Section 20

LECTURE

Jaundice and Disorders of Bilirubin Metabolism

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Bilirubin is an orange-colored tetrapyrrole formed by the degradation of heme. This water-insoluble product is excreted in bile after its conversion (i.e. conjugation) to water-soluble glucuronides in the liver. Hyperbilirubinemia simply means an abnormal increase in the serum bilirubin concentration (normally 0.3-1.0 mg/100ml); this may result from enhanced bilirubin production (via hemolysis or ineffective erythropoiesis) and/or impaired hepatic disposition (via defective uptake, conjugation or secretion into bile). Jaundice or icterus is evident when the serum bilirubin level exceeds 2.5-3.0 mg/100ml.

I. Bilirubin Chemistry

A. Bilirubin-IX(Z,Z) is the naturally occurring bilirubin isomer.

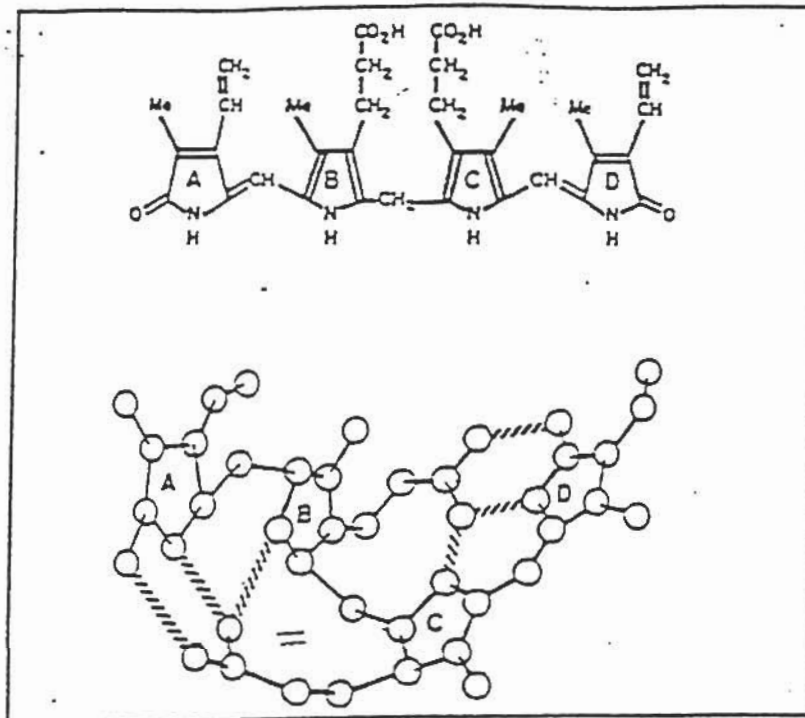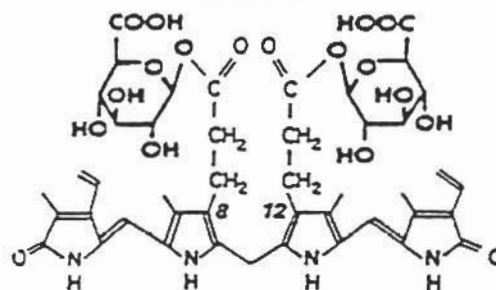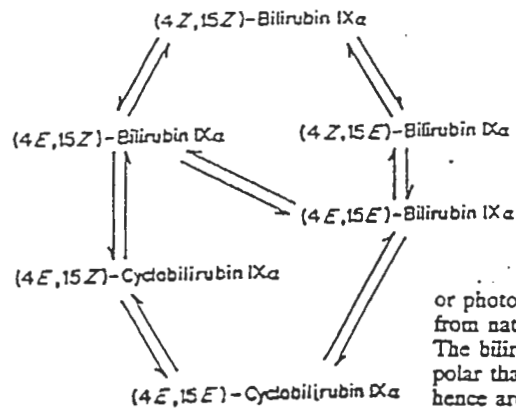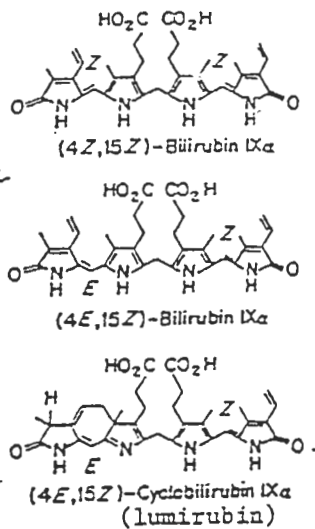Fig. 1

Structure of bilirubin-IXα diglucuronide. The two molecules of glucuronic acid attached to the propionic acid groups prevent intramolecular hydrogen bonding and thus account for the enhanced aqueous solubility.

