Clinical Chemistry of the Liver and Hemolytic Anemias

I. Anatomy and Function of the Liver
Figure 1 Location of the liver in the body.

Figure 2 Names for direction orientations in the body: superior (top), inferior (bottom), anterior (front), posterior (back)
Figure 3 Figure showing peritoneum surrounding the abdominal cavities and abdominal organs. The peritoneum also holds the organs in place. Double layer peritoneum called ligaments connect liver to the abdominal wall (falciform ligament), to diaphragm (coronary ligament), to the stomach (lesser omentum).


Figure 3 Views of liver showing areas of liver (porta hepatis and bare area) not covered by the peritoneum and showing the falciform ligament, dividing the liver into the right and left lobes.

Figure 5 showing front view of right and left lobes and Figure 6 showing sections of the right lobe (quadrate lobe, caudate lobe, and right lobe proper) seen on the visceral side.


Figure 7 Blood vessels into (portal vein and hepatic artery) and out of (hepatic vein) the liver. Also shows bile duct coming out of the liver.

Figure 8  Figure showing portal vein moving blood from the digestive organs directly into the liver.


Figure 9  Figure showing drainage of bile from the liver, exiting the liver from the left and right hepatic ducts into the common hepatic duct into common bile duct (which the cystic duct also drains into from the gall bladder), into the duodenum through the ampulla of vater (which the pancreatic duct also drains into).
Figure 10  Figure showing liver lobule and portal triad.
W.F. Ganong "Review of Medical Physiology" 10th Ed; Lange Medical Publications; Los Altos, CA, 1981

Figure 11  Another figure showing more clearly the liver lobule and portal triad structures.
Figure 12  Figure showing flow of blood from branches of hepatic arteries and portal vein (called distributing vein) into sinusoids.


Figure 13  Figure showing bile canaliculi surrounding the hepatocyte cells which drain into the intrahepatic ductules which drain into interlobular bile ducts (labeled as bile ducts here) which eventually empty into the hepatic ducts (either right or left).

Figure 16  Figure showing the two principal cells in the liver: the hepatocyte (parenchymal liver cell, 1) and the Kupffer cell (3) in relation to the sinusoid (8).


Figure 17  Diagram showing various chemical processes occurring in the liver and the handling of nutrients carried to the liver from the intestines via the portal vein and the flow of bile (green) from the liver into the intestines.

F.H. Netter "The CIBA Collection 3-Digestive System III; 2nd Ed; R.R. Donnelley & Sons Company; New York, 1964
II. Bilirubin Formation and Excretion

Basic Porphyrin Structure

Cyclic structure with conjugated double bonds consisting of 4 pyrole rings connected to each other by 4 methylene bridges
Basic Heme Structure

Porphyrin

+ 
Fe$^{2+}$

Structure of protoheme showing:
1) the base porphyrin structure (4 pyrole ring structure);
2) the side chains of the specific porphyrin, protoporphyrin IX;
3) and the bound Fe$^{2+}$

Heme is always associated with proteins as a prosthetic group

Hemoproteins in mammals (examples)

- Hemoglobin - \( \text{O}_2 \) transport (blood)
- Cytochromes – electron transport
- Catalase – \( \text{H}_2\text{O}_2 \) utilization
- Myoglobin – \( \text{O}_2 \) transport (muscle)

6 Coordinate Bonding Positions for \( \text{Fe}^{2+} \) in Heme

1) 4 coordinate bonds to nitrogen of 4 pyroles (in plane)

2) 2 Others (above and below plane)
   a) Hemoglobin
      1 histidine of the hemoglobin protein
      1 ligand (either \( \text{CO}_2 \) or \( \text{O}_2 \))
   b) Cytochromes
      2 R groups of amino acid residues of the hemoglobin protein
Methemoglobin

- Oxidized hemoglobin
  \[ \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} \]

- Does not carry \( O_2 \)

- Normally 1% of hemoglobin in erythrocyte

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Steps 1 – 7 in Bilirubin Processing

Formation Sources of Bilirubin

1) **75-80%** - breakdown of heme from hemoglobin in senescent red blood cells

2) **20-25%** - early labeled fraction
   - Breakdown of heme from other proteins
   - Breakdown of heme from hemoglobin in defectively formed red blood cells
   - Free heme (in liver and marrow erythroid cells)
Location of the spleen in the body


Spleen Functions

a) **Defense**
   Macrophages

b) **Hematopoiesis**
   Produces monogranular leukocytes (monocytes, lymphocytes)

c) **Blood Resevoir**
   - Holds up to 350 mL blood
   - Can release 200 ml blood

d) **Immunologic**
   Source of production of antibodies

e) **Cleanser of blood cellular components**
   - Senescent Red Blood Cells (RBCs)
   - Imperfect Platelets
Parenchyma of Spleen

1) **White pulp** – lymphoid tissue

2) **Red Pulp**
   a) Reticular meshwork (Reticuloendothelial cells)
   b) Venous sinuses (lined with macrophages)
   c) Splenic cords (collapsed sinuses or endothelial cells that have become phagocytic)
   d) Histiocytes and Erythrocytes

Diagram showing white and red pulp areas and trabecular arteries and veins in the spleen.

Destruction of Senescent RBC’s by Spleen

1) Arterioles dump blood into macrophage containing splenic cords and venous sinuses in the red pulp

2) Percolation of red blood cells (7 um) through macrophage environment exposes red blood cells to harsh conditions of the macrophage environment (hypoxic, acidic, free radical, etc.). The RBCs must pass through narrow passage ways (fenestrations, 3 um) to get into venous circulation.

3) Time traversing this environment in single pass through the spleen:
   - New RBCs  →  30 seconds
   - Old RBCs  →  minutes to hours

Diagram of the red pulp of spleen showing the end point of the arterial blood emptying into harsh chemical meshwork of splenic cords and venous sinuses. For red blood cells to re-enter the venous circulation they need to squeeze through narrow (3 um) fenestrations (pathways) that separate the venous circulation from the splenic cords and the venous sinuses.

The metabolism of hemin to bilirubin

1) Reactions of microsomal heme oxygenase system (oxidation - incorporation of 2 oxidation oxygen atoms)

- Hemin
- Oxyheme (not shown)
- Biliverdin IX-a

2) Reaction in cytosol (Reduction reaction)

- Biliverdin IX-a
- Bilirubin IX-a


Heme Oxygenase Reactions: Heme to Oxyheme to Biliverdin IX-a

**Heme Oxygenase System**

a) Microsomal  
b) Substrate – free heme  
c) Rate limiting step in conversion of heme to bilirubin  
d) Reaction

\[
\begin{align*}
302 \text{NADPH} & \quad \text{Heme} \\
& \quad \text{Biliverdin} \\
& \quad \text{CO} \quad \text{Fe}^{3+}
\end{align*}
\]

**Bilirubin Generation Reaction:**  
Note the enzymes and the reduction of the center methylene bridge

---

Is bilirubin soluble in aqueous solutions?

Intra-molecular hydrogen bonds tie up propionic acid groups making bilirubin (Z-Z confirmation) hydrophobic

Importance of Bilirubin Solublization

1) How is bilirubin solubilized in aqueous blood?

2) Purpose of conjugation reaction with glucuronic acid: makes bilirubin soluble in bile

3) Purpose of solubilizers in the bilirubin assays
Bilirubin made soluble in body

A. Physiologic
1) In blood – bound to albumin
2) In hepatocytes - bound to ligandin
3) In hepatocytes - conjugation of glucuronic acid to bilirubin in order to be soluble in bile

B. Therapy
1) UV light treatment in neonates to solubilize bilirubin

Diagram showing solubilization of bilirubin in blood (bound to albumin) and in the hepatocyte cell (bound to ligandin and then formation of conjugated bilirubin)

### Binding of Bilirubin to Albumin

1) All unconjugated bilirubin (except for small amount) is bound reversibly to albumin

2) Two binding sites
   a) High affinity (one)
   b) Low affinity (two)

### Binding of Bilirubin to Albumin in Neonate

Less binding capacity of albumin leading to increased free bilirubin

Due to

Unidentified agent competing with bilirubin for binding sites
Causes of $[\text{Bilirubin}]_{\text{Free}}$ Increases

1) Increased Fatty Acids or Organic Anions
   - Administration of anionic drugs to pregnant women or newborns can lead to increased free bilirubin in fetus or newborn (salicylates or sulfonamides)
   - High concentration of fatty acids

2) Others

Other Binding Species of Bilirubin

- B-lipoproteins, globulins, hemopexin, erythrocytes
- Not physiologically significant
Binding of Conjugated Bilirubin to Albumin

1) Normally weakly bound
   - Has much weaker binding constant with albumin than unconjugated form
   - 0.6% free bilirubin (i.e., not bound) in circulation

