Bilirubin and Jaundice

Pathways of Bilirubin Synthesis and Catabolism

- Bone Marrow
  - Hgb
  - Globin
  - Heme
  - Fe
  - Porphogens

- RBCs
  - Hgb
  - Fe

- Reticuloendothelial System (RES)
  - Hgb
  - Fe
  - Globin
  - Biliverdin
  - Bilirubin

- Conjugated Bilirubin
  - 68mg
  - 70mg

- Free Bilirubin – Albumin Complex
- Urobinogen
- Stercobilin
  - Fecal Pigments

- Urobinogen
- Shunt Pathway
  - Urine Urobilinogen
  - 2mg

- Heme Precursors
  - Myoglobin
  - Non-Hgb Heme Proteins

- 2mg
- 68mg
- 70mg
Labeling of RBC hemoglobin and fecal stercobilin

Labeling of red cell hemoglobin and fecal stercobilin in a normal human given $^{15}$N orally

Sources of bilirubin production in the rat

Early Bilirubin
15%
0-3 Days

Late Bilirubin
65%
40-80 Days

Sources of bilirubin production in the rat, as adduced from studies of the labeling of plasma bilirubin in Gunn rats and bile bilirubin in normal rats.
Reaction Catalyzed by Biliverdin Reductase

Biliverdin

\[ \text{NADPH} + \text{H}^+ \rightarrow \text{NADP}^+ \]

Bilirubin
OATP MDR2

A.

B.
BILIRUBIN CONJUGATION IN MICROSOMES INVOLVES TWO STEPS, MEDIATED BY ONE BILIRUBIN-UDPGA TRANSFERASE

BILIRUBINS:

1. UNCONJUGATED (UCB)

2. MONO-GLUCURONIDE (BMG)

3. DIGLUCURONIDE (BDG)

Conjugation prevents internal H-bonding by the -COOH groups of Bilirubin
UROBILINOGENS ARE FORMED BY DECONJUGATION AND REDUCTION OF BILIRUBINS BY INTESTINAL BACTERIA

**Conjugated Bilirubin**

- COOGA

**Unconjugated Bilirubin**

- COOH

**A Urobilinogen**

- COOH

**Deconjugation**

- M = Methyl
- E = Ethyl
- V = Vinyl

**Reduction**

- \(2 \text{ GAL} + 8 \text{H} = \text{Methyl E = Ethyl V = Vinyl} \)
FRACTIONAL DIAZO REACTION OF PLASMA

\[
\text{SO}_3H \quad \begin{array}{c} \text{N}^+ \\ \text{N} \quad \text{NN} \end{array} \quad \text{+} \quad \begin{array}{c} \text{R} \\ \text{R} \end{array} \quad \quad \begin{array}{c} \text{CONJUGATED} \\ \text{BILIRUBIN} \end{array} \quad \rightarrow \quad \text{Direct reaction}
\]

\[
\text{SO}_3H \quad \begin{array}{c} \text{N}^+ \\ \text{N} \quad \text{NN} \end{array} \quad \text{+} \quad \begin{array}{c} \text{UNCONJUGATED} \\ \text{BILIRUBIN} \end{array} \quad \rightarrow \quad \text{Indirect reaction}
\]

Fast

Slow
**Bilirubinuria in Jaundice**

<table>
<thead>
<tr>
<th>UNCONJUGATED</th>
<th>PLASMA BILIRUBIN (B)</th>
<th>CONJUGATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BB</td>
<td>BB</td>
</tr>
<tr>
<td>A</td>
<td>BB</td>
<td>BB</td>
</tr>
<tr>
<td>A</td>
<td>BB</td>
<td>BB</td>
</tr>
<tr>
<td>A</td>
<td>BB</td>
<td>BB</td>
</tr>
</tbody>
</table>

**TOTAL DIAZO REACTION OF PLASMA**

\[
\begin{align*}
\text{SO}_3\text{H} + \text{N}^+ \text{N} \rightarrow \text{CONJUGATED BILIRUBIN} \\
+ \text{ACCELERATOR} \rightarrow \text{UNCONJUGATED BILIRUBIN} \\
\text{TOTAL} - \text{DIRECT} = \text{INDIRECT}
\end{align*}
\]
Phototherapy for neonatal jaundice

Treatment for Neonatal Jaundice

Prevention of kernicterus
Structure of the $\alpha$ and $\beta$ isomers of bilirubin IX. In the IX$\beta$ isomer (and in the $\gamma$ and $\delta$ isomers, not shown) the propionic acid groups are moved to positions other than those indicated on the central pyrole rings B and C of bilirubin IX$\alpha$.

