BILIRUBIN

Hemoglobin Catabolism

Hemoglobin → Globin → Protein pool

Heme

NADPH → O₂ → CO → Fe

Heme oxygenase

NADP⁺

Biliverdin

NADPH

NADP⁺

Bilirubin

Biliverdin reductase
Bilirubin Transport

- Free Bilirubin insoluble in water → binds to albumin for transport
- Circulates in plasma
- Uptake by hepatocytes

Hepatocyte Metabolism of Bilirubin

1) Uptake  2) Conjugation  3) Excretion
Bilirubin Metabolism Cont’d

- After excretion into bile duct, conjugated bilirubin passes into the intestinal lumen.

- In the distal small intestine and colon, anaerobic bacteria hydrolyze the glucuronic acid residues and reduce bilirubin to a variety of compounds known collectively as urobilinogens.

- 80% of Urobilinogens excreted in feces in oxidized form (urobilin, stercobilin, mesobilin).

- 20% reabsorbed; majority taken up by liver; remainder excreted in urine.
Bilirubin Transport

• Delta Bilirubin
• Covalently Bound to Albumin
• Formed when hepatic excretion of bilirubin is impaired

Hyperbilirubinemia

• Jaundice, a yellowish discoloration of the skin and sclera, appears at high serum bilirubin concentrations
• Hyperbilirubinemia indicates an abnormality in the production or subsequent metabolism of bilirubin
• Disorders classified as:
  - **Unconjugated Hyperbilirubinemia**
    - Prehepatic
    - Defective Conjugation (Gilbert’s Disease, Crigler- Najjar Syndrome, Physiologic Disease of the Newborn)
  - **Conjugated Hyperbilirubinemia**
    - Hepatic (Hepatocyte Injury)
    - Hepatic with Intrahepatic Obstruction
    - Defective Hepatic Excretion
      (Dubin Johnson Syndrome, Rotor’s Syndrome)
    - Post-hepatic (Obstruction)
Unconjugated Hyperbilirubinemia

Defective Conjugation
- Gilbert’s Disease (genetic deficiency)
- Crigler-Najjar Syndrome (genetic deficiency)
- Lucey-Driscoll Syndrome (inhibitor to conjugation)
- Physiological Jaundice of the Newborn
  - immature glucuronyl transferase
  - kernicterus can develop
  - risk increases in:
    - premature infants
    - problems with albumin binding
SUMMARY OF EFFECTS OF ABNORMAL BILI RUBIN METABOLISM

<table>
<thead>
<tr>
<th>Test</th>
<th>Unconjugated</th>
<th>Hepatic</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre-Hepatic</td>
<td>Hepatic</td>
</tr>
<tr>
<td></td>
<td>(over-load)</td>
<td></td>
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<tr>
<td>Serum bilirubin:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unconjugated</td>
<td>↑↑</td>
<td>↑</td>
</tr>
<tr>
<td>(Free, Indirect)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugated</td>
<td>→sl↑</td>
<td>→, ↓</td>
</tr>
<tr>
<td>(Direct)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal Urobilinogen</td>
<td>↑↑</td>
<td>Normal, ↓</td>
</tr>
<tr>
<td>Urine Urobilinogen</td>
<td>↑</td>
<td>Normal, ↓</td>
</tr>
<tr>
<td>Urine Bilirubin</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>(Bile)</td>
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</table>

Conjugated Hyperbilirubinemia
Conjugated Hyperbilirubinemia

Intrahepatic Obstruction

Fecal and urine urobilinogens ↓

Conjugated bilirubin ↑

Conjugated Hyperbilirubinemia

Defective Hepatic Excretion
ie. Dubin Johnson Syndrome
Rotor’s syndrome
# Conjugated Hyperbilirubinemia

![Diagram of bilirubin metabolism](image)

**SUMMARY OF EFFECTS OF ABNORMAL BILI RUBIN METABOLISM**

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<th>Test</th>
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<th>Hepatic</th>
<th>Hepatic with Intrahepatic Obstruction</th>
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</thead>
<tbody>
<tr>
<td>Serum bilirubin: Unconjugated (Free, Indirect)</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td>Conjugated (Direct)</td>
<td>↑↑</td>
<td>↑↑</td>
<td></td>
<td>↑↑ Delta Bilirubin Present</td>
<td>↑↑ Delta Bilirubin Present</td>
</tr>
<tr>
<td>Fecal Urobilinogen</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Urine Urobilinogen</td>
<td>N, ↑ or ↓</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

Fecal urobilinogen: ↓ (clay-colored feces)
Measurement

**Direct Spectrophotometric Method**

- Abs at 455nm due to Bili + Hb
- Abs at 540nm due to Hb
- Difference due to Bili
- Absorbance scan of Oxyhemoglobin
- Absorbance scan of Bilirubin

- Affected by lipochromes such as carotenes

Measurement

**Chemical Diazot Methods**

- Bilirubin reacts with diazotized sulfanilic acid → azobilirubin
- Direct reacting fraction – reflects Conjugated Bilirubin (both mono- and di-gluronides) and Delta Bilirubin
- Unconjugated Bilirubin (Indirect reacting fraction) requires an accelerator
- Examples of accelerators: Caffeine, Methanol
- All fractions react with the addition of accelerator → Total Bilirubin
- Total Bilirubin – Direct = Indirect Bilirubin
Measurement

Jendrassik-Grof Procedure

• **Direct:** serum mixed with HCl
diazo reagent added
conjugated and delta bilirubin forms azobilirubin
reaction halted with ascorbic acid
azobilirubin converted to blue green color with alkaline tartrate
absorbance measured at 600 nm

• **Total**
serum mixed with caffeine Na benzoate + HCl + diazo rg
all fractions of bilirubin react to form azobilirubin
remainder of procedure the same as above

Measurement

**Specimen:**

• serum or plasma
• protect from light
• store at low temperatures
• fasting preferred to avoid lipemia