Bilirubin Metabolism

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INTRODUCTION

The metabolism of bilirubin has been under intense investigation for many years; research has been directed at both understanding the metabolism of this pigment in relation to clinical liver disorders and understanding the physical-chemical parameters of the bilirubin molecule (including bilirubin conjugates) and its interaction with other lipids and proteins. As it turns out, these lines of investigation often intersect, with the physical properties often explaining the clinical disorders. This article will concentrate on those areas of bilirubin metabolism that have clinical correlates.

Not only is it important to understand the metabolism of bilirubin because hyperbilirubinemia is a common pediatric problem, but also because hyperbilirubinemia can be a manifestation of a multitude of disorders that are not primarily associated with liver disease (Table 1). Also, with an increasing percentage of pediatric in-patients requiring intensive care, the causes of hyperbilirubinemia in any given patient may often be multifactorial. Thus, a premature baby may develop hyperbilirubinemia from a relative deficiency of glucuronyl transferase, hemolysis, sepsis, metabolic disease or occasionally even cholelithiasis. Understanding the metabolism of bilirubin will often help the clinician sort through the myriad of diagnostic possibilities and decide what additional tests are required.

MEASUREMENT AND INTERPRETATION OF SERUM BILIRUBIN LEVELS

Although the preceding article discusses in detail the approach to the interpretation of liver function tests, several points on the measurement and interpretation of serum bilirubin levels require particular emphasis here. Measurement of bilirubin varies considerably from laboratory to laboratory and clinical decisions should be based upon the experience of an individual laboratory. Notwithstanding the fact that Van den Berg began measuring direct (conjugated) bilirubin from indirect (unconjugated) bilirubin more than 75 years ago, measured serum values of direct bilirubin are notoriously inaccurate, particularly with total serum bilirubins of less than 5 mg%. Thus if one measures the bilirubin of an infant on one day and the total is 10 mg% with a direct fraction of 6 mg%, and the next day the total is 8.5 mg% and the direct is 4 mg%, one cannot assume that the baby is showing evidence of improvement and has resolving cholestasis. The small decrement in bilirubin may simply be due to the day to day variation in bilirubin measurement or represent an increased urinary clearance of conjugated bilirubin. We cannot solely blame these problems with bilirubin measurement on the clinical laboratories. Although it is well-known that bilirubin is light-sensitive and that bilirubin conjugates will undergo hydrolysis to unconjugated bilirubin, in most if not all hospitals, no attempts are made to protect bilirubin samples from light or to prevent hydrolysis by placing these blood samples on ice and processing them immediately. Although the serum bilirubin levels seemed to decrease slightly in the aforementioned infant, this baby is just as likely to have biliary atresia as an intrahepatic etiology of cholestasis. Also, one cannot assume that low serum levels of total bilirubin exclude the diagnosis of biliary atresia or neonatal hepatitis, especially during the first 3 weeks of life. Thus we have seen infants less than 1 month of age with either biliary atresia or hepatitis that have had total bilirubins less than 3.0 mg%.

Recent evidence suggests that bilirubin conjugates may be converted to bilirubin-protein conjugates through a non-enzymatic covalent linkage of bilirubin to albumin. Although conjugated bilirubin is cleared by the kidneys, this covalently linked bilirubin conjugate is not, similar to the way unconjugated bilirubin non-covalently linked to albumin is not cleared by the kidney. Thus, in patients with extrahepatic biliary obstruction that is relieved by surgery or, for example by removal of a common duct bile stone, direct hyperbilirubinemia may persist for several weeks after the obstruction has been relieved because of delayed clearance of bilirubin-protein conjugates (Table 2).
TABLE 1
MECHANISMS OF UNCONJUGATED HYPERBILIRUBINEMIA IN THE NEWBORN