Effect of Sn-protoporphyrin (Sn-PP) and CO-protoporphyrin (Co-PP) when administered once at a dose of 50 $\mu$mol/kg body wt on hepatic heme oxygenase activity in the rat.
Effect of Sn-protoporphyrin (Sn-PP) (100 μmol/kg body wt) administered at 4d before and on the day of surgery on hyperbilirubinemia in the bile-duct-ligated rat. Effect of Sn-PP (2 x 0.25 μmol/kg body wt) on the levels of serum bilirubin in a patient with primary biliary cirrhosis (PBC).
# Unconjugated Hyperbilirubinemia

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Crigler-Najjar Type I</th>
<th>Gilbert Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma bilirubin</td>
<td>340-860 μM</td>
<td>50-85 μM (fluctuates)</td>
</tr>
<tr>
<td>Plasma BSP retention at 45 min</td>
<td>Normal</td>
<td>Usually normal; elevated in a minority of cases</td>
</tr>
<tr>
<td>Hepatic bilirubin-UDPGT activity</td>
<td>Undetectable</td>
<td>30-50% of normal</td>
</tr>
<tr>
<td>Effect of Phenobarbital on plasma bilirubin</td>
<td>No effect</td>
<td>Reduction</td>
</tr>
<tr>
<td>Pigments in bile</td>
<td>Small amounts of unconjugated bilirubin</td>
<td>Increased proportion of monoglucuronide</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Rare</td>
<td>2-7% of population</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Kernicterus</td>
<td>Benign</td>
</tr>
<tr>
<td>Animal Model</td>
<td>Gunn rat</td>
<td>Bolivian Squirrel Monkey</td>
</tr>
<tr>
<td>Mutation</td>
<td>UDPGT null</td>
<td>UDPGT promoter A(TA)_6 TAA</td>
</tr>
</tbody>
</table>

# Chronic conjugated hyperbilirubinemia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dubin-Johnson Syndrome</th>
<th>Rotor Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance of liver</td>
<td>Grossly black</td>
<td>Normal</td>
</tr>
<tr>
<td>Histology of liver</td>
<td>Dark pigment; predominantly in centrilobular areas; otherwise normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>Elevated, usually between 2 and 5 mg%, occasionally as high as 20 mg%; predominantly direct-reacting</td>
<td>Elevated, usually between 2 and 5 mg%, occasionally as high as 20 mg%; predominantly direct-reacting</td>
</tr>
<tr>
<td>Routine liver function tests</td>
<td>Normal except for bilirubin</td>
<td>Normal except for bilirubin</td>
</tr>
<tr>
<td>45-min plasma BSP retention</td>
<td>Normal or elevated; secondary rise at 90 min</td>
<td>Elevated; no secondary rise at 90 min</td>
</tr>
<tr>
<td>Urinary coproporphyrin</td>
<td>Normal total &gt;80% as coproporphyrin I</td>
<td>Elevated total; elevated proportion of coproporphyrin I but &lt;80%</td>
</tr>
<tr>
<td>Mode of inheritance</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Uncommon (1:1300 in Persian Jews)</td>
<td>Rare</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Benign</td>
<td>Benign</td>
</tr>
<tr>
<td>Mutation</td>
<td>MRP2</td>
<td></td>
</tr>
</tbody>
</table>
Jaundice

Unconjugated bilirubin
- Hemolysis
- Defect in Conjugation

Conjugated bilirubin
- Hepatocellular
- Intrahepatic
- Extrahepatic

Increased Bilirubin Production Beyond the Liver’s Capacity to Conjugate It

Blood

Cells
- endoplasmic reticulum

Conalculus
- conjugated bilirubin
Jaundice

- Unconjugated bilirubin
  - Hemolysis
  - Defect in Conjugation

- Conjugated bilirubin
  - Hepatocellular
    - Intrahepatic
    - Extrahepatic
  - Cholestatic

