

Serum bilirubin levels increase when an imbalance exists between bilirubin production and clearance. A logical evaluation of the patient who is jaundiced requires an understanding of bilirubin production and metabolism.

### PRODUCTION AND METABOLISM OF BILIRUBIN

(See also Chap. 359) Bilirubin, a tetrapyrrole pigment, is a breakdown product of heme (ferroprotoporphyrin IX). About 70–80% of the 250–300 mg of bilirubin produced each day is derived from the breakdown of hemoglobin in senescent red blood cells. The remainder comes from prematurely destroyed erythroid cells in bone marrow and from the turnover of hemoproteins such as myoglobin and cytochromes found in tissues throughout the body.

The formation of bilirubin occurs in reticuloendothelial cells, primarily in the spleen and liver. The first reaction, catalyzed by the microsomal enzyme heme oxygenase, oxidatively cleaves the  $\alpha$  bridge of the porphyrin group and opens the heme ring. The end products of this reaction are biliverdin, carbon monoxide, and iron. The second reaction, catalyzed by the cytosolic enzyme biliverdin reductase, reduces the central methylene bridge of biliverdin and converts it to bilirubin. Bilirubin formed in the reticuloendothelial cells is virtually insoluble in water due to tight internal hydrogen bonding between the water-soluble moieties of bilirubin—i.e., the bonding of the propionic acid carboxyl groups of one dipyrrolic half of the molecule with the imino and lactam groups of the opposite half. This configuration blocks solvent access to the polar residues of bilirubin and places the hydrophobic residues on the outside. To be transported in blood, bilirubin must be solubilized. Solubilization is accomplished by the reversible, noncovalent binding of bilirubin to albumin. Unconjugated bilirubin bound to albumin is transported to the liver. There, the bilirubin—but not the albumin—is taken up by hepatocytes via a process that at least partly involves carrier-mediated membrane transport. No specific bilirubin transporter has yet been identified (Chap. 359, Fig. 359-1).

After entering the hepatocyte, unconjugated bilirubin is bound in the cytosol to a number of proteins including proteins in the glutathione-S-transferase superfamily. These proteins serve both to reduce efflux of bilirubin back into the serum and to present the bilirubin for conjugation. In the endoplasmic reticulum, bilirubin is solubilized by conjugation to glucuronic acid, a process that disrupts the internal hydrogen bonds and yields bilirubin monoglucuronide and diglucuronide. The conjugation of glucuronic acid to bilirubin is catalyzed by bilirubin uridine diphosphate-glucuronosyl transferase (UDPGT). The now-hydrophilic bilirubin conjugates diffuse from the endoplasmic reticulum to the canalicular membrane, where bilirubin monoglucuronide and diglucuronide are actively transported into canalicular bile by an energy-dependent mechanism involving the multidrug resistance-associated protein 2 (MRP2).

The conjugated bilirubin excreted into bile drains into the duodenum and passes unchanged through the proximal small bowel. Conjugated bilirubin is not taken up by the intestinal mucosa. When the conjugated bilirubin reaches the distal ileum and colon, it is hydrolyzed to unconjugated bilirubin by bacterial  $\beta$ -glucuronidases. The unconjugated bilirubin is reduced by normal gut bacteria to form a group of colorless tetrapyrroles called *urobilinogens*. About 80–90% of these products are excreted in feces, either unchanged or oxidized to orange derivatives called *urobilins*. The remaining 10–20% of the urobilinogens are passively absorbed, enter the portal venous blood, and are re-excreted by the liver. A small fraction (usually <3 mg/dL) escapes hepatic uptake, filters across the renal glomerulus, and is excreted in urine.