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Details</th>
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<tbody>
<tr>
<td>I. Increased &quot;load&quot; of bilirubin</td>
<td>A. Increased RBC volume—especially with delayed clamping of the umbilical cord</td>
</tr>
<tr>
<td>B. Increased RBC turnover</td>
<td>1. Normal—half life of RBC</td>
</tr>
<tr>
<td>2. Abnormal—primarily extravascular erythrocyte destruction in:</td>
<td>a. Isoimmunization—Rh or ABO incompatibility</td>
</tr>
<tr>
<td>3. Erythrocyte biochemical defects—G6PD, pyruvate kinase or hexokinase deficiency</td>
<td>c. Structural abnormalities of erythrocytes: spherocytosis, elliptocytosis, pyknocytosis</td>
</tr>
<tr>
<td>C. Sequestrated blood</td>
<td>1. Subdural hematoma/cephalocelema</td>
</tr>
<tr>
<td>2. Echymoses</td>
<td>3. Hemangiomas</td>
</tr>
<tr>
<td>II. Enterohepatic circulation of bilirubin</td>
<td>A. Bilirubin with abnormal breast milk</td>
</tr>
<tr>
<td>B. Bilirubin with gastrointestinal obstruction</td>
<td></td>
</tr>
<tr>
<td>III. Decreased activity of glucuronyl transferase (GT)</td>
<td>A. Transient in every newborn</td>
</tr>
<tr>
<td>B. Total absence of GT in Crigler-Najjar, type I</td>
<td>C. Permanent deficiency in Crigler-Najjar, type II, and Gilbert's syndrome</td>
</tr>
<tr>
<td>D. Hypopituitarism or hypothyroidism</td>
<td>V. Multifactorial</td>
</tr>
<tr>
<td>A. Sepsis—hemolysis, uptake, excretion</td>
<td></td>
</tr>
<tr>
<td>B. Prematurity—GT, acidosis, sepsis, TPN drugs, patent ductus venous, transfusions</td>
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TABLE 2
TYPES OF BILIRUBIN

<table>
<thead>
<tr>
<th>Type of Bilirubin</th>
<th>Description</th>
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<tbody>
<tr>
<td>I. Unconjugated bilirubin</td>
<td>A. &quot;Free&quot; bilirubin</td>
</tr>
<tr>
<td></td>
<td>1. In Z-Z configuration: insoluble</td>
</tr>
<tr>
<td></td>
<td>2. Diffuses across blood-brain barrier</td>
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<tr>
<td></td>
<td>3. In Z-E, E-E, or E-Z configuration (photobilirubin): water soluble and excreted without conjugation</td>
</tr>
<tr>
<td>B. Albumin or &quot;bound&quot; bilirubin</td>
<td>1. Not readily available</td>
</tr>
<tr>
<td></td>
<td>2. Not cleared by the kidney</td>
</tr>
<tr>
<td></td>
<td>3. Does not readily cross the blood-brain barrier</td>
</tr>
<tr>
<td>II. Conjugated bilirubin (free)</td>
<td>A. Predominantly conjugated with glucuronic acid</td>
</tr>
<tr>
<td></td>
<td>B. Mono conjugates in Gilbert's disease, Crigler-Najjar type II and newborns</td>
</tr>
<tr>
<td></td>
<td>C. Diconjugates are more soluble than monoconjugates</td>
</tr>
<tr>
<td></td>
<td>D. Diconjugates predominant in human bile</td>
</tr>
<tr>
<td></td>
<td>E. Cleared by the kidney in cholestasis</td>
</tr>
<tr>
<td>III. Protein-bilirubin conjugates</td>
<td>A. Form in plasma by nonenzymatic covalent linkage to albumin in patients with cholestasis</td>
</tr>
<tr>
<td></td>
<td>B. Not excreted in urine</td>
</tr>
<tr>
<td></td>
<td>C. Remain in serum for several weeks after etiology of cholestasis is resolved</td>
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HEME CATABOLISM

Heme, the ultimate source of bilirubin, is derived from a variety of sources or pools which vary in their rates of turnover. Heme containing enzymes in the liver such as cytochrome P450, catalase, and peroxidases are degraded to bilirubin in a matter of a few hours to a few days after their synthesis from the heme precursor, aminoolevulnic acid. On the other hand, heme incorporated in red cells turns over more slowly, with half-lives of approximately 120 days in adults and 80 days in newborns.

Heme oxygenase is the enzyme present in the cell smooth endoplasmic reticulum that converts heme to biliverdin by splitting heme at the alpha carbon bridge. During this enzymatic step, carbon monoxide is released. Since this is the only reaction in the body in which carbon monoxide is produced, the production of carbon monoxide production indicate the rate of heme turnover. Such studies indicate that the newborn is capable of producing 8.5 ± 2.5 mg of bilirubin per kg per day—more than twice the rate observed in the adult. This enzyme, although present in many different types of cells (eg, kidney and gastrointestinal epithelial cells) has its highest activity in organs involved in the sequestration of red cells such as spleen and liver. The activity of heme oxygenase is enhanced in hemolytic states but it is still the rate-limiting enzyme in the conversion of heme to bilirubin.