2) In pathology (cholestasis) there is a form of bilirubin that is covalently bound to albumin
   δ-bilirubin

Question

How will decreased albumin concentration in blood affect the total bilirubin concentration in blood?
Uptake of bilirubin involves a 54 K dalton bilirubin carrier protein and possibly an albumin receptor


Uptake of Bilirubin by Hepatocyte

1) Facilitated Diffusion- 54K dalton protein in membrane
   a) Obeys Michaelis-Menton Kinetics
   b) Some types organic anions compete with bilirubin for binding to this protein and are facilitated uptaken into the hepatocyte (but not bile acids)

2) Not rate-limiting step in bilirubin metabolism (greatly exceeds excretion rate into bile)

3) Fixed fraction taken up 5% regardless of load

4) Bi-directional flux
   40% refluxes back into plasma
Bilirubin Binding Proteins in Hepatocyte

1) Ligandin (Glutathione S-transferases)
2) Protein Z

Role of Binding Proteins in Hepatocyte

1) Solubizes bilirubin
2) Controls capacity of liver to process bilirubin (uptake, store, transport, detoxify)
3) Storage of Bilirubin
4) Transport of Bilirubin to ER
5) Detoxification
Conjugation of Reaction of Bilirubin in the Hepatocyte


Purpose of Conjugation of Bilirubin

1) Solubilizes bilirubin (breaks intramolecular hydrogen bonds) so can be excreted in bile

2) Impairs reabsorption in GI tract and gallbladder
Glucuronyl-transferase reaction takes place in the endoplasmic reticulum of the hepatocyte


---

**Fun Facts about UDP- Glucuronyl Transferase**

1) Different UDP-glucronyl transferases with different substrate specificity
2) High reserve activity except in the following:
   a) Neonate (causing jaundice)
   b) Severe hemolytic conditions
   c) Congenital UDP-Glucuronyl Deficiencies
3) Microsomal enzyme inducers induce UDP-transferase
   - phenobarbital
   - glutethimide
   - antipyrene
   - clofibrate
4) Inhibitors – are glucuronide forming compounds which are acted upon by bilirubin UDP-transferases
Bilirubin in Bile

Glucuronides (95%) + Glucosides/ Xylosides (5%)

90%  10%

Diglucuronide  Monoglucuronide

There is a small percentage of mixed glucuronides present in bile: glucuronide/glucoside or glucuronide/xyloside. There is also a small percentage unconjugated bilirubin 1%.

Bilirubin in Serum (Health)

96% unconjugated

4% conjugated
Excretion of Conjugated Bilirubin into Bile

1) Active transport
   (susceptible to hypoxia, shock, circulatory insufficiency)

2) IOOK dalton transporter protein in canalicular membrane

3) - Bile acids and bilirubin have different excretory systems
   - Bilirubin excretory system is shared by other organic ions (listed pg 34 of chapter 2)

4) Rate limiting step in bilirubin processing from the point after its formation to its transfer into the bile
### Consequence of impaired excretion of conjugated bilirubin into bile

1) **↓ Excretion bilirubin in feces**

2) **↑ Conjugated bilirubin in blood**

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<table>
<thead>
<tr>
<th>Overview of Bilirubin Processing in Intestines</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Bile enters duodenum. Conjugated bilirubin is not reabsorbed in intestines.</td>
</tr>
<tr>
<td>2) In terminal ileum and colon</td>
</tr>
<tr>
<td>a) Conjugated bilirubin $\xrightarrow{\text{bacterial \ B-glucuronidases}}$ Unconjugated bilirubin + glucuronic acid</td>
</tr>
<tr>
<td>b) Reduction of bilirubin to <strong>urobilinogens</strong></td>
</tr>
</tbody>
</table>
Overview of Bilirubin Processing in Intestines (cont.)

3) In colon

3) In colon

\[
\text{urobilinogens} \xrightarrow{\text{spontaneous oxidation}} \text{urobilins} \xrightarrow{\text{spontaneous oxidation}} \text{unknown substance}
\]

4) Excretion of urobilins in feces

Summary of reduction reactions of bilirubin to form urobilinogens and oxidation reactions of urobilinogens to form urobilins


Fig. 32-9 Conversion of bilirubin to urobilinogens in the intestine. It is not known whether glucuronic acid is hydrolyzed before or after the reduction steps.

Structures of urobilinogens

Highlighted areas show sites in the molecule which are successively reduced in going from bilirubin to the various urobilinogens.

Properties of Urobilinogens and Urobilins

**Urobilinogens**

1) Lipophilic (soluble in aqueous solutions only at high pH)
2) Unstable, easily oxidized

**Urobilins**

Polar, water soluble
Excretion of Bilirubin Metabolites

Bilirubin → urobilinogens and urobilins

50% → 50% → ?

Urobilinogen in Urine increased in:
(normally < 4mg/day)

1) Hepatocellular disease (impaired hepatic excretory mechanism)
2) Hemolytic disease
3) Others
   - fasting
   - menstruation
   - pregnancy
   - labor
Urobilinoids in Feces

1) Normal
   47 – 276 mg/day

2) Increased
   ➢ Diagnostic for bilirubin overproduction disorders

3) Decreased
   ➢ Indicative of biliary obstruction or defect in glucuronidation of bilirubin

Urobilinoids in Serum

<table>
<thead>
<tr>
<th>Condition</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.30 ug/dL</td>
</tr>
<tr>
<td>Liver Cirrhosis</td>
<td>55 ug/dL</td>
</tr>
<tr>
<td>Acute Hepatitis</td>
<td>60 ug/dL</td>
</tr>
<tr>
<td>Hemolytic Anemia</td>
<td>50 ug/dL</td>
</tr>
</tbody>
</table>
III. Bilirubin Assays

A. Colorimetric Determination of Bilirubin

Diazotized Sulfanilic Acid Method
Bilirubin Math

Direct + Indirect = Total

conjugated bilirubin + δ-bilirubin

unconjugated bilirubin

combined determined by diazotized sulfanic acid method (without accelerator)

Calculated as total – direct

Determined by diazotized sulfanilic acid method (with accelerator)

Diazotized Sulfanilic Acid Method

Determination of different bilirubin fractions

1) Total bilirubin determined by adding “accelerator” to assay
   - accelerators solubilizes the unconjugated bilirubin (the conjugated bilirubin is already soluble in aqueous solution)
   - Example accelerators
     Alcohol
     Benzoate/Caffeine

2) Direct fraction of bilirubin determined in the absence of accelerator
3) Indirect = Total - Direct
% bilirubin in normal serum that is unconjugated by various bilirubin methods

<table>
<thead>
<tr>
<th>15%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>by diazotized sulfanilic acid methods (inaccurate method)</td>
<td>by HPLC (accurate method)</td>
</tr>
</tbody>
</table>

What are the reasons for the positive error of the diazotized sulfanilic acid method?

Various Diazotized Sulfanilic Acid Methods

1) Malloy and Evelyn Method
   - Performed at pH 1.2; uses methanol as accelerator
   - Measured at 560 nm

2) Jendrassik – Grof Method
   - Reaction at pH 6.5, measurement at pH 13
   - Measured at 600 nm
   - Most utilized
   - Advantages over Malloy and Evelyn
     - Low hemoglobin interference
     - Better sensitivity
     - Better precision (at lower bilirubin concentrations)
Figure 1 Chemical reactions of diazotized sulfamic acid with unconjugated bilirubin (solubilized by accelerator) generating colored product

Figure 2 Chemical reactions of diazotized sulfamic acid with conjugated bilirubin generating colored product
B. Other Methods for Determining Bilirubin

<table>
<thead>
<tr>
<th>Table 62-2</th>
<th>Methods of bilirubin analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td><strong>Type of analysis</strong></td>
</tr>
<tr>
<td>1. Mallory-Edelson</td>
<td>Kinetic, end point, with or without blank</td>
</tr>
<tr>
<td>2. Jaiani-Le-Goff</td>
<td>Kinetic, end point, with or without blank</td>
</tr>
<tr>
<td>3. Bilirubinometer</td>
<td>Direct spectrophotometric</td>
</tr>
<tr>
<td>4. High-performance liquid chromatography</td>
<td>Chromatographic separation</td>
</tr>
<tr>
<td>5. Bilirubin reductase</td>
<td>Kinetic, end point</td>
</tr>
<tr>
<td>6. Spectral shift</td>
<td>End point</td>
</tr>
</tbody>
</table>