Fig. 3

Formation of geometric or photoisomers ('photobilirubin') from natural bilirubin-IXα (Z,Z). The bilirubin photoisomers are more polar than bilirubin-IXα (Z,Z) and hence are readily excreted into bile without prior conjugation. Once in bile, they rapidly revert to the stable Z,Z form.

B. The conventionally written structure (Fig. 1, top panel) shows the methene bridge double bonds adjacent to the outer pyrrole rings (A and D) in the Z,Z position. However, bilirubin-IX(Z,Z) is not a linear tetrapyrrole, but an involuted structure (Fig. 1, bottom panel) which is stabilized by 6-intramolecular hydrogen bonds linking the propionic acid groups to the nitrogens and oxygen of the opposite pyrrole rings.

C. The H-bonding accounts for the water insolubility of bilirubin. Conjugation of the propionic acid groups with glucuronic acid (Figure 2) prevents H-bond formation and results in water-soluble derivatives (bilirubin glucuronides), which are readily excreted in bile.

D. During phototherapy with blue light (to reduce plasma bilirubin levels in jaundiced neonates),the outer pyrrole rings of bilirubin-IX(Z,Z) flip over resulting in formation of unstable geometric isomers (termed "photobilirubin", (Figure 3). Cyclization of the E,Z photoisomer results in the more stable "lumirubin". These isomers are unable to form intramolecular H-bonds, and hence are more polar and are excreted into bile without the need of conjugation. Once in bile, the unstable photoisomers rapidly revert to the more stable bilirubin-IX (Z,Z). By this mechanism of photoisomerization, phototherapy results in a reduction of total serum bilirubin concentration.

II. Formation of Bilirubin

A. Heme is dissociated from its aproprotein (e.g. globin) and transferred to the microsomal enzyme, heme oxygenase.

B. The protoporphyrin (heme) ring is cleaved selectively at the α-methene carbon bridge. The reaction requires molecular O_2 and NADPH and yields carbon monoxide.

$$\text{Heme} + O_2 \xrightarrow{\text{heme oxygenase}} \text{Biliverdin} + \text{Fe} + \text{CO}$$

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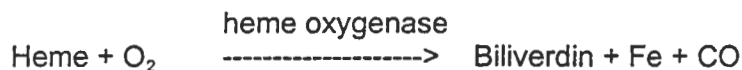
C. The green pigment, biliverdin, is converted to bilirubin by the cytosolic enzyme, <u>biliverdin reductase</u>.

$$\text{Biliverdin} \xrightarrow{\text{2H}} \text{Bilirubin}$$

III. Sources of Bilirubin

A. Total daily bilirubin <u>production</u> averages 250-300 mg in adults.

B. Destruction of the heme moiety of <u>hemoglobin</u> in the RE system (bone marrow, spleen, liver) accounts for 70-80% of total bilirubin production. An increased rate of red cell breakdown (hemolysis) may lead to unconjugated hyperbilirubinemia (≤ 3 mg/100 ml).

C. An early-labeled <u>bilirubin fraction</u> (20-30%) is apparent in studies using the heme precursor, glycine of Θ-aminolevulinic acid isotopically labeled. This consists of two or more components.

    1. Hepatic - the major portion arises from turnover of hemoproteins (e.g. cytochrome P-450, catalase).

    2. Erythropoietic - release of heme from destruction of erythrocyte precursors in marrow. Of little importance in physiologic red cell production, but is greatly enhanced, causing unconjugated hyperbilirubinemia, in disorders associated with <u>ineffective erythropoiesis</u> (e.g. pernicious anemia, thalassemia).