The next enzyme involved in the heme degradative pathway, biliverdin reductase, is present in the cytosol and converts biliverdin, a green compound, to bilirubin, a yellow-orange compound.

Clinical Importance of the Heme Degradative Pathway

Recently, Kappas and co-workers have determined that
tin-protoporphyrin, a potent inhibitor of heme oxygenase will decrease bilirubin levels in jaundiced animals and in adult humans. This compound inhibits heme oxygenase by binding to the enzyme active site; since tin is substituted for the iron portion, tin-protoporphyrin is not converted to biliverdin and remains bound to the active site, thereby preventing further heme degradation. The excess heme which is not converted into bilirubin is excreted as free heme in bile.

Obviously, this discovery may have implications in the future treatment of neonatal hyperbilirubinemia. To date, this compound has no significant toxicity when administered at doses necessary to inhibit heme oxygenase. There have been no clinical trials in newborns, but it seems likely that this compound, if found safe, will be particularly useful in situations where access to phototherapy is either nonexistent or severely limited.

If heme catabolism is accelerated by hemolysis from any etiology (Table 1), unconjugated bilirubin levels may rise. In the adult human, unconjugated bilirubin levels will rarely be over 6 mg% with hemolysis unless there is underlying liver disease; the normal liver is capable of converting heme to bilirubin, and perhaps excreting free heme in bile at a rate fast enough to prevent accumulation. The newborn who is relatively deficient in glucuronyl transferase may have unconjugated hyperbilirubinemia that increases to levels that are toxic to the central nervous system. In a full-term newborn, this represents levels that are greater than 20 mg%. Whether newborns excrete free heme in bile with hemolytic stress is not known.

BILIRUBIN TRANSPORT IN PLASMA

Once bilirubin is formed in the reticuloendothelial system, it is transported to the liver tightly bound to serum albumin. The hepatocytes have a highly efficient capacity of removing unconjugated bilirubin and other organic ions from the plasma. Bilirubin bound to albumin interacts with an as-yet uncharacterized binding site on the hepatocyte plasma membrane surface. The albumin becomes detached and returns to the circulation. The presence of an albumin receptor provides an explanation for the efficient hepatic extraction of a variety of ligands which are tightly bound to albumin.

Transport of bilirubin across the plasma membrane appears to be carrier-mediated and exhibits saturation and competitive inhibition. The transport mechanism for unconjugated bilirubin is shared by conjugated bilirubin, indophenol (x-ray contrast agent) and BSP, but is distinct from that of bile acids.

Intracellular Transport and Conjugation of Bilirubin

After bilirubin passes across the hepatocyte plasma membrane, it binds to hydrophobic cytosolic proteins, principally glutathione-S-transferase B, also known as ligandin. Within the cell, ligandin serves as the major transport protein for bilirubin, and its high affinity for bilirubin may prevent bilirubin from diffusing into mitochondria where it could inhibit mitochondrial respiration. Ligandin may also diminish the efflux of bilirubin from the hepatocyte.

Since bilirubin is a non-polar compound with poor aqueous solubility, it must be rendered polar before excretion into bile. Although cholesterol and lecithin are also non-polar lipids that are excreted in non-polar forms, bilirubin, in the concentrations usually present in bile would be insoluble unless conjugated, while cholesterol and lecithin are solubilized in mixed micelles. Thus, it is the function of UDP-glucuronyl transferase to conjugate (esterify) one or both of the propionic acid groups of bilirubin with a polar sugar. In humans, the most abundant sugar group is glucuronic acid, but other less polar sugars conjugates such as glucose and xylose are found in human bile.

UDP-glucuronyl transferase is a microsomal (smooth endoplasmic reticulum) enzyme that catalyzes the glucuronidation of a variety of lipophilic compounds other than bilirubin such as thyroxin, sex steroids, certain bile acids and xenobiotics (eg, lorazepam, morphine, chloramphenicol). It appears that there are several different forms of glucuronyl transferase present and that the genetic absence of the form that conjugates bilirubin (ie, Crigler-Najjar, type 1 syndrome) does not prevent the glucuronidation of xenobiotics.