IV. Hyperbilirubinemia:
General Considerations

Figure 3 Serum bilirubin concentrations for males and females versus age

**Definitions**

**Hyperbilirubinemia** –
bilirubin in serum exceeds 1mg/dL

**Jaundice (Icterus)** -
yellow pigmentation of skin and sclera (usually with serum bilirubin at 2 - 2.5 mg/dL)

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**Clinical Aspects of Hyperbilirubinemia**

1) Hyperbilirubinemia is **NOT** pathologic in adults

2) Hyperbilirubinemia **CAN BE** pathologic in neonate because of under-developed blood-brain barrier leading to bilirubin accumulation in brain, resulting in **kernicterus**

- Neonatal conditions that lead to severe kernicterus
  - Erythroblastosis Fetalis (Hemolytic Disease of New Born)
  - Crigler – Nijar, Type I

- Neonatal condition that leads to mild kernicterus
  - Severe physiologic jaundice of new born
Kernicterus

1) Condition of severe neural symptoms due to binding of unconjugated bilirubin to lipophilic components of the brain leading to cell destruction and encephalopathy (term for any degenerative condition of the brain)

2) Outcomes
a) Death
b) Some surviving infants develop severe symptoms
   Mental retardation symptoms and/or
   Hearing Defects and/or
   Cerebral Palsy

c) Others – no neonatal mental retardation symptoms. However later in childhood there can develop hearing defects and/or perceptual handicaps and/or hyperkinesis (increased motor

Two General Causes of Hyperbilirubinemia

1) Production of more bilirubin than liver can excrete

2) Failure of damaged liver or damaged biliary system to excrete bilirubin produced in normal amounts
Two Types of Hyperbilirubinemia

1) Unconjugated Hyperbilirubinemia

2) Conjugated Hyperbilirubinemia
Two Types of Hyperbilirubinemia

1) Unconjugated Hyperbilirubinemia
   - Due to defects up to and including conjugation

2) Conjugated Hyperbilirubinemia
   - Due to impairment of secretion of bilirubin into biliary system
     or
   - Due to impairment of biliary flow

Simple test for whether unconjugated or conjugated hyperbilirubinemia?

Detection of bilirubin in urine
Serum Bilirubin Concentrations for the Two Types of Hyperbilirubinemia

1) **Unconjugated Hyperbilirubinemia**
   - Increased bilirubin (both total and unconjugated)
   - No more that 15% direct

2) **Conjugated Hyperbilirubinemia**
   - Increased bilirubin (total, conjugated, and unconjugated)
   - At least > 30% direct, usually > 50% direct
   - δ-bilirubin present in conditions of increased conjugated bilirubin
   - Also significant elevation of indirect fraction
     Due to hydrolysis of bilirubin conjugates by tissue glucuronidases, and impaired delivery, uptake and storage of unconjugated bilirubin with liver cell damage.

---

**Table 52-1. Conjugated Versus Unconjugated Hyperbilirubinemia**

<table>
<thead>
<tr>
<th>Finding</th>
<th>Unconjugated Hyperbilirubinemia</th>
<th>Conjugated Hyperbilirubinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retained bilirubin</td>
<td>UCB</td>
<td>CB and UCB</td>
</tr>
<tr>
<td>Bilirubin in urine</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Diazo reaction (direct/total)</td>
<td>&lt;15%</td>
<td>&gt;30% (usually &gt;50%)</td>
</tr>
<tr>
<td>Abnormal steps</td>
<td>1–5 (Fig. 52-5)</td>
<td>6 or 7 (Fig. 52-6)</td>
</tr>
<tr>
<td>Usual causes</td>
<td>Hematologic, circulatory, or functional hepatic disorders</td>
<td>Hepatocellular or biliary tract disease</td>
</tr>
<tr>
<td>Compensatory mechanism</td>
<td>Catabolism of UCB to polar derivatives</td>
<td>Renal excretion of CB</td>
</tr>
</tbody>
</table>

CB, conjugated bilirubin; UCB, unconjugated bilirubin.
V. Hemolytic Anemias

A. Hemolytic Anemias:
General Considerations
## Definitions

1) **Hemolytic Anemia** – disorder that leads to a premature breaking up of red blood cell

2) **Anemia** – Reduction of circulating red cell mass. Can be measured as a reduction of hematocrit or reduction of hemoglobin

### General causes of anemia
- Blood loss
- Increased hemolysis
- Diminished erythropoiesis

## Classification Systems of Hemolytic Anemias

### A) Intravascular versus Extravascular
- **Intravascular**
  - Breakup within vascular system
- **Extravascular**
  - Breakup outside vascular system (i.e.- spleen)

### B) Congenital versus Acquired
- **Congenital**
  - Defect inherent in red blood cell (membrane, enzyme or hemoglobin defect)
- **Acquired**
  - Due to noxious factor in factor in blood
Hemolytic Anemias Cause:

1) Increased production of red blood cells

2) Bone marrow changes

Stem Cell Differentiation into Blood Cellular Components

Stem Cells (in bone marrow)

- Erythrocytes
- Monocytes
- Granulocytes
- Lymphocytes
- Platelets

Erythropoetin
(released by kidney, acts on stem cells to produce erythrocytes)
Precursor Cells of Erythrocytes

Stem Cell
\[\rightarrow\]
Pronormoblast
\[\rightarrow\]
Normoblast
\[\rightarrow\]
Reticulocyte
\[\rightarrow\]
Erythrocyte

Note: not all precursors are shown

Blood Cell Production in Developing Fetus

3rd Week \rightarrow Cluster of Stem Cells

3rd Month \rightarrow Liver principal site of blood cell production

4th Month \rightarrow Bone marrow increasing contribution to blood cell production from 4 months to birth

Birth \rightarrow Bone marrow is sole site of blood cell production from birth onward (in health)

Puberty \rightarrow Minor sites from 4 months to birth: spleen, lymph nodes, thymus

Liver decreasing contribution to blood cell production from 4 months to birth

Bone Marrow (all skeletal bones are active up to puberty)
Bone Marrow Beyond 18 Years

1) **Red Marrow** (active, i.e. synthesizing RBCs)
   - Vertebrae
   - Ribs
   - Sternum (breastbone)
   - Skull
   - Pelvis
   - Parts of humerus and femur

2) **Inactive Marrow** (yellow, fatty and inactive)

---

**Common Characteristics of all Hemolytic Anemias**

1) Premature destruction of RBCs
2) Retention of iron by body
3) Hyperactive bone marrow
4) Increased bilirubin (unconjugated)
General Manifestations of Hemolytic Anemias

1. Pallor skin
2. Hemosiderosis
3. Compositional fatty increases in heart, liver, proximal tubular, and ganglion cells
4. Bone marrow hyperplasia, activation of fatty marrow
5. Extramedullary hematopoiesis
6. Decreased hematocrit
7. Elevated reticulocyte count
8. Jaundice; unconjugated hyperbilirubinemia

Hemosiderosis: Excess Retention of Iron by Body

1) Iron stored in reticulo-endothelial (RE) cells as hemosiderin. Principal tissues having RE cells storing iron
   - Liver
   - Bone Marrow
   - Spleen
   - Lymph Nodes
   - Also skin, pancreas, kidneys

2) Causes little, if any, tissue injury
Manifestations of Hyperactive Bone Marrow

1) Reticulocytosis
   Increased number of young red blood cells (reticulocytes) in blood

2) Neo-osteogenesis
   Expansion of bone marrow volume

3) Activation of fatty marrow

Some Facts About Hyperactive Marrow

- Bone marrow can increase RBC production by 7-8X

- Anemia occurs only if bone marrow compensatory mechanisms outstripped
  i.e., hemolysis is greater than hyperactive marrow production of RBCs
Extramedullary Hematopoiesis
(formation of blood cells outside bone marrow)

Tissues that produce red blood cells in response to hemolytic anemia (extramedullary hematopoiesis)

- Liver*
- Spleen
- Lymph Nodes

*First to kick in

Intravascular Hemolysis
Manifestations

- Decreased haptoglobin in blood
- Hemoglobinemia
- Hemoglobinuria
- Methemalbuminemia
- Jaundice
- Hemosiderinuria
- Increased bilirubin in blood
- Increased LDH
Haptoglobin in Plasma

1) Binds hemoglobin (Hb)

2) \(\uparrow\text{Hb in plasma} \rightarrow \downarrow\text{Haptoglobin in plasma}\)

because Hb-haptoglobin complex removed within minutes \((t_{1/2} \text{ uncomplexed haptoglobin} = 4 \text{ days})\)

Mechanism of Hemosiderinuria

1) Hemoglobin (Hb) (α-β dimer, 32,000 MW) appears in filtrate when haptoglobin binding capacity exceeded.