IV. Plasma Transport of Bilirubin

A. Unconjugated bilirubin is mostly bound to <u>albumin</u> (one high-affinity and at least two low-affinity sites).

B. The small amount of free or <u>unbound</u> bilirubin in plasma is presumed to be the fraction available for transit across the blood-brain barrier. <u>Kernicterus</u> (neonatal brain damage) may occur when the unbound bilirubin level is increased by the displacement of albumin-bound bilirubin by drugs (e.g. certain sulfonamides, analgesics, diuretics), free fatty acids or acidosis. No accurate method is available for determination of unbound bilirubin fraction in plasma.

C. Conjugated bilirubin is less tightly bound to albumin. The non-protein-bound, water-soluble, conjugated pigment (<1%) is freely filtered by the glomeruli (in contrast to the unconjugated fraction).

D. In pathologic states, a fraction of the plasma conjugated bilirubin is <u>covalently bound</u> to albumin (bilirubin or BIL-ALB), and hence is not excreted in urine. This plasma bilirubin fraction is not present in normal individuals or patients with unconjugated hyperbilirubinemia, but occurs in hepatobiliary or cholestatic conditions.

V. Hepatic Transport and Conjugation

A. <u>Uptake</u> of bilirubin across the hepatocyte sinusoidal membrane occurs by a carrier-mediated mechanism, which is shared by other organic anions, such as sulfobromophthalein or indocyanine green, but not by bile acids.

B. Once inside the liver cell, bilirubin partitions into membranes or binds to <u>cytoplasmic</u> proteins (e.g. glutathione-S-transferase B or ligandin). These cytosolic macromolecules appear to limit the efflux of bilirubin from liver cells back to plasma, and hence influence net hepatic bilirubin uptake.

C. In the process microsomal <u>conjugation,</u> the COOH groups of one or both propionic acid side chains of bilirubin are esterified with glucuronic acid, to form bilirubin mono- or diglucuronide. The reaction is catalyzed by the enzyme, <u>UDP-glucuronosyltransferase</u>, which is responsible for the glucuronidation of a variety of other substrates; e.g. estradiol, testosterone, morphine, chloramphenicol. However, bilirubin conjugation appears to be catalyzed by a specific isoform of the enzyme.

　　1. Bilirubin + UDP-glucuronic acid → bilirubin monoglucuronide (BMG)

　　2. BMG + UDP-glucuronic acid → bilirubin diglucuronide (BDG)

D. Under normal conditions, reaction 2 appears to be rate-limiting, so that in UDP-glucuronosyltransferase deficiency states (e.g., <u>Gilbert's syndrome)</u>, there is relatively more BMG formed than BDG.

E. Methods are available for the measurement of UDP-glucuronosyltransferase activity in needle biopsy specimens of human liver, although this is rarely utilized clinically.


VI. Biliary Excretion of Bilirubin

A. Normal bile contains about 80% bilirubin diglucuronide and 20% monoglucuronide. The proportion of these glucuronides varies between species (i.e. sheep and guinea pigs excrete BMG only). Less than 1% of the bilirubin excreted in normal bile is in the unconjugated form.

B. The processes involved in the canalicular transport of conjugated bilirubin and other organic anions are poorly understood, although a membrane carrier appears to be involved (which is defective in <u>Dubin-Johnson syndrome)</u>. Excretion is enhanced by micelle-forming bile acids, suggesting that conjugated bilirubin may be physically associated with biliary micelles (bile acid, phospholipid, cholesterol).

C. The process of biliary secretion is susceptible to damage form a variety of <u>acquired liver diseases</u>, which lead to an increased serum conjugated bilirubin concentration (e.g. sensitivity to drugs, such as chlorpromazine, estrogens and methyltestosterone; hepatocellular necrosis, such as hepatitis or cirrhosis).

VII. Fate of Bilirubin in the Intestine

A. Conjugated bilirubin is not reabsorbed by the gallbladder or intestine so that, in normal individuals, there is no appreciable enterohepatic circulation of bilirubin.

B. However, bilirubin is reduced by intestinal bacteria (mostly in the colon) to a series of colorless tetrapyrroles, termed urobilinogen.

$$Bilirubin \xrightarrow{H^+} Urobilinogen$$
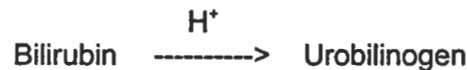
Oral administration of broad-spectrum antibiotics greatly diminishes the formation of urobilinogen.