The action of glucuronyl transferase on bilirubin may result in two forms of bilirubin—bilirubin monoglucuronide and bilirubin diglucuronide. Since bilirubin is an asymmetric molecule, the monoglucuronide form of bilirubin actually exists as a pair of isomers. The aqueous solubility of the monoglucuronide is intermediate between that of the unconjugated insoluble bilirubin and bilirubin diglucuronide which is fairly water soluble. Adult human bile usually contains 80% to 90% diconjugated bilirubin. The majority is in the form of the diglucuronide, with small amounts of other sugar conjugates, 10% to 20% monoglucuronides and 1% or less of unconjugated bilirubin. The neonate may have 50% or more of its bile pigments in the form of monoconjugates and patients with Gilbert's syndrome and Crigler Najjar, type II also have an increased percentage of monoconjugates. In addition, people with hemolytic anemias may have an increased proportion of monoconjugated bilirubin.

On a biochemical level, it appears that either a relative deficiency of glucuronyl transferase or a relative excess of bilirubin substrate results in a higher percentage of bilirubin in the form of the monoconjugate. This is striking evidence that there is only one enzyme (perhaps with two or more subunits) that is involved in the process of conjugating bilirubin. Presumably, when excess bilirubin substrate is present, the bilirubin does not stay in contact with the enzyme long enough to become diconjugated; this implies that the affinity of the enzyme for unconjugated bilirubin is greater than for the monoconjugated bilirubin, even if the mono-form is already at the active site.

A different mechanism for formation of bilirubin diglucuronide has also been postulated. According to this scheme, the monoglucuronide and small amounts of diglucuronide are formed from glucuronyl transferase, however, the majority of the diglucuronide is formed by a hepatocyte canalicular isomerase that catalyzes the formation of the diglucuronide and unconjugated bilirubin from two
molecules of monoglucuronide. Although this conversion can occur, it now appears that this conversion is a non-enzymatic reaction that occurs through dipyrrole exchange and has no physiologic significance.

BILIARY SECRETION OF BILIRUBIN

The mechanism of transport of bilirubin from the cytosol of the hepatocyte across the canalicular membrane and into bile has not been well-delineated. This process is believed to be the rate-limiting step in the overall transport of bilirubin from blood to bile and is presumably responsible for hyperbilirubinemia associated with hepatocellular and hepatocanalicular disorders.

A direct correlation exists between bile acid flow and bilirubin output. Intravenous infusions of bile acids result in increased excretion of bilirubin conjugates. Bile acids in canalicular bile may exert such an effect by providing a "micellar sink" such that bilirubin within the micelle is not readily available for back diffusion through the canalicular membrane.

DISORDERS OF BILIRUBIN METABOLISM

Neonatal Hyperbilirubinemia

The normal serum bilirubin of an adult should not exceed 2 mg%. By this standard, every newborn infant has hyperbilirubinemia. This transient hyperbilirubinemia is usually benign in normal full-term infants and has been referred to as "physiologic jaundice" of the newborn. In the absence of hemolysis, hyperbilirubinemia of the neonate can be characterized as "physiologic" if the maximal serum bilirubin does not exceed 12 mg% in the full-term neonate and 15 mg% in the premature within the first week of life. About 50% of all neonates have clinically apparent jaundice, characteristically with serum bilirubin concentrations that peak at an average of 5 to 6 mg% within 72 hours of birth and then gradually decrease until normal levels are obtained in 7 to 10 days. However, in a series of 4,000 consecutive infants, 16% had maximal serum bilirubin levels of 10 mg% or above and 5% had levels that exceeded 20 mg%. It is well worth repeating that any baby jaundiced enough to require a serum bilirubin should have a direct bilirubin performed and that if the direct exceeds 20% of the total, the infant does not have physiological jaundice but rather, neonatal cholestasis.

A host of mechanisms is responsible for neonatal jaundice. On a per kg basis, the newborn produces an average of twice as much bilirubin as the adult. This increased production of bilirubin is the result of an increased circulating red blood cell volume and a shorter mean red cell life span. A variety of hemolytic disorders may affect the neonate and further increase the load of bilirubin required for excretion (Table 1). Furthermore, at least in the neonatal thalassemia major, the hepatic uptake of bilirubin is impaired and seems to correlate with the maturation of hepatic ligandin. Glucuronyl transferase activity is diminished in the newborn and as a result, the newborn has an impaired ability to handle the excessive load of bilirubin. The combination of an increased load of bilirubin and a decreased level of conjugating enzyme results in a higher proportion of bilirubin secreted in the monoconjugated form as mentioned previously. Finally, either as a result of decreased canalicular excretion or as a result of precipitation of bilirubin in the canaliculus, further increases in the bilirubin load may result in the accumulation of conjugated bilirubin in the serum.

