Bilirubin Secretion, Jaundice and Evaluation of Liver Function

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Jaundice occurs as a result of excess bilirubin in the blood. It is a hallmark of liver disease but not always present in liver disease. Jaundice occurs when the liver fails to adequately secrete bilirubin from the blood into the bile. To understand how jaundice occurs, you must first understand bilirubin synthesis, metabolism and secretion.

Evaluation of Liver Disease and Hepatic Function

History
Physical Examination
Laboratory Tests
Sometimes Radiological/Nuclear Medicine
Sometimes Liver Biopsy

Heme Oxygenase

Bilivirdin Reductase
Kikuchi et al. Nature Structural Biology 8, 221 - 225 (2001)
Bilirubin is frequently depicted as a linear tetapyrrole.

However, intramolecular hydrogen bonding fixes it in a rigid structure that blocks exposure of its polar groups to aqueous solvents, making it very insoluble in blood.

Bilirubin in Blood is Bound to Albumin: Uptake into Hepatocyte at Basolateral (Sinusoidal) Membrane

Some bilirubin stored in cytosol bound to proteins

Bilirubin UDP-glucuronosyltransferase is localized to the endoplasmic reticulum; it catalyzes conjugation to a diglucuronide, making it more water soluble.

A: Labeling of periphery of cell hepatocyte nucleus
B: Labeling of ER with antibody to UDP-glucuronosyltransferase

Alternative RNA splicing of different first exons of UGT1 gives different isoforms with different substrate specificities, some for bilirubin and others to different substrates, such as phenol.

**UGT1 GENE COMPLEX**

- **BILIRUBIN-UGT1 mRNA**: 1245
- **BILIRUBIN-UGT2 mRNA**: 1245
- **PHENOL-UGT mRNA**: 1245
Bilirubin glucuronide is secreted from hepatocytes by an ATP-binding cassette protein. This is the rate limiting step in hepatocyte bilirubin metabolism and disrupted in most acquired liver diseases.

**Bilirubin is Only Approximately 2% of Bile**

- **SOLID COMPONENTS OF BILE** (by weight)
  - Bile Salts 51.3%
  - Cholesterol 4.1%
  - Phospholipid 12.9%
  - MISC. 17.4%
  - Other 3%

**Diagnostic Consequences of Enterohepatic Circulation of Bilirubin**

- In hepatocyte dysfunction (hepatocellular)
  - May see increased urobilinogen in urine because it is less efficiently reabsorbed by hepatocytes
- In biliary obstruction
  - Stools may appear white because bilirubin does not get into intestine and therefore not converted to stercobilins/urobilins
  - No urobilinogen detected in urine

**Measurement of Bilirubin in Blood**

- Normally ≤17 μM (1 mg/dl)
  - >35 μM can begin to detect jaundice clinically, (sclera, mucus membranes early)
  - Discoloration of skin with higher concentrations
- When measured precisely (e.g. by HPLC), around 96% of serum bilirubin is unconjugated
- Clinical laboratory generally “overestimates” amount of conjugated bilirubin (up to 30%) because of method
  - reported as “total,” “direct” (approximates conjugated) and “indirect” (approximates unconjugated)

**van den Bergh and Muller Reaction (1916)**

Using this method, 20% to 30% serum direct bilirubin is normal value
Excess conjugated bilirubin in serum may be excreted by kidneys (dark urine). Albumin-bound unconjugated bilirubin cannot be excreted by kidneys.

With longstanding elevated serum conjugated bilirubin, less is in urine because of covalent binding to albumin.

**Causes of Hyperbilirubinemia and Jaundice**

**DISORDERS CAUSING UNCONJUGATED HYPERBILIRUBINEMIA**
- Overproduction
- Impaired Uptake
- Impaired Conjugation

**UNCONJUGATED HYPERBILIRUBINEMIA CAUSED BY OVERPRODUCTION OF BILIRUBIN**
- Hemolysis
  - Intravascular—hemolytic anemia, transfusion reactions
  - Extravascular—resorption of hematoma
- Ineffective Erythropoiesis
  - Megaloblastic anemia, Thalassemia
- Impaired Uptake
  - Fast, Seppals, Gilbert syndrome?, Some drugs (e.g., probenecid)

**UNCONJUGATED HYPERBILIRUBINEMIA CAUSED BY IMPAIRED BILIRUBIN CONJUGATION**
- Crigler-Najjar Syndrome Type I
- Crigler-Najjar Syndrome Type II
- Gilbert Syndrome

**CRIGLER-NAJJAR SYNDROME**
- Autosomal recessive inheritance
- Mutations in UGT1 gene resulting in decreased to absent bilirubin conjugation
- Type 1: absent activity
- Type 2: decreased activity with serum bilirubin concentrations from 8 to 20 mg/dl
- Animal model: Gunn rat
Most Common Cause of Unconjugated Hyperbilirubinemia in Western Countries

**Gilbert Syndrome**
- Mildly decreased UGT1 activity
- Mutations in the UGT1 gene promoter have been described
- Unconjugated serum bilirubin can range from 1.5 mg/dl to 6.0 mg/dl
- Other deficits besides deconjugated conjugation such as decreased uptake may contribute
- Exacerbated by stress, fasting, and infection
- Serum total bilirubin concentrations decreased by phenobarbital
- Fairly common and familial tendency

Gilbert syndrome is not really a “disease” but a normal variant.