2) Hb dimer reabsorbed in proximal tubule

3) In proximal tubule iron from heme stored as ferritin and hemosiderin

4) Cells sloughed off – hemosiderin detected in sediment with prussian blue dye
Hemoglobinuria

Only appears in rapid intravascular hemolysis – when Hb amount exceeds tubular capacity to reabsorb

Methemalbuminemia

Hemoglobin unstable in plasma. If kidneys’ capacity to excrete is exceeded then:

- Hemoglobin (in plasma) → Heme + Globin → Hemopexin in plasma
  - Heme oxidation
  - Dissociation
  - Binding to albumin
  - Binding to hemopexin
  - Complex cleared rapidly

methemalbuminemia
Other Manifestations of Intravascular Hemolysis

↑ LDH in blood

↑ Bilirubin (Unconjugated) in blood

Extravascular Hemolysis

1) Mechanism
   Red cells sequestered by spleen and liver

2) Manifestations
   - No hemosiderinuria, no hemoglobinuria, no hemoglobinemia
   - Haptoglobin in blood decreases (not as much as intravascular hemolysis)
   - Increased bilirubin in blood
   - Increased LDH in blood (not as much as intravascular hemolysis)
   - Hypertrophy of RE and enlargement of spleen (vulnerability to infection)
Bilirubin gallstones can be also formed in hemolytic conditions

B. Hemolytic Anemias: RBC Membrane Defects
### TABLE 137–1. CLASSIFICATION OF THE CAUSES OF HEMOLYTIC ANEMIA

<table>
<thead>
<tr>
<th>I.</th>
<th>Congenital hemolytic disorders (see Ch. 138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Membrane defects</td>
</tr>
<tr>
<td>B.</td>
<td>Enzyme defects</td>
</tr>
<tr>
<td></td>
<td>1. Embden-Meyerhof pathway defects</td>
</tr>
<tr>
<td></td>
<td>2. Hexose monophosphate shunt defects</td>
</tr>
<tr>
<td>C.</td>
<td>Hemoglobin defects</td>
</tr>
<tr>
<td></td>
<td>1. Structural (hemoglobinopathies) (see Ch. 143)</td>
</tr>
<tr>
<td></td>
<td>2. Synthetic (thalassemias) (see Ch. 142)</td>
</tr>
<tr>
<td>D.</td>
<td>Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II.</th>
<th>Acquired hemolytic disorders (see Ch. 139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Sequestrational hemolysis (hypersplenism)</td>
</tr>
<tr>
<td>B.</td>
<td>Immune hemolytic disorders</td>
</tr>
<tr>
<td></td>
<td>1. Alloimmune</td>
</tr>
<tr>
<td></td>
<td>2. Autoimmune</td>
</tr>
<tr>
<td></td>
<td>3. Drug-induced</td>
</tr>
<tr>
<td>C.</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
</tr>
<tr>
<td>D.</td>
<td>Due to toxins and metabolic abnormalities</td>
</tr>
<tr>
<td>E.</td>
<td>Due to red cell parasites</td>
</tr>
<tr>
<td>F.</td>
<td>Due to red cell trauma</td>
</tr>
</tbody>
</table>

---


---

![Schematic illustration](image)

**FIGURE 138–1.** Schematic illustration of the organization of the major proteins of the red cell membrane and membrane skeleton.

Hereditary Spherocytosis

1) Defect in spectrin membrane protein (either quantitative or qualitative) (forms skeleton network - present only in RBC neurons, cardiac myocytes skeletal myocytes) or deficiency in protein 4.1 causing RBC to have:
   a) Spherical Shape
   b) Fragile Membrane (thus vulnerable to sequestration)

2) Most common in North Europeans (1:5000)

3) Neonatal symptoms – excessive jaundice, sometimes requires transfusion.

Hereditary Spherocytosis (cont.)

4) Adult groups
   a) Partially compensated hemolysis
      ➢ Most adult patients
      ➢ Symptoms: mild to moderate anemia, jaundice and splenomegaly
   b) Mild
      ➢ 25% of patients
      ➢ Symptoms: no anemia, no jaundice, minimal splenomegaly
   c) Transfusion Dependent
      ➢ Small portion of patients
      ➢ Symptoms: anemia, jaundice, splenomegaly, fully compensated anemia except in combination with illnesses that lead to hypertrophy of spleen]
Hereditary Spherocytosis (cont.)

5) Clinical course interrupted by crises
   a) Hemolytic Crisis
      - Wave of hemolysis (patient becomes jaundiced, fever, abdominal pain, nausea, vomiting, tachycardia, low blood pressure)
      - Brought on by infection, which leads to hyperplasia of RE cells
   b) Aplastic Crisis
      - Suppression of RBC production
      - Worsens anemia
      - Caused by parvovirus which invades stem cells
      - Requires transfusion
   c) Megaloblastic Crisis
      - Dietary intake of folic acid inadequate

Hereditary Spherocytosis (cont.)

6) Extravascular Hemolysis
Laboratory Tests for Hereditary Spherocytosis

1) ↑ Reticulocyte count
2) ↑ Hyperbilirubinemia (50-60% cases)
3) ↑ Spherocytes
4) ↑ Osmotic fragility
   Measure of RBC cell’s ability to swell in graded series of hypotonic solutions: HS RBCs tolerate less swelling
5) ↑ Mean corpuscular hemoglobin concentration (due to dehydration)

Hereditary Spherocytosis: Treatment

1) Splenectomy – in patients with significant hemolysis
2) Unsplenectomized patients – folic acid supplementation to prevent megaloblastic crisis
C. Hemolytic Anemias:

RBC Enzyme Defects
Most Common RBC Enzyme Deficiencies Leading to Hemolysis

*1) Glucose –6– Phosphate Dehydrogenase
(Hexose Monophosphate Shunt Enzyme)

2) Pyruvate Kinase
(Embden-Meyerhoff Enzyme)

*Most common: affects millions
Two Metabolic Pathways Important to Maintaining Integrity of RBC

1) **Embden – Meyerhof Pathway (Glycolysis)**

Oxidation pathway of glucose → Lactic acid → ATP

2) **Hexose Monophosphate (HMP) Shunt Pathway**

Pathway that synthesizes NADPH

Reducive biosynthesis such as fatty acids and cholesterol

Synthesis of glutathione (provides protection from free radical oxidation)

---

Figure showing Embden-Meyerof and Hexose Monophosphate Shunt pathways and the enzymes promoting pathway reactions

HMP Shunt Reaction Generating NADPH

Glucose 6-phosphate

\[ 3 \text{G6P} + 6\text{NADP}^+ + 3\text{H}_2\text{O} \leftrightarrow \]

Ribulose 5-phosphate

\[ 6 \text{NADPH} + 6\text{H}^+ + 3\text{CO}_2 + 3 \text{Ru5p} \]

Fructose 6-Phosphate

Glyceraldehyde 3-phosphate

Both enter glycolytic pathway
How HMP Shunt Maintains Integrity of RBC

Generated NADPH maintains glutathione in reduced form (GSH) – which reacts with free radicals, preventing their reaction with red cell components.

Glucose - 6 - Phosphate Dehydrogenase Deficiency

Insufficient GSH in RBC

Infection or oxidant drug

Acute Intravascular Hemolysis
Various Mechanisms of Hemolysis for G-6-PD Deficiency

1) Formation of Heinz Bodies
   a) Free radicals oxidize hemoglobin to methemoglobin and sulfhemoglobin – which precipitates as heinz bodies.
   b) Heinz bodies adhere to cell walls, leading to decrease deformability, leakiness and osmotic fragility
   c) Heinz bodies plucked out by RE cells – RBC lysed in spleen

2) Oxidative cross-linking of spectrin which decreases RBC flexibility and promotes splenic trapping of RBCs

3) Peroxidation of membrane lipids

Genetics of G-6-PD

G-6-PD gene on X chromosome leading to:

- **Affected Females (XX):**
  Have mosaic – some RBC cells are affected (G-6-PD X chromosome gene that is activated is disease variant), others are not (G-6-PD X chromosome gene that is activated is normal gene) – mild hemolysis

- **Affected Males (XY):**
  All RBC cells are affected, since there is only one X chromosome gene, being the disease variant – severe hemolysis
Variants of G-6-PD

Gd^B - normal enzyme
70% American Blacks
99% Whites

Gd^A+ - normal enzyme
20% American Black

Gd^A – Abnormal variant associated with hemolysis
10% of American blacks

Gd^Med – Abnormal variant associated with hemolysis
Mediterranean, India, SE Asian populations