The Enterobacterial Circulation of Bilirubin

One potential pathway leading to increased bilirubin levels in the newborn is through an enterobacterial circulation of bilirubin. The newborn is particularly susceptible to this mechanism of hyperbilirubinemia since the newborn lacks the normal bacterial flora that break bilirubin down to polar, non-absorbable compounds (sterocobin or urobilinogen) which are excreted in feces or in the urine. As a result of the transient absence of this bacterial flora, bilirubin conjugates are presumably hydrolyzed by intestinal beta-glucuronidase to lipophilic unconjugated bilirubin that can diffuse across the lipophilic enterocyte membrane and be absorbed into portal blood. This absorbed bilirubin is then picked up by the liver, conjugated and re-excreted. The process of excretion, absorption and re-excretion represents an enterohepatic circulation of bilirubin, similar to the familiar cycling of bile salts through the gastrointestinal tract and the liver. Thus, neonatal intestinal obstruction, which provides a prolonged time for reabsorption of bilirubin, is associated with high serum levels of unconjugated bilirubin. For the same reason, oral charcoal or agar which interfere with bilirubin absorption in the neonate may reduce serum UCB levels by preventing the enterohepatic circulation of bilirubin.

Breast Milk Jaundice

Breast milk jaundice (Table 3) is a well-recognized form of unconjugated hyperbilirubinemia in which serum bilirubin concentrations rise rapidly after the fourth day of life and peak at the end of the second week of life. This phenomenon occurs in about 0.5% to 2% of otherwise healthy breast fed infants, and although bilirubin levels may occasionally reach levels that require phototherapy, kernicterus has not been reported with this entity alone. Even with continued ingestion of the "abnormal milk," serum bilirubin levels decline gradually over a period of 3 to 16 weeks. If serum bilirubin levels are high enough to require phototherapy, it is prudent to temporarily discontinue breast feeding; a 24-hour cessation of breast feeding will usually be sufficient to allow a significant reduction in serum bilirubin levels—a test which is both diagnostic and therapeutic.

Based on studies in rats, normal cow's milk and normal human milk inhibit the absorption of bilirubin from the GI tract. However, "abnormal" breast milk not only failed to inhibit absorption of bilirubin but actually enhanced the absorption of bilirubin from the GI tract. The mechanism for this enhanced absorption has not been defined but may be a result of an increased concentration of free fatty acids found in these abnormal milks. Similar to the way free fatty acids displace bilirubin from serum albumin, free fatty acids in the gut could potentially displace bilirubin from the surface of undigested proteins, thereby increasing the concentration of free bilirubin that can diffuse across the
cytoplasmic membrane of the intestinal epithelial cell.

Crigler-Najjar Syndrome, Type I
This is a rare form of neonatal hyperbilirubinemia in which there is total absence of glucuronyl transferase. As a result, there is severe unconjugated hyperbilirubinemia without evidence of hemolytic anemia. Kernicterus usually occurs unless the affected patients are treated with continuous phototherapy during early childhood. After the age of 3 or 4 years, phototherapy becomes less effective as a result of thickening of the skin and decreased surface area to body mass. During acute exacerbations, especially if neurologic symptoms are present, plasmapheresis is indicated. A number of affected people with this syndrome survive the neonatal period intact only to succumb to neurologic sequelae during adolescence or early adulthood. The syndrome is transmitted as an autosomal recessive trait and is often associated with consanguinity.

Gunn rats, the animal model for Crigler-Najjar type I, completely lack the ability to conjugate bilirubin. However, when transplanted with either hepatoma cells or with Wistar (normal) rat hepatocytes, these animals have the ability to conjugate bilirubin. Therefore, it appears that hepatic transplantation for patients with severe uncontrollable hyperbilirubinemia may soon be in order. In addition, this disease lends itself to the possibility of future enzyme replacement when the biotechnology for this type of therapy becomes available.