C. A portion of urobilinogen undergoes oxidation to orange-colored urobilin (stercobilin).

D. About 20% of urobilinogen is reabsorbed via the portal circulation, and most is promptly re-excreted by the liver into bile (i.e., enterohepatic circulation). A small fraction (2% of daily urobilinogen production) is excreted in the urine.

E. Urinary urobilinogen is measured using Ehrlich's aldehyde reagent (red color extractable by chloroform, c.f. porphobilinogen). Increased urinary concentrations are associated with hemolytic disorders and hepatocellular disease.

VIII. Serum Bilirubin Determination

A. Van den Bergh reaction-- formation of stable red-violet derivatives (dipyrroles) with diazonium salts of aromatic amines such as sulfanilic acid. Conjugated bilirubin gives an immediate "direct" reaction, whereas unconjugated bilirubin ("indirect") requires addition of an accelerator (e.g., methanol, caffeine benzoate). Estimation of conjugated bilirubin is less accurate than unconjugated pigment. Normal serum values with the standard diazo reaction are: total is <1.0 mg/100 ml (<17 μmol/1) and direct reaction is <0.3 mg/100 ml (<5 μmol/).

B. More accurate methods using high performance liquid chromatography (HPLC) have been developed for measurement of bilirubin and its conjugates (BDG and BMG). Although these procedures have not yet been adopted in routine hospital laboratories, they have show that conjugated bilirubin is virtually undetectable in normal serum, and that in hepatobiliary disease, unconjugated bilirubin, BDG and BMG are present, with latter predominating.

IX. Disorders of Bilirubin Metabolism

May be classified as disorders associated with increased production or decreased clearance of bilirubin. Inherited disorders may be broadly considered as causing unconjugated hyperbilirubinemia (Table 1) or a predominantly conjugated hyperbilirubinemia (Table 2), only the latter being associated with bilirubinuria. In diffuse hepatocellular injury (e.g., hepatitis or cirrhosis), plasma conjugated and unconjugated bilirubin are both elevated (the former predominates), reflecting a variety of possible defects in hepatic transport and biliary secretion, coupled with deconjugation of the conjugated pigments (due to the action of hepatic ß-glucuronidase).

A. Increased Production

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1. <u>Hemolysis</u> - most common, either intravascular (hemolytic anemias) or extravascular (hematomas). Serum unconjugated bilirubin rarely exceeds 4 mg/100 ml except in hemolytic crises (sickle cell disease) or unless hepatic function is impaired.

2. <u>Ineffective erythropoiesis</u> -pernicious anemia, thalassemia, sideroblastic anemia.

TABLE 1. Chronic Nonhemolytic Unconjugated Hyperbilirubinemic Syndromes

	Crigler-Najjar Syndrome, Type I	Crigler-Najjar Syndrome, Type II	Gilbert's Syndrome
Incidence	Rare	Uncommon	$\leq$ 7% population
Mode of inheritance	Autosomal recessive with variable penetrance	? Autosomal dominant	? Autosomal dominant
Plasma bilirubin (mg/100ml)	Usually > 20	Usually < 20	< 6, usually < 3
Clinical Features	Jaundice and kernicterus in infancy or early adulthood	Kernicterus rare: usually asymptomatic jaundice	Onset usually early adult-hood: may be first recog-nized with fasting Occasionally mild jaundice, usually asymptomatic
Bilirubin UDP-GT activity	Undetectable	Markedly decreased	Decreased
Associated defects	None	? Decreased hepatic uptake of bilirubin	Mild hemolysis ($\leq$50); decreased hepatic uptake of bilirubin?, sulfobromophthlein ($\leq$40% of patients), and indocyanine green ($\leq$20% of patients)
Bile	Trace of unconjugated bilirubin and BMG	Mostly BMG	Relative decrease in BDG and increase in BMG
Effect of pheno-barbital on serum bilirubin concentration	None	Marked decrease	Decrease

B. <u>Impaired Hepatic Uptake</u>

Defects in hepatic uptake are rare and ill-defined. Flavaspidic acid may compete with bilirubin for binding to cytosolic proteins. Patients with Gilbert's syndrome may have an uptake defect in addition to impaired conjugation. Fasting (400 kcal/day for 2 days), particularly in Gilbert's syndrome, produces a rise in serum unconjugated bilirubin, which may be associated in part with reduced uptake.