### MEASUREMENT OF SERUM BILIRUBIN

The terms *direct* and *indirect* bilirubin—i.e., conjugated and unconjugated bilirubin, respectively—are based on the original van den Bergh reaction. This assay, or a variation of it, is still used in most clinical chemistry laboratories to determine the serum bilirubin level. In this assay, bilirubin is exposed to diazotized sulfanilic acid and splits into two relatively stable dipyrromethene azopigments that absorb

maximally at 540 nm, allowing photometric analysis. The direct fraction is that which reacts with diazotized sulfanilic acid in the absence of an accelerator substance such as alcohol. The direct fraction provides an approximation of the conjugated bilirubin level in serum. The *total* serum bilirubin is the amount that reacts after the addition of alcohol. The indirect fraction is the difference between the total and the direct bilirubin levels and provides an estimate of the unconjugated bilirubin in serum.

With the van den Bergh method, the normal serum bilirubin concentration usually is 17  $\mu\text{mol/L}$  (<1 mg/dL). Up to 30%, or 5.1  $\mu\text{mol/L}$  (0.3 mg/dL), of the total may be direct-reacting (conjugated) bilirubin. Total serum bilirubin concentrations are between 3.4 and 15.4  $\mu\text{mol/L}$  (0.2 and 0.9 mg/dL) in 95% of a normal population.

Several new techniques, although less convenient to perform, have added considerably to our understanding of bilirubin metabolism. First, studies using these methods demonstrate that, in normal persons or those with Gilbert's syndrome, almost 100% of the serum bilirubin is unconjugated; <3% is monoconjugated bilirubin. Second, in jaundiced patients with hepatobiliary disease, the total serum bilirubin concentration measured by these new, more accurate methods is lower than the values found with diazo methods. This finding suggests that there are diazo-positive compounds distinct from bilirubin in the serum of patients with hepatobiliary disease. Third, these studies indicate that, in jaundiced patients with hepatobiliary disease, monoglucuronides of bilirubin predominate over diglucuronides. Fourth, part of the direct-reacting bilirubin fraction includes conjugated bilirubin that is covalently linked to albumin. This albumin-linked bilirubin fraction (*delta fraction*, or *biliprotein*) represents an important fraction of total serum bilirubin in patients with cholestasis and hepatobiliary disorders. The delta fraction (delta bilirubin) is formed in serum when hepatic excretion of bilirubin glucuronides is impaired and the glucuronides accumulate in serum. By virtue of its tight binding to albumin, the clearance rate of delta bilirubin from serum approximates the half-life of albumin (12–14 days) rather than the short half-life of bilirubin (about 4 h).

The prolonged half-life of albumin-bound conjugated bilirubin accounts for two previously unexplained enigmas in jaundiced patients with liver disease: (1) that some patients with conjugated hyperbilirubinemia do not exhibit bilirubinuria during the recovery phase of their disease because the bilirubin is covalently bound to albumin and therefore not filtered by the renal glomeruli, and (2) that the elevated serum bilirubin level declines more slowly than expected in some patients who otherwise appear to be recovering satisfactorily. Late in the recovery phase of hepatobiliary disorders, all the conjugated bilirubin may be in the albumin-linked form.

### MEASUREMENT OF URINE BILIRUBIN

Unconjugated bilirubin is always bound to albumin in the serum, is not filtered by the kidney, and is not found in the urine. Conjugated bilirubin is filtered at the glomerulus, and the majority is reabsorbed by the proximal tubules; a small fraction is excreted in the urine. Any bilirubin found in the urine is conjugated bilirubin. The presence of bilirubinuria implies the presence of liver disease. A urine dipstick test (Ictotest) gives the same information as fractionation of the serum bilirubin and is very accurate. A false-negative result is possible in patients with prolonged cholestasis due to the predominance of delta bilirubin, which is covalently bound to albumin and therefore not filtered by the renal glomeruli.

## APPROACH TO THE PATIENT:

### Jaundice

The goal of this chapter is not to provide an encyclopedic review of all of the conditions that can cause jaundice. Rather, the chapter is intended to offer a framework that helps a physician to evaluate the patient with jaundice in a logical way (Fig. 58-1).