundice.

**Bilirubin in the Fetus**

Bilirubin appears in the human fetus at 14 weeks of gestation. (31) At 16 weeks, unconjugated bilirubin-IX-alpha appears in bile. By 38 weeks’ gestation, bilirubin-IX-alpha is the primary isomer. (29) Umbilical vein blood samples show total bilirubin concentrations in-
Bilirubin metabolism is different in the fetus compared with the neonate. Unconjugated bilirubin in the fetus can be disposed of either by crossing the placenta into the maternal circulation or by passing through the fetal liver and being excreted into fetal bile. Conjugation and excretion into bile is associated with accumulation of a quantity of bilirubin in meconium corresponding to 5 to 10 times daily production. (19) However, most fetal bilirubin production must be handled differently.

Placental membranes are essentially impermeable to polar compounds such as biliverdin and bilirubin conjugates, but nonpolar compounds such as unconjugated bilirubin can diffuse across the membranes. A study in rhesus monkeys, which have a placenta structurally very similar to that in humans, found that more than 50% of bilirubin infused into the fetus was transferred intact across the placenta and excreted in maternal bile, with only 3% to 6% found in fetal bile. (32) Bilirubin also is transferred rapidly into and removed from the amniotic fluid. (33) The umbilical artery contains bilirubin in a concentration nearly twice that of the umbilical vein, showing that bilirubin is cleared from fetal blood when it passes through the placenta. (34)

**Bilirubin in Fetal Hemolytic Disease**

When hemolysis occurs in the fetus in maternal alloimmunization, the concentration of TSB may increase from approximately 1.5 mg/dL (25 mcg/mL) at 20 weeks of gestation to approximately 4.1 mg/dL (70 mcg/mL) at 32 weeks’ gestation. In a study of fetuses that developed anemia (hematocrit <30% [0.30]), 82% had serum bilirubin concentrations that exceeded the 97.5th percentile. (35) The increase in serum bilirubin was detectable weeks before anemia developed. Thus, although bilirubin can pass the placenta, hemolytic disease in the fetus causes some hyperbilirubinemia. Some of the bilirubin is conjugated and cannot cross the placenta, but the major fraction is unconjugated. (36) Therefore, it follows that the placenta has a limited capacity for transfer of bilirubin and that this capacity is exceeded in fetal hemolytic disease.

**Bilirubin in the Amniotic Fluid**

Although bilirubin has been found in amniotic fluid in early gestation, normally there is no bilirubin in amniotic fluid near term. Bilirubin initially was found in the amniotic fluid in cases of Rhesus immunization, (37) and the concentrations of bilirubin in amniotic fluid have been shown to correlate with the degree of fetal effects. Because amniotic fluid is primarily a product from the fetus, bilirubin in amniotic fluid probably comes from the fetus itself.

**Mechanisms of Physiologic and Nonphysiologic Jaundice**

Most newborns develop a serum unconjugated bilirubin value greater than 1.8 mg/dL (30 mcg/mL) during the first postnatal week. The incidence of significant neonatal jaundice varies among populations and geographic locations. For example, neonatal jaundice is more prevalent in populations living at high altitudes, likely due to increased red cell mass. Noninvasive bilirubin measurements on 490 term and near-term infants from a racially diverse background showed that 80% had bilirubin concentrations greater than 5 mg/dL (85 mcg/mL). (38) Approximately the value at which the human eye can detect jaundice in the skin. The course of hyperbilirubinemia in neonates is characterized by a peak between the second and fourth postnatal days, followed by a decline that initially is rapid and subsequently tapers. Jaundice is a very common phenomenon in neonates and a frequent reason for clinical concern and investigation.

The term physiologic jaundice should be applied to jaundice in newborns due to the normal occurrences of increased breakdown of red cells in the presence of a low capacity for uptake, conjugation, and excretion of bilirubin in the liver. However, the distinction between physiologic and nonphysiologic jaundice in newborns often is not clear. Thus, the normally occurring mechanisms that produce hyperbilirubinemia may be exaggerated by both endogenous and exogenous factors.