---

**TABLE 138–4. CLINICAL COMPARISON OF THE TWO COMMON FORMS OF G6PD DEFICIENCY**

<table>
<thead>
<tr>
<th></th>
<th>Gd^A⁻</th>
<th>Gd^Med</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Common in black populations</td>
<td>Common in Mediterranean populations</td>
</tr>
<tr>
<td>Chronic hemolysis</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Degree of acute</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>hemolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6PD defect</td>
<td>Old red cells</td>
<td>All red cells</td>
</tr>
<tr>
<td>Hemolysis with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs</td>
<td>Unusual</td>
<td>Common</td>
</tr>
<tr>
<td>Infection</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>Need for transfusions</td>
<td>Rare</td>
<td>Sometimes</td>
</tr>
</tbody>
</table>

How Embden-Meyerhof Pathway Maintains Integrity of RBC

Pathway generates ATP which maintains RBC
Most Common RBC Enzyme Deficiency in Embden-Meyerof Pathway

Pyruvate Kinase

Figure showing Embden-Meyerof and Hexose Monophosphate Shunt pathways and the enzymes promoting pathway reactions

Enzyme Defects in Embden-Meyerof Pathway

1) Hemolysis is chronic and not affected by drugs

2) Laboratory Diagnosis – determine enzyme activity in serum sample

D. Hemolytic Anemias: Hemoglobinopathies
**Hemoglobinopathies**

1) **Background: Types of Hemoglobin and Hemoglobin in Development**

---

**TABLE 137-1. CLASSIFICATION OF THE CAUSES OF HEMOLYTIC ANEMIA**

<table>
<thead>
<tr>
<th>I. Congenital hemolytic disorders (see Ch. 138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Membrane defects</td>
</tr>
<tr>
<td>B. Enzyme defects</td>
</tr>
<tr>
<td>1. Embden-Meyerhof pathway defects</td>
</tr>
<tr>
<td>2. Hexose monophosphate shunt defects</td>
</tr>
<tr>
<td>C. Hemoglobin defects</td>
</tr>
<tr>
<td>1. Structural (hemoglobinopathies) (see Ch. 143)</td>
</tr>
<tr>
<td>2. Synthetic (thalassemias) (see Ch. 142)</td>
</tr>
<tr>
<td>D. Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Acquired hemolytic disorders (see Ch. 139)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sequestrational hemolysis (hypersplenism)</td>
</tr>
<tr>
<td>B. Immune hemolytic disorders</td>
</tr>
<tr>
<td>1. Alloimmune</td>
</tr>
<tr>
<td>2. Autoimmune</td>
</tr>
<tr>
<td>3. Drug-induced</td>
</tr>
<tr>
<td>C. Paroxysmal nocturnal hemoglobinuria</td>
</tr>
<tr>
<td>D. Due to toxins and metabolic abnormalities</td>
</tr>
<tr>
<td>E. Due to red cell parasites</td>
</tr>
<tr>
<td>F. Due to red cell trauma</td>
</tr>
</tbody>
</table>

Hemoglobin (Hb) Structure – Tetramer consisting of four globin monomer chains, comprising two pairs of similar monomer chains (different hemoglobins have different pairs).

Six monomer globin chains:

- α
- β
- γ
- δ
- ε
- ζ

Table giving structure and percent of hemoglobin types in blood samples.

HbA (α₂β₂)

1) Predominant Hb in adult (95-98%)

2) First appears last six weeks of fetal life

3) By 6-12 months after birth - 95-98% of Hb

HbA₂ (α₂δ₂)

1) First appears at term

2) Low concentration throughout life
Hemoglobin in Developing Fetus

1) Gower I ($\zeta_2 \epsilon_2$) and Gower II ($\alpha_2 \epsilon_2$) present in first month (not detectable after that)

2) Portland ($\zeta_2 \gamma_2$) – small amounts persist throughout fetal life

3) Fetal Hemoglobin (HbF; $\alpha_2 \gamma_2$) - major hemoglobin in fetus after first months
(90% hemoglobin in fetus after first months is HbF)
### HbF after birth

<table>
<thead>
<tr>
<th>Time</th>
<th>% HbF of all Hemoglobins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>70-90%</td>
</tr>
<tr>
<td>End of 1 month</td>
<td>50-70%</td>
</tr>
<tr>
<td>End of 2 months</td>
<td>25-60%</td>
</tr>
<tr>
<td>End of 3 months</td>
<td>10-30%</td>
</tr>
<tr>
<td>6-12 months</td>
<td>Falls from 8% to 2%</td>
</tr>
<tr>
<td>12-24 months</td>
<td>1.8%</td>
</tr>
<tr>
<td>By third year</td>
<td>1.0%</td>
</tr>
<tr>
<td>After that</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

### Hemoglobinopathies

2) Different Types
Hemoglobinopathies- genetic disorder of structure or synthesis of one or more of the globin chains of Hb

1) **Structural Variants** - substitutions deletion, or addition.

2) **Thalassemia** – group of disorders in which quantitative defect in α or β chain production.

3) **Complex** – Combination of both α + β

4) **Hereditary persistence of fetal hemoglobin**

Hemoglobinopathies

3) Structural Variants
Structural Hb Variants

1) Approximately 300 discovered

2) Most that are clinically significant involve changes in β chain

3) Clinical manifestations (vary with variant type)
   a) Hemolytic Anemia
   b) Cyanosis - lack of O₂ delivered to tissue, blue color of blood.
      This is caused by one of the following defects resulting from the defective hemoglobin:
      - Methemoglobinemia (M)
      - Decreased O₂ affinity of Hb
   c) Polycythemia
      Increase number of RBCs, also called erythrocytosis
   d) Hypochromic Anemia – decreased Hb concentration leading to “less” color

---

Table 33-2 Clinical manifestations associated with some abnormal hemoglobins

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Abnormal Hb</th>
<th>Structural change</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic anemia</td>
<td>H</td>
<td>alpha(bΑ₂)β₄ → β₄</td>
<td>Unable hemoglobin occurring in some forms of alpha-thalassemia,</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>beta 6 gln → val</td>
<td>precipitation of hemoglobin and hemolysis are accelerated by certain drugs</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>beta 6 gln → lys</td>
<td>Forms molecular aggregates when deoxygenated, producing sickle cell anemia in homozygotes</td>
</tr>
<tr>
<td></td>
<td>Davenport</td>
<td>beta 121 gln → gln</td>
<td>Low solubility low plasticity of red cells, causing hemolytic anemia in homozygotes</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>beta 26 glu → lys</td>
<td>Mechanism unknown</td>
</tr>
<tr>
<td></td>
<td>Zurich</td>
<td>beta 63 his → arg</td>
<td>Unable hemoglobin precipitated by certain drugs, producing hemolytic anemia in homozygotes</td>
</tr>
<tr>
<td></td>
<td>Köln</td>
<td>beta 98 val → met</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sydney</td>
<td>beta 67 val → ala</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Santa Ana</td>
<td>beta 88 leu → pro</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Philadelphia</td>
<td>beta 35 tyr → phe</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gun Hill</td>
<td>beta deletion of 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>residues between 90 and 96</td>
<td></td>
</tr>
<tr>
<td>Cytocytosis caused by methemoglobinemia</td>
<td>M₈S₁₀₄</td>
<td>alpha 38 his → tyr</td>
<td>Methemoglobin causes cyanosis in homozygotes; precipitated hemoglobin tends to form inclusion bodies within red cells, under certain conditions</td>
</tr>
<tr>
<td></td>
<td>M₈S₁₀₄</td>
<td>alpha 87 his → tyr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M₈S₈N²/N₁₀⁻</td>
<td>beta 92 his → tyr</td>
<td></td>
</tr>
<tr>
<td>Cytocytosis caused by increased deoxyhemoglobin</td>
<td>Kansas</td>
<td>beta 102 asn → dir</td>
<td>Increased oxygen affinity of hemoglobin causes cyanosis in heterozygotes; some also have evidence of hemolytic anemia</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>Lancaster</td>
<td>alpha 92 arg → gln</td>
<td>Methemoglobin causes cyanosis in homozygotes; some also have evidence of hemolytic anemia</td>
</tr>
<tr>
<td></td>
<td>Cheapestake</td>
<td>alpha 92 arg → leu</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raisner</td>
<td>beta 145 tyr → cys</td>
<td>Increased oxygen affinity of hemoglobin hinders release of oxygen to tissues, causing compensatory polycythemia in heterozygotes</td>
</tr>
<tr>
<td></td>
<td>Hydrops feticus</td>
<td>bart's alpha, gamma₉ → gamma₉</td>
<td>Unable hemoglobin with high oxygen affinity occurring in high concentration in stillborn fuses with homozygous alpha-thalassemia</td>
</tr>
</tbody>
</table>

Press Schmidt, RM, and Buxton, RM: Basic laboratory methods of hemoglobinopathy detection, Atlanta, 1978, Centers for Disease Control