Crigler-Najjar Syndrome, Type II
Patients with this syndrome, when compared with type I patients, have a milder form of unconjugated hyperbilirubinemia. Typically serum bilirubins are less than 20 mg/dL but can increase to as high as 40 mg/dL with fasting or intercurrent illness. The hyperbilirubinemia is secondary to reduced hepatic levels of glucuronyl transferase; for the same reason, the bile of patients with this syndrome contains predominantly unconjugated bilirubin. Phenobarbital, an inducer of microsomal enzymes, will dramatically decrease the hyperbilirubinemia in these patients. The mode of inheritance of this syndrome is uncertain.

Gilbert's Syndrome
This is a common benign syndrome affecting perhaps as much as 6% of the normal population. It is characterized by mild, unconjugated hyperbilirubinemia with normal serum transaminases in an otherwise healthy individual. The syndrome is most commonly diagnosed in young adults, but may be one of the etiologies of hyperbilirubinemia of the newborn. The unconjugated hyperbilirubinemia is exaggerated during periods of fasting and perhaps with stress or fatigue. Laboratory studies are normal except for the presence of unconjugated hyperbilirubinemia. Although this is classically a nonhemolytic form of jaundice, many of these patients have a very subtle form of hemolysis that is characterized by a decreased red cell survival and a mild reticulocytosis. Serum bilirubin levels may vary at times from normal to 8 mg/dL, although values of 2 to 3 mg/dL are most common. People with this syndrome have reduced hepatic levels of glucuronyl transferase and, as a result, have an increased proportion of bilirubin monoglucuronide in their bile. Gilbert's syndrome may represent a less severe phenotypic expression of a similar biochemical defect that this syndrome shares with Crigler-Najjar, type II.

Dubin Johnson and Rotor's Syndrome
Both these syndromes are autosomal recessive disorders characterized by conjugated hyperbilirubinemia. Both disorders are associated with a benign course and with normal laboratory tests except for conjugated hyperbilirubinemia. The livers of patients with Dubin-Johnson syndrome are grossly black and contain a dark pigment in centrilobular areas, whereas the appearance and pathology of the liver of patients with Rotor's syndrome is normal. The 45-minute plasma BSP retention study shows a characteristic secondary rise in Dubin-Johnson syndrome but not in Rotor's syndrome. The oral cholecystogram usually visualizes in Rotor's but not in Dubin-Johnson syndrome. Characteristic abnormalities are seen in urinary porphyrin excretion in both these disorders. The etiology of these disorders is unknown.

THE CHEMISTRY AND PHYSIOLOGY OF PHOTOTHERAPY
Phototherapy has been used widely in this country as treatment for severe neonatal hyperbilirubinemia for the past 20 years. Before this mode of therapy for hyperbilirubinemia, exchange transfusion was the only means of significantly lowering serum bilirubin levels in the neonate; now exchange transfusion is rarely required. The emphasis on the aggressive treatment of hyperbilirubinemia stems from the damage this seemingly innocuous compound can do when deposited in the central nervous system. Thirty years ago, kernicterus, as a result of central nervous system damage from hyperbilirubinemia, was not an uncommon pediatric problem. Today, as a result of rigorous monitoring of serum bilirubin levels and aggressive phototherapy, kernicterus is extremely rare.

Despite the fact that bilirubin contains two propionic acid groups that should render it slightly water soluble through ionization of these acid moieties, bilirubin in the native state has virtually no aqueous solubility and is a lipophilic molecule. This lipophilic hydrophobic state is a direct result of intramolecular hydrogen bonding of the acid groups of continued on page 457
bilirubin with its own pyrrole nitrogens and carbonyl groups. X-ray diffraction and NMR studies have shown that bilirubin exists in the Z,Z or all-trans state and that only in this state can bilirubin form these intramolecular hydrogen bonds.

Phototherapy changes the state of bilirubin from the Z,Z (trans) state to an E or cistrans configuration through a process called photoisomerization. In this state bilirubin is unable to undergo intramolecular hydrogen bonding and the proponic acid groups are therefore free to ionize and interact with the aqueous environment. Thus, since it is a polar compound, this "photobilirubin" can be excreted without conjugation. Photo-oxidation of bilirubin is no longer felt to be the main pathway of excretion during phototherapy. During the administration of phototherapy to neonates, these photobilirubins have been detected in bile, but are unstable and quickly revert back to an all-trans state. Recently, intramolecular cyclized forms of photobilirubin have been reported.

REFERENCES