C. <u>Impaired Conjugation</u> (Bilirubin UDP-glucuronyltransferase activity)
1. <u>Neonatal ("physiological") jaundice</u> -- serum unconjugated bilirubin is normal at birth, but rises in some neonates to 3-5 mg/100 ml in the first week, returning to normal in the second week. The disorder is probably due to immaturity of the conjugating and possibly the hepatic transport steps (e.g., ligandin). When associated with hemolysis (e.g., erythroblastosis fetalis), the increase in serum unconjugated bilirubin may result in kernicterus. Treatment includes phototherapy (see Bilirubin Chemistry) and exchange transfusion, and

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avoidance of acidosis or drugs which displace bilirubin from albumin (e.g., sulfa, aspirin).

TABLE 2. Hereditary Conjugated Hyperbilirubunemias

	Dubin-Johnson Syndrome	Rotor's Syndrome*	Hepatic Storage* Syndrome
Incidence	Uncommon	Rare	Rare
Mode of inheritance	Autosomal recessive	Autosomal recessive	Not known
Plasma bilirubin concentration	Usually 2-5 (-60% conjugated): increased by estrogens and pregnancy	Usually 2-5 (60% conjugated	3-7 (50% conjugated)
Clinical features	Usually asymptomatic jaundice in early adulthood. Occasionally, hepatosplenomegaly	Asymptomatic jaundice	Asymptomatic jaundice
Gross and histologic appearance of liver	Black-brown color; pigment granules in centrilobular areas	Normal	Normal
Oral cholecystography	Gallbladder usually not visualized	Normal	Normal: delayed visualization of gall bladder
Plasma sulfobromophthlein (BSP) disappearance	Initially slow (45 min retention ≤ 20%), secondary increase at 90 min	Initially very slow (45 min retention, 30-50%). no secondary increase	36% in one case: no secondary increase
BSP transport maximum	Markedly decreased	Moderately decreased	Decreased
BSP hepatic storage capacity	Normal	10% of normal	Markedly decreased
Urinary coproporphyrin excretion and isomer pattern (coproporphyrin III is normally 75% of total)	Normal total; coproporphyrin > 80% of total	Increased total; increased (< 80%)	Not known

* Rotor's Syndrome and Hepatic Storage Syndrome may be identical disorders.

2. *Crigler-Najjar Type I Syndrome* (see Table I) -- associated with undetectable hepatic glucuronyltransferase activity. Invariably fatal kernicterus, usually within first few years after birth. Serum unconjugated bilirubin levels may be reduced transitorily by phototherapy or plasmapheresis.

3. *Crigler-Najjar Type II Syndrome* (see Table I)-- due to a moderate deficiency of glucuronyltransferase activity. Activity of the enzyme is enhanced (induced) by treatment with phenobarbital, with an associated fall in plasma unconjugated bilirubin concentration. Patients rarely, if ever, develop brain damage.

4. *Gilbert's Syndrome* -- relatively common, benign disorder, male: female (4:1), due to a mild ↓ in glucuronyltransferase activity. As in Crigler-Najjar syndrome, liver histology and all liver function tests are normal, except for mild unconjugated hyperbilirubinemia (usually 1-3 mg/100 ml). The hyperbilirubinemia is more evident in patients who have associated hemolysis. Fasting (400 kcal/day for 2 days) causes a 2- to 3-fold rise in serum unconjugated bilirubin level, which may be useful in distinguishing patients with Gilbert's syndrome from those with acquired liver disease and/or hemolysis.

D. Impaired Biliary Secretion

1. *Dubin-Johnson Syndrome* (see Table 2)-- benign, mostly conjugated hyperbilirubinemia associated with bilirubinuria. Other liver function tests are

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normal, including alkaline phosphatase. Brown pigment in centrilobular hepatocytes is a non-toxic melanin-like compound. Defect in transport of bilirubin and other organic anions across the canaliculus (rebound rise in plasma Bromosulfophthlein at 90 min) and cholecystographic dyes (non-visualized gallbladder).