Hemoglobinopathies
3) Most Common Structural Variant – Sickle Cell Syndromes

Sickle Cell Syndromes

Defective Hemoglobin - HbS \( (\alpha_2\beta^S_2) \)

Results from substitution of valine in place of the glutamic acid in the 6th position of the \( \beta \) chain
Sickling of RBC results from aggregation of HbS in RBC
Aggregation Process

1) HbS aggregates when deoxygenated (gelation or crystallization)
2) Aggregates grow - linear arrangement form viscous gel.
3) Sickling of red blood cell (conforms to viscous interior), RBC has decreased flexibility
4) With reoxygenation, the HbS aggregate dissociates (gel “liquifies”)

Repeated Cycles of Aggregation

1) Damaged membrane – cells lose K⁺ and water

2) Irreversible sickled RBC formed

3) These irreversibly sickled RBCs are sequestered in spleen

4) Occlusion of microvasculature occurs

5) Mean life of sickled RBC is 20 days

Gel Formation Favored

1) High concentration of deoxyhemoglobin S such as will arise with increased mean corpuscular hemoglobin concentration (MCHC)

2) Acidosis (pH 6.8 optimum)

3) High concentration 2,3 DPG

4) Low ionic strength

5) Low state of oxygenation

2,3 diphosphoglycerate – most abundant glycolytic intermediate in red blood cells, reduces affinity of Hb for O₂
Sickle Cell Syndromes

1) Sickle Cell Trait
   - Heterozygous in βS gene
   - 8-10% prevalence for African Americans

2) Sickle Cell Disease
   - Homozygous in βS gene
   - 1 in 400 prevalence for African Americans
   - Also found in lower frequency in Mediterranean, Saudi Arabian, India populations

Sickle Cell Trait

1) Asymptomatic – No anemia

2) Vaso-occlusion can occur in extreme circumstances of severe hypoxia (flying unpressurized aircraft)

   - Mechanism: Dehydration of RBC in renal medulla, leading to increased MCHC causing increased sickling, which then gets stuck in microvasculature causing diminished/stopped blood flow in the medulla, causing cell death in medulla
   - Unable to concentrate urine
   - Small percentage of traits (3%) experience hematuria due to infarction of renal medulla
Pathology of Sickle Cell Disease

Results from sickling of RBCs

Signs and Symptoms of Sickle Cell Disease

1) Chronic compensated anemia

2) Painful vaso-occlusive events
   - Pain in bones of trunk (back and chest) and extremities
   - Lasts for hours to days
Signs and Symptoms of Sickle Cell Disease (cont.)

3) Acute, chronic, and progressive tissue damage (can include following):
   a) Cerebrovascular accidents (partial paralysis and seizures)
   b) Acute chest syndrome (occlusion of pulmonary vessels)
   c) Acute renal papillary infarction
      > Hematuria
      > Polyuria
   d) Microinfarction of peripheral retina (can cause retinal detachment and blindness)
   e) Chronic manifestations
      > From repeated bone infarcts; degenerative arthritis and necrosis of hip
      > Refractory skin ulcers
      > Variable degrees of renal insufficiency

Signs and Symptoms of Sickle Cell Disease (cont.)

4) Others
   a) Increased infection susceptibility to
      > Due to, in part, absence of splenic function
   b) Cholelithiasis (gallstones)
   c) Abnormal growth and development

5) Hemolytic crisis triggered by:
   - Exertion
   - Hypertonic environment of renal medulla
   - Lower O_2
   - Acidosis
   - Infections
   - Pregnancy
   - Sleep
   - Hemoconcentration
Signs and Symptoms of Sickle Cell Disease (cont.)

6) General Manifestations of Hemolytic Anemias
   a) Pallor skin (paleness, absence of skin color)
   b) Hemosiderosis
   c) Composition fatty increase in heart, liver and tubules of kidney
   d) Bone marrow hyperplasia; activation of fatty marrow
   e) Extramedullary hematopesis
   f) Decreased hematocrit
      Normal 40-50%
      Sickle Cell disease 20-30%
   g) Elevated Reticulocyte count
      Normal 0.5-1.5%
      Sickle Cell Disease 10-25%
   h) Jaundice - mild unconjugated hyperbilirubinemia

Progression of Sickle Cell Disease

1) Not evident at birth – manifests itself 3-6 months until γ chains are replaced by βs chains. Appears as hemolytic anemia.

2) Prognosis
   Significant number of infants with sickle cell anemia die in the first 2-3 years due to sepsis or acute splenic sequestration crisis. Mean survival is to 4th decade with death due to cardiopulmonary complications and/or renal insufficiency

3) Treatment – Supportive
   a) Large volumes of intravenous fluids
   b) Analgesics for pain
   c) Antibiotics to treat infections
   d) Oxygen for hypoxia
   e) Transfusions - for life-threatening vaso-occlusive events
   f) Young children receive penicillin because of high risk of septicemia
Lab Findings in Sickle Cell Disease

1) Sodium Metabisulfate Test
   - Totally deoxygenates blood, induces sickling of any Sickle RBCs seen under microscope

2) Solubility Test
   - Blood mixed with solution at high ionic strength and observe turbidity
     - Normal Hb – clear
     - HbS - precipitate

3) Hemoglobin Electrophoresis
   - Specific mobility for HbS

---

Table 33.3  Varying clinical severity of the different sickle syndromes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% of hemoglobin S</th>
<th>% of non-S hemoglobin</th>
<th>Clinical severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>30-40</td>
<td>60-70 (A)</td>
<td>0</td>
</tr>
<tr>
<td>SF*</td>
<td>70</td>
<td>30 (F)</td>
<td>0</td>
</tr>
<tr>
<td>SS</td>
<td>80-90</td>
<td>5-15 (F)</td>
<td>++/++++</td>
</tr>
<tr>
<td>S-thalassemia</td>
<td>80</td>
<td>20 (A + F)</td>
<td>+++</td>
</tr>
<tr>
<td>SC</td>
<td>50</td>
<td>50 (C)</td>
<td>+++</td>
</tr>
<tr>
<td>SO.SD</td>
<td>30-40</td>
<td>60-70 (O.D)</td>
<td>+++/++++</td>
</tr>
</tbody>
</table>


*Double heterozygous state for hemoglobin S and hereditary persistence of fetal hemoglobin.

Drug offers relief for sickle cell anemia

Plain Dealer, January 31, 1995

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Hemoglobinopathies

4) Thalassemias
Hemoglobinopathies- genetic disorder of structure or synthesis of one or more of the globin chains of Hb

1) Structural Variants - substitutions deletion, or addition.

2) Thalassemia – group of disorders in which quantitative defect in α or β chain production.

3) Complex – Combination of both a + b

4) Hereditary persistence of fetal hemoglobin

Thalassemia

Hemoglobinopathy that is characterized by decreased (or absent) synthesis of α or β chain
Prevalence of Thalassemia Syndromes

1) α-Thalassemia
   - 30% African Americans are carriers
   - Also in Mediterranean populations (lower prevalence)

2) β-Thalassemia
   - Mediterranean and Asian
   - 4-6% of India, Chinese, Southeast Asian have thalassemia trait

Classification of Thalassemias

\[ \alpha \leftrightarrow \text{Type} \rightarrow \beta \]

(Deficient Chain)

\(\alpha\) Thalassemia Phenotypes/Mutations
1) Silent Carrier 1*
2) Trait 2*
3) Hemoglobin H Disease 3*
4) Hydrops Fetalis 4*
* number of defective α genes

\(\beta\) Thalassemia Mutations
- \(\beta^0\) chain synthesis absent
- \(\beta^+\) 1/2 normal chain synthesis
- \(\beta^{++}\) 2/3 normal chain synthesis

\(\beta\) Thalassemia Phenotypes
- Major
- Intermedia
- Trait
\(\beta^0\beta^0\) \(\beta^{0+}\) \(\beta^{0++}\)
\(\beta^0\beta^+\) \(\beta^{++}\)
\(\beta^0\beta^{++}\)
\(\beta^+\beta^{++}\)
\(\beta^{+}\) \(\beta^{++}\)
\(\beta^0, \beta^+, \text{ or } \beta^{++}\)
Cause of Hemolysis in Thalassemias

Precipitation of counterpart chain damages erythrocyte, causing sequestration by spleen

β-Thalassemia - precipitation of α chains
α-Thalassemia - precipitation of β chains

Course of β-Thalassemia Major

Severe Anemia and Poor Growth
(appears at 6-9 months)

No Blood Transfusion
Severe expansion of bone marrow (Deformity in skull in particular and susceptibility to fractures)
Death by 2-3 yrs

With Blood Transfusion
Normal development until age 10-12
**Course of β-Thalassemia Major**  
(Treated with Blood Transfusions)