Defective Secretion of Conjugated Bilirubin from Liver Cells

Blood

- unconjugated bilirubin

Cells

- endoplasmic reticulum

Canaliculus

- conjugated bilirubin
Liver Function Tests

- Bilirubin
- PT (Prothrombin time)
- Glucose
- Cholesterol
- ALT (alanine aminotransferase)
- AST (aspartate aminotransferase)
- Alkaline phosphatase
- GGT (γ-glutamyltranspeptidase)

Imaging

- Ultrasound
- CT scan
- Liver-spleen scan
- Radionuclide biliary scan
- ERCP (endoscopic retrograde cholangiopancreatography)
- Transhepatic cholangiography
Jaundice

Unconjugated bilirubin
- Hemolysis
- Defect in Conjugation

Conjugated bilirubin
- ↑ ALT
- ↑ AP

Hepatocellular

Cholestatic
- ↑ BR
- Dilated BD
- NI BD

Intrahepatic

Extrahepatic

↑ BR

ALT

AP

↑
Bilirubin Metabolism

- Blood
- Conjugated & Conjugated
- Urine – Urobilinogen
- Stool – Stercobilin

Conversion of protoheme (ferroprotoporphyrin IX) to bilirubin IXα. Cleavage of the protoporphyrin ring occurs selectively at the α-methene bridge. The bridge carbon atom is oxidized to carbon monoxide.
BILIRUBIN IS PRODUCED BY OXIDATION OF HEME AND REDUCTION OF THE RESULTANT BILIVERDIN

BILIVERDIN IXα

M = Methyl
E = Ethyl
Pr = Propionyl

Heme Oxygenase

BILIRUBIN IXα

Biliverdin Reductase

O₂ & NADPH

NADP⁺

Fe³⁺

CO

NADPH

Conjugation of Bilirubin

Two separate steps

Two UDP-glucuronic acid

Two UDP

Bilirubin diglucuronide
C.T. 5559 is a 45 y.o. Native American
et al abuse c/o abdominal pain and fever.
05/15 — psychotropic admission for et al,
with SGOT 192, SGPT 162, BR 1.9/.5,
Ni during hospitalization.
12/28: — Alcohol hepatitis, alcoholic ketoacidosis
04/86: — Alcohol hepatitis, alcoholic ketoacidosis
05/86: — Diarrhea, alcohol related seizure
12/86: — Alcohol hepatitis, ketoacidosis, seizure
04/87: — Alcohol related seizure. No ascites, encephalopathy.
SGOT 144, SGPT 169, BR 4.3/2.3, Albumen 3.4, PT 14.3/12.5
2/8/88: — Jaundice with dark urine x 3
wks., fevers x 2 m with chills
and sweats. RUQ constant pain
unrelated to eating x 1 wk.
Denies diarrhea, hematemesis,
hematochezia. No travel, exposures.
PE: Jaundiced, sl e obese female in NAD
T-95, P95, R20, BP 130/80
HEENT:
Skin + spider angiomes
Pulm—clear to P&A
CV- VS, S2, no S3, S4, murmur
Abd—Active BS. Liver 22 cm span
moderately tender, — spleen, ascites
Initial Hospital Course
Dx severe alcoholic hepatitis. Rx vitamin K, thiamine
Temp 101 max. WBC: Diarrhea.
Tox screen: -- HepB: -- Hep A --
Cultures: -- LP: --
AbdUS: no evidence of biliary obstruction, dense liver, no lesions
Hemoccult stools: HCT, Tx RBC
EGD: small varices, no estimate of bleeding.
Develops edema and ascites
Paracentesis: 30 WBC, 80% PMN. TP: 50
Develops hepatic encephalopathy – asterixis, oriented 0.
Rx: lactulose
Develops renal insufficiency BUN: creat 22/1.8
Coagulopathy worsens
Gunn Rats

* Absent bilirubin UDP-glucuronyltransferase
* Inherited as autosomal recessive trait
* Normal liver function
* Prototype of Crigler-Najjar type I
* No bilirubinuria, small amount of bilirubin in bile
* Excrete bilirubin IXα into bile
* Excrete unconjugated bilirubin into bile after phototherapy
* Defect corrected by hepatoma cells or kidney transplant

Diagram:
- Alb
- Bilirubin
- Bilirubin Y protein
- Bilirubin UDP-glucuronyltransferase
- Bilirubin diglucuronide
- Active transport into gut
- Bilirubin diglucuronide
- Beta-glucuronidase
- Stool
- Enterohepatic circulation