TABLE 3. Causes Of Hyperbilirubinemia and Associated Serum Bilirubin Patterns

Disorder	Defects in Bilirubin Metabolism	Serum Bilirubin Pattern*		
		Unconjugated	Conjugated	Covalently Bound
Hemolysis (hemolytic anemias)	Increased production	(Rarely exceeds 4 mg/dl)	--	--
Hematomas	Increased production (1 L blood = 150 mg hemoglobin = 5 gm bilirubin = 20 x normal daily production	•	--	--
Ineffective erythropoiesis (pernicious anemia, thalassemia)	Increased production	•	--	--
Neonatal ("physiologic") jaundice	Increased production, decreased glucuronyltransferase activity, dec. cytosolic ligandin, inc. intestinal bilirubin absorption	• (6 mg/dl in full term, 10-12 mg/dl in premature)	--	--
Breast milk jaundice	? Inhibition of glucuronyltransferase activity (pregnane-3α, 20β-diol and long-chain fatty acids, inc. intestinal bilirubin absorption	• (up to 20-30 mg/dl)	--	--
Crigler-Najjar syndrome, type 1	Absent glucuronyltransferase	• (usually >20mg/dl)	--	--
Crigler-Najjar syndrome, type II	Markedly decreased glucuronyl-transferase activity	• (usually <20mg/dl)	--	--
Gilbert Syndrome	Decreased glucuronyltransferase activity, ? decreased hepatic uptake, associated hemolysis or dyserythropoiesis in about 50%	• (<6mg/dl)	--	--
Fasting hyperbilirubinemia (marked response in Gilbert syndrome)	Increased production, decreased hepatic clearance. ? decreased hepatic uptake ? decreased conjugation	•	--	--
Dubin-Johnson syndrome	Impaired biliary secretion, (? canalicular membrane-carrier defect)	-- (usually <5mg/dl)	•	--
Rotor syndrome and hepatic storage syndrome	Decreased hepatic uptake and storage, ?decreased biliary secretion	--	•	--
Intrahepatic cholestasis (canalicular or ductule damage)	Decreased biliary secretion, deconjugation leads to increased serum unconjugated bilirubin leads	•	•	•
Extrahepatic cholestasis (mechanical obstruction)	Decreased biliary secretion	•	•	•
Hepatocellular injury	Decreased biliary secretion (conjugation) and other steps usually remain intact)	•	•	•

* Symbols: -- Absent or minimal   • Present

2. *Rotor's Syndrome and Hepatic Storage Disease* (see Table 2) -- rare disorders with poorly defined defects in hepatic uptake and storage of bilirubin and other organic anions. May well be the same condition, but both are distinct from Dubin-Johnson syndrome.

E. Intrahepatic Cholestasis (Bile canalicular or ductule).

1. Drugs - chlorpromazine, C-17 alkylated steroids (anabolic and contraceptive steroids)
2. Primary biliary cirrhosis
3. Cholestatic jaundice of pregnancy -- occurs in third trimester.
4. Benign recurrent cholestasis - rare benign disorder which runs a fluctuating course.
5. Post-operative jaundice (cholestasis) -- results from the combination of a variety of factors, including increased bilirubin load (transfusions, hematomas), hypoxemia (?transitory liver cell and bile canalicular damage) and possibly impaired renal excretion of serum conjugated bilirubin.

F. Extrahepatic Cholestasis (obstruction of extrahepatic bile ducts). As in intrahepatic cholestasis, elevation of serum alkaline phosphatase, 5'-nucleotidase, bile acids, and conjugated bilirubin (with bilirubinuria). Due to gallstones or stricture, carcinoma of head of pancreas or bile ducts, or congenital atresia of bile ducts.

G. Hepatocellular Disease

Usually involves several phases of bilirubin transport, although hepatic uptake and conjugation are rarely affected. The typical serum bilirubin pattern is conjugated:unconjugated = 6:4. Most commonly seen in viral or drug induced hepatitis or chronic active hepatitis or cirrhosis.

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