High blood concentrations of lipid soluble unconjugated bilirubin in infants that also have poorly developed blood-brain barrier can lead to kernicterus (brain damage caused by bilirubin deposition). Treatments include exchange transfusion and phototherapy. Heme oxygenase inhibitors are also being studied for this indication.

Treatment of Neonatal Jaundice by Phototherapy

Less intramolecular hydrogen bonding of E diastereomers make them more aqueous soluble for renal excretion.

**Disorders Causing Primarily Conjugated Hyperbilirubinemia**

- Impaired secretion of conjugated bilirubin
- Intrahepatic and extrahepatic biliary tree obstruction/cholestasis

**Primarily Conjugated Hyperbilirubinemia Caused by Impaired Secretion of Bilirubin**

- **Hepatocellular Diseases**
  - Viral, drug, and alcoholic hepatitis, various metabolic diseases, cirrhosis
- **Pregnancy**
  - Presumably related to estrogen sensitivity, similar to jaundice induced by birth control pills
- **Inherited Disorders**
  - Dubin-Johnson syndrome,Rotor syndrome

Caused by mutation in ABCG2 encoding canicular transporter.
With abnormal secretion from hepatocytes, most excess bilirubin in blood is conjugated by can get “mixed picture” because of “backup” of unconjugated bilirubin.

Laboratory Tests in Liver Diseases

Bilirubin
- From breakdown of heme (mostly from old red blood cells)
- Hyperbilirubinemia can occur in liver disease
- Not specific for liver disease – increased production (e.g. hemolysis)

Albumin
- Synthesized in hepatocytes and secreted into blood
- Half-life about 20 days
- Hypoalbuminemia can occur with hepatic synthetic dysfunction in chronic liver disease, especially advanced cirrhosis
- Not specific for liver disease (renal, cardiac, gut)
- Usually measured in mg/dl

Prothrombin Time
- Reflects a type of blood clotting
- Several blood clotting factors are synthesized in the liver, most with half-lives of 9 to 26 hr
- Prothrombin time can be prolonged within a day in severe acute liver disease
- Also prolonged in severe chronic liver disease
- Not specific for liver disease
- Usually measured in seconds or INR
Glucose
- Glycogenolysis and gluconeogenesis take place in the liver
- In severe liver dysfunction, hypoglycemia can occur
- Cirrhosis sometimes associated with insulin resistance and elevated serum glucose
- Usually measured in mg/dl

Cholesterol
- Synthesized in the liver and secreted complexed as lipoproteins
- Serum cholesterol concentration will be low in severe hepatocellular dysfunction
- Biliary obstruction can lead to elevations in serum cholesterol, which will be part of an abnormal lipoprotein called lipoprotein X
- Usually measured in mg/dl

Alanine aminotransferase (ALT)
- Present in cytosol of hepatocytes
- Catalyzes transamination between amino and α-keto acids
- Serum activity is elevated when hepatocytes are damaged or destroyed (e.g. hepatitis, hepatic necrosis)
- Fairly specific for liver disease
- Measured in IU/L (activity)

Aspartate aminotransferase (AST)
- Present in cytosol and mitochondria of hepatocytes and muscle cells
- Catalyzes transamination between amino and α-keto acids
- Serum activity is elevated when hepatocytes are damaged or destroyed (e.g. hepatitis, hepatic necrosis)
- Also elevated in muscle disease and acute MI
- Measured in IU/L (activity)

Alkaline Phosphatase
- Present predominantly near the microvilli of the bile canaliculi
- Serum activity is elevated with intrahepatic cholestasis, extrahepatic biliary obstruction, or invasion of the liver (e.g., tumor, mycobacterial infections, etc.)
- Also present in bone and placenta and serum activity may be elevated in bone diseases
- Measured in IU/L (activity)

“Liver Enzymes”

Liver enzymes are not liver function tests!
- In chronic hepatitis, there is poor correlation between the magnitude of the liver enzyme elevations and the degree of liver injury
Liver Biopsy in the Evaluation of Liver Disease

Dr. Lefkowitch

\[ \gamma \text{ glutamyltranspeptidase} \]

- Present predominantly near the microvilli of the bile canaliculi
- Serum activity is elevated in same conditions as those that increase alkaline phosphatase
- Also induced by alcohol and other drugs that may cause serum elevations in activity
- Helpful to differentiate biliary disease from bone disease when serum alkaline phosphatase activity is elevated
- Measured in IU/L (activity)

Radiological/Nuclear Medicine

- Ultrasound
- Computerized Tomography
- Magnetic Resonance Imaging
- Liver–Spleen Scan
- Endoscopic Retrograde Cholangiopancreatography
- Tagged Red Blood Cell Scan
- Oral and Transhepatic Cholecystography
- Radionuclide Biliary Scans