### After Age 10-12

<table>
<thead>
<tr>
<th>Endocrine Problems</th>
<th>Liver Problems</th>
<th>Cardiac Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Growth Failure</td>
<td>- Liver Enlarged</td>
<td>- Pericarditis</td>
</tr>
<tr>
<td>- Hypogonadism</td>
<td>- Fibrosis which may</td>
<td>- CHF</td>
</tr>
<tr>
<td>- Diabetes Mellitus</td>
<td>progress to cirrhosis</td>
<td>- Arrhythmias</td>
</tr>
<tr>
<td>- Hypothyroidism</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Death by age 17  
(from CHF or Arrhythmias)

**Cause of Clinical Problems of β-Thalassemia Major**

Iron overload resulting from:
- Blood transfusions
- Increased Fe absorption in GI Tract with increased erythropoiesis
Hemosiderosis
(excess hemosiderin in tissues)

Can develop to

Hemochromatosis
(pathologic excess of iron in tissue leading to tissue damage and dysfunction)

Tissues susceptible to develop hemochromatosis
- Endocrine glands
- Heart
- Liver

β-Thalassemia Major
Laboratory Findings

1) Hemoglobins
   - HbF 70-90%
   - HbA₂ Up to 20%

2) Elevated serum iron
3) Red blood cells – small, hypochromic
4) Usual hemolytic anemia findings
   - increased bilirubin
   - decreased hematocrit
   - decreased hemoglobin (3-6g/dL)
     normal 12-18g/dL
Treatment of β-Thalassemia Major

1) Blood Transfusion

2) Chelation Therapy – Desferal
   - Delays cardiac dysfunction

3) Splenectomy
   - Done at 4 years age
   - Antibiotics given to prevent sepsis
   - Necessary because splenic enlargement often cause functional hypersplenism

β-Thalassemia Trait

1) Genotypes
   - Heterozygous: β with β⁰, β⁺, or β⁺⁺

2) Asymptomatic
   - For the most part, may have mild anemia, splenomegaly

2) β-thalassemia trait more severe than α-thalassemia trait
Laboratory Findings in Thalassemia Trait  
(for both $\alpha$ and $\beta$)

1) Hemoglobins

<table>
<thead>
<tr>
<th>$\beta$-Thalassemia Trait</th>
<th>$\alpha$-Thalassemia Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA$_2$</td>
<td>4-8%</td>
</tr>
<tr>
<td>HbF</td>
<td>1.5-2.5%</td>
</tr>
<tr>
<td>HbA</td>
<td>remainder</td>
</tr>
</tbody>
</table>

2) RBCs – small and hypochromic

3) $^{\downarrow}$ hematocrit

4) Serum iron is normal

β-Thalassemia Intermedia

1) Genotypes

- $\beta^{+}\beta^{+}$
- $\beta^{+}\beta^{-}$
- $\beta^{-}\beta^{+}$
- $\beta^{-}\beta^{-}$

2) Clinical Course

- Much better prognosis than major condition
- Most survive into adulthood
- Accumulation of iron leads to cardiac and endocrine dysfunction

3) Laboratory values

- Hemoglobin concentration (above 6-7 g/dL)
- Hemoglobin types
  - HbF - large amounts
  - HbA$_2$ - significant amounts
  - HbA - variable amounts
α-Thalassemia Syndromes

1) Silent Carrier
   - One defective α gene
   - Asymptomactic
α-Thalassemia Syndromes (cont.)

2) α-Thalassemia Trait
   a) Mild microcytosis only manifestation
      A condition in which RBCs are smaller than normal
   b) Types
      1’) α Thalassemia-1-trait
         ➢ 2 defective α genes on same chromosome
         ➢ Prevalent in Oriental populations
      2’) Homozygous – α Thalassemia -2-trait
         ➢ One defective α gene on each chromosome
         ➢ Prevalent in Black populations

α-Thalassemia Syndromes (cont.)

3) Hemoglobin H Disease
   a) Three defective α genes
   b) Clinical Manifestations
      1’) Mild hemolysis and anemia compared to
          β thalassemia major
           - Normal longevity
           - Requires only intermittent transfusions
      2’) Occurrence of hemolytic and aplastic crises can
          be fatal
      3’) Moderate splenomegaly
α-Thalassemia Syndromes (cont.)

3) Hemoglobin H Disease (cont.)
   c) Laboratory Findings
      1’) Hemoglobin (7-10g/dL)
         - HbA 70-90%
         - HbH (\(\beta_4\)) 10-30%
         - HbA_2 low
      2’) Microcytosis and hypochromia

Mechanism of Hemolysis in Hemoglobin H Disease

1) Excess pf \(\beta\) chain leads to formation of \(\beta_4\) (HbH)

2) \(\beta_4\) precipitates as heinz bodies as RBC ages
   (loses ability to withstand oxidative “attack”)

3) \(\beta_4\) more stable and soluble than \(\alpha_4\) thus
   hemolysis in HBH disease much less than \(\beta\) Thalassemia
α-Thalassemia Syndromes (cont.)

4) **Hydrops Fetalis** (Hemoglobin Bart’s Disease)
   a) Four defective α genes
      ➢ No HbA, HbA₂, HbF synthesis
   
   b) Clinical Course
      Infants still born. Infants are edematous (hydropic) because of CHF resulting from severe anemia
   
   c) γ₄ (Bart’s) has extremely high affinity for O₂ (functionally useless for O₂ transport)
   
   d) β₄ also has high affinity for O₂ and useless for O₂ transport (only produced at last part of gestation)

---

Hemoglobinopathies

5) Methodology – Hemoglobin Electrophoresis
Electrophoresis

Amino acids with charged polar side chains

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Structure</th>
<th>pKᵢ of side chain group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>[Structure]</td>
<td>10.00</td>
</tr>
<tr>
<td>Arginine</td>
<td>[Structure]</td>
<td>12.48</td>
</tr>
<tr>
<td>Histidine</td>
<td>[Structure]</td>
<td>6.00</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>[Structure]</td>
<td>3.95</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>[Structure]</td>
<td>4.32</td>
</tr>
</tbody>
</table>
Electrical Force ($F_E$)

$$F_E = Q E$$

- $Q$: Charge on ion (Coulombs)
- $E$: Electrical Field Strength ($\text{V/m}$ or $\text{Joule/Coulomb-m}$)
- $F$: Work done

Frictional Force ($F_F$)

$$F = 6 \pi r \eta \nu$$

- $r$: Wall radius (m)
- $\eta$: Viscosity ($\text{kg/m-s}$)
- $\nu$: Velocity of solute ($\text{m/s}$)

Electro-osmosis

(Solvent Flow)
Figure 18-30. A schematic of a typical electrophoresis apparatus. Two buffer boxes (1) with buffer plates contain the buffer used in the process. In each buffer box is an electrode (2), either platinum or carbon, the polarity of which is fixed by the mode of connection to the power supply. The electrophoresis support (3) on which separation takes place is in contact with buffer by means of wicks (4). In some systems, the support dips directly into the buffer solution. The whole apparatus may be covered (5) to minimize evaporation and protect the system. Direct current power supply may be either constant current (adjustable) or constant voltage (adjustable), or both.

---

Table 8-3 Common effects of electrophoretic parameters on separation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect on electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Changes charge of analyte and hence effective mobility; can affect structure of analyte, such as denaturing or dissociating a protein.</td>
</tr>
<tr>
<td>Ionic strength</td>
<td>Changes voltage or current; increased ionic strength usually reduces migration velocity and increases heating.</td>
</tr>
<tr>
<td>Ions present</td>
<td>Can change migration velocity if interaction is strong; can cause tailing of bands.</td>
</tr>
<tr>
<td>Current</td>
<td>Too high a current causes overheating.</td>
</tr>
<tr>
<td>Voltage</td>
<td>Migration velocity is proportional to voltage. Temperature gradients in support medium cause banded bands. Overheating can denature (precipitate) proteins. Lower temperatures reduce diffusion but also reduce migration velocity; there is no effect on resolution.</td>
</tr>
<tr>
<td>Temperature</td>
<td>Separation of bands (resolution) increases linearly with time, but diffusion of bands (diffusion) increases with the square root of time.</td>
</tr>
<tr>
<td>Time</td>
<td>Major factors are osmosis and pore-size effects, which affect migration velocities.</td>
</tr>
</tbody>
</table>
Common Electrophoresis Media

1. Cellulose Acetate

2. Agarose

3. Polyacrylamide

Cellulose Acetate Electrophoresis

1. Cellulose treated with acetic anhydride

2. Advantages
   - Speed of separation 20 min-lh
   - Reproducibility
   - Easy storage

3. Disadvantages
   - Resolution (maximum of 8-9 serum protein bands)

4. Most popular media in clinical lab
Agarose Electrophoresis

1. Polymer of galactose and 3,6-anhydrogalactose
2. Very large pore size
3. Low amount of absorption low electro-osmotic effect
4. Second most popular media in clinical lab
5. Higher resolution than cellulose acetate