**Increased Heme Catabolism**

The fetus lives in an oxygen-poor environment. To compensate for this, the fetus has a hemoglobin that is structurally different from the one formed in postnatal life as well as a higher hemoglobin concentration. These compensatory mechanisms are not needed after birth. Breakdown of fetal red cells, resulting in production of bilirubin, may be increased in congenital hemolytic anemias, in immunologic conditions such as maternal-fetal blood group incompatibilities, and due to drugs or toxins. Infections or bruising/hematomas also can shorten red cell survival.

**Decreased Hepatic Uptake, Conjugation, and Excretion of Bilirubin**

The neonate is deficient in ligandin, the hepatocellular binding protein for bilirubin. There is no evidence that...
deficiency of ligandin beyond the physiologic degree is implicated in nonphysiologic jaundice. However, deficiency of UDPGT occurs in several hereditary disorders.

**Increased Enterohepatic Circulation**

Enterohepatic circulation occurs in the presence of delayed or interrupted passage of intestinal contents. This can happen in intestinal atresias, in infants in whom oral feedings are held for reasons of severe illness, and in infants receiving inadequate nutrition due to difficulties in establishing lactation (“lack-of-breast-milk jaundice”). It is also possible that increased enterohepatic circulation is involved in so-called human milk jaundice. (20) On the other hand, enterohepatic circulation is reduced following the successful establishment of enteral nutrition, by increasing the frequency of feeding, and following supplementation with human milk substitutes. (39)

**Genetics and Bilirubin Metabolism**

It is increasingly recognized that the risk for neonatal jaundice may be modulated by genetics. Crigler-Najjar syndrome and Arias syndrome are well-recognized causes of extreme jaundice in neonates. The impact of Gilbert syndrome on jaundice in the newborn also has become apparent. Crigler-Najjar syndrome is caused by a stop or nonsense mutation in the gene for UDP-GT1A1, Arias syndrome is due to a missense mutation in the same gene, and Gilbert syndrome is caused by mutations in the promoter sequence. (40) Recently, genetic polymorphism for the organic anion transporter protein OATP-2 was shown to be associated with a threefold increased risk for developing marked neonatal jaundice. (41) Glucose-6-phosphate dehydrogenase deficiency and other hereditary hemolytic anemias also are associated with increased risk of neonatal jaundice. In a recent study across three genes that had an impact on bilirubin metabolism (G6PD, UGT1A1, and OATP1B1), coexpression of two or more of these genes was common. (42) Accordingly, genetic polymorphisms probably are important in explaining some of the clinically observed variability in the course and degree of neonatal jaundice.

**The Impact of Bilirubin Isomers**

Isomers of bilirubin differ in their polarity. The predominant IX-alpha (Z,Z) isomer is almost insoluble in water, but the isomers that arise when bilirubin is exposed to light, primarily IX-alpha (Z,E) and lumirubin, are more polar and more soluble in water. (43) These water-soluble isomers can be excreted in bile and urine without conjugation, accounting for the therapeutic effect of phototherapy. By virtue of its lipophilic nature, bilirubin IX-alpha (Z,Z) can enter the brain. However, the presence of P-glycoprotein in the blood-brain barrier appears to block bilirubin entry to some extent, (44) so the concentration of bilirubin in brain is less than might be expected, given its solubility characteristics. It has been speculated that the more polar bilirubin photoisomers should be less able to diffuse across the blood-brain barrier. However, no experimental evidence to date supports this theoretically based hypothesis.

**Conclusion**

Bilirubin metabolism undergoes significant changes during fetal and neonatal life in terms of the balance of isomers, the processes involved in uptake into the hepatocyte, binding and conjugation inside the hepatocyte, and excretion into the bile. Several steps in the process that causes neonatal jaundice can be modulated both by endogenous and exogenous compounds. Drugs that increase the concentration of ligandin or the activity of bilirubin UDPGT are well documented but appear not to be used widely. Drugs that limit bilirubin production by inhibiting heme oxygenase are undergoing trials, but their eventual place in the routine management of neonatal jaundice remains to be determined. Enterohepatic circulation contributes to neonatal jaundice and is amenable to therapeutic intervention by formula feedings or other bilirubin binding or degrading agents.

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