Polyacrylamide Electrophoresis

1. Polymerization of acrylamide and N,N-methylene bisacrylamide (cross-linking agent)
   - Pore size controlled by amount of cross-linking agent (75% gives 50Å pore size)
2. Separates by charge and size
3. Minimal electro-osmosis
4. High Resolution (20 or more serum bands)
5. Disadvantages: preparation gel reproducibility
6. Research tool
Relative Mobilities of Hemoglobins on Cellulose Acetate (T.E.B., pH 8.4)

Relative Mobilities of Some Hemoglobins on Citrate Agar (pH 6.0-6.2)
E. Hemolytic Anemias: Acquired Disorders

TABLE 137–1. CLASSIFICATION OF THE CAUSES OF HEMOLYTIC ANEMIA

I. Congenital hemolytic disorders (see Ch. 138)
   A. Membrane defects
   B. Enzyme defects
      1. Embden-Meyerhof pathway defects
      2. Hexose monophosphate shunt defects
   C. Hemoglobin defects
      1. Structural (hemoglobinopathies) (see Ch. 143)
      2. Synthetic (thalassemias) (see Ch. 142)
   D. Other

II. Acquired hemolytic disorders (see Ch. 139)
   A. Sequestrational hemolysis (hypersplenism)
   B. Immune hemolytic disorders
      1. Alloimmune
      2. Autoimmune
      3. Drug-induced
   C. Paroxysmal nocturnal hemoglobinuria
   D. Due to toxins and metabolic abnormalities
   E. Due to red cell parasites
   F. Due to red cell trauma

E. Hemolytic Anemias: Acquired Disorders

1. Hypersplenism

Hypersplenism

1. Accelerated function of spleen due to splenomegaly
   How spleen operation is accelerated:
   - hypertrophy of phagocytic elements
   - congestion of spleen slowing transit time

2. Hypersplenic condition can lead to destruction of as much as:
   - 90% platelets
   - 45% RBC
Criteria for Diagnosis of Hypersplenism

1. **Cytopenia**
   Deficiency in cellular elements of the blood

2. **Compensatory marrow hyperplasia**

3. **Splenomegaly**

4. **Correction by splenectomy**

---

**TABLE 164—1. CAUSES OF SPLENOMEGALY**

<table>
<thead>
<tr>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection (lymphoid hyperplasia)</td>
</tr>
<tr>
<td>Viral, parasitic, bacterial, fungal</td>
</tr>
<tr>
<td>Inflammation (lymphoid hyperplasia)</td>
</tr>
<tr>
<td>Rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus, renal dialysis, beryllium</td>
</tr>
<tr>
<td>Neoplasms (infiltrative or myeloproliferative)</td>
</tr>
<tr>
<td>Leukemia, lymphoma, polycythemia vera, myeloid metaplasia, cysts, metastatic tumors, primary tumors</td>
</tr>
<tr>
<td>Hemolytic Disease (phagocytic hyperplasia)</td>
</tr>
<tr>
<td>Spherocytosis, thalassemia major, pyruvate kinase deficiency</td>
</tr>
<tr>
<td>Deficiency Diseases</td>
</tr>
<tr>
<td>Iron deficiency, pernicious anemia</td>
</tr>
<tr>
<td>Infiltration</td>
</tr>
<tr>
<td>Gaucher’s disease, Neiman-Pick disease, amyloidosis, extramedullary hematopoiesis</td>
</tr>
<tr>
<td>Splenic Vein Hypertension (vascular congestion)</td>
</tr>
<tr>
<td>Cirrhosis, splenic or portal vein thrombosis, hepatic schistosomiasis</td>
</tr>
<tr>
<td>Endocrine</td>
</tr>
<tr>
<td>Graves’ disease, Hashimoto’s thyroiditis</td>
</tr>
<tr>
<td>Hemophilia (subsequent to intensive therapy with clotting-factor concentrate)</td>
</tr>
</tbody>
</table>

Classification of Causes of Splenomegaly

1. Proliferation of lymphoid cells into spleen
2. Infiltration of spleen by neoplastic cells or lipid-laden macrophages
3. Extramedullary hematopoiesis
4. Hypertrophy & Hyperplasia
5. Vascular congestion

Splenomegaly conditions leading to hypersplenism

1) Hypertrophy and hyperplasia of phagocytic cells (inflammatory disease and infections)

2) Congestive splenomegaly
Positive feedback mechanism leads to accelerated splenic activity

\[ \uparrow \text{Splenic activity (hypersplenism)} \rightarrow \uparrow \text{phagocytosis} \rightarrow \uparrow \text{hyperplasia of splenic cells} \]

Hypersplenism is particularly threatening to patients with a compensated RBC abnormality
### TABLE 137-1. CLASSIFICATION OF THE CAUSES OF HEMOLYTIC ANEMIA

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<td></td>
</tr>
<tr>
<td>D. Other</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Acquired hemolytic disorders (see Ch. 139)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sequestrational hemolysis (hypersplenism)</td>
<td></td>
</tr>
<tr>
<td>B. Immune hemolytic disorders</td>
<td></td>
</tr>
<tr>
<td>1. Alloimmune</td>
<td></td>
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<tr>
<td>2. Autoimmune</td>
<td></td>
</tr>
<tr>
<td>3. Drug-induced</td>
<td></td>
</tr>
<tr>
<td>C. Paroxysmal nocturnal hemoglobinuria</td>
<td></td>
</tr>
<tr>
<td>D. Due to toxins and metabolic abnormalities</td>
<td></td>
</tr>
<tr>
<td>E. Due to red cell parasites</td>
<td></td>
</tr>
<tr>
<td>F. Due to red cell trauma</td>
<td></td>
</tr>
</tbody>
</table>


---

**E. Hemolytic Anemias:**

**Acquired Disorders**

**2. Immune Hemolytic Disorders**
Basic Immunology

Basics of Immune Response

1. Differentiation of B and T Cells in Primary Lymphoid Tissues
2. Mature B and T Cells Settle in Secondary Lymphoid Tissues
3. Antigen Stimulates Clonal Proliferation of B and T Cells in Secondary Lymphoid Tissues
4. T Cells Act on B Cells to Increase (T Helper) or Suppress (T Suppressor) Production of Monoclonal Subset of B Cells in Secondary Lymphoid Tissues
5. The B Cells Produce Plasma Cells Which Secrete Large Quantities of Antibodies in the Secondary Lymphoid Tissues
A. Overview Facts

1) There are two types of lymphocytes: B lymphocytes and T lymphocytes

2) Primary lymphoid tissues
   - Location where mature B cells and T cells are formed from stem cells and then released into the blood and the lymph. This process happens without antigen (antigen independent)
   - The primary lymphoid tissues are:
     - B Cells: Bone marrow partially and other unknown location
     - T cells: Thymus

A. Overview Facts (cont.)

3) Secondary Lymphoid Tissues
   - Place where the antigen is concentrated and the specific immune response is generated
   - Location:
     - Spleen
     - Lymph Nodes
B. The Clonal Selection Theory  The actual production of Ab (occurs in secondary lymphoid tissue)

- Antigen stimulated proliferation of monoclonal subset of mature lymphocytes (see diagram)
  - **B Cells:** There is a specific B cell for each antibody produced of given specificity. Thus there are countless B cell subtypes each dedicated to the production of plasma cells producing one clone of antibodies with one epitope specificity. Antigen binds to the one subtype of B cell that has antibodies on the surface that specifically bind it causing a proliferation of this particular subset of B cells.
  - **T Cells:** The antigen also interacts with T cells (although how, is not been defined, as there is not a specific receptor on the cell surface for antigen) that cause the proliferation of T cells that either promote the further proliferation of the B cell subset (T helper cells) or suppress it (T suppressor cells)

- The antibody receptors on the B cells are either monomers of IgM or IgD. These antibodies have identical specificity to IgG and IgM antibodies produced by the plasma cells (the cells that produce the Ab)

C. Primary response to antigen

- This is response when body is first exposed to antigen
- The response is primarily production of IgM (small amount of IgG) by the plasma cells
- Memory clonal B cells specific for that antigen are produced which prime the body for next exposure to the antigen
D. Secondary response to antigen

- Subsequent exposure of the body to antigen activates the clonal memory cells specific for that antigen to produce plasma cells.
- The antibody response gives a higher concentration (titer) of antibody than the primary response with the production of IgG primarily (some IgM).
- This is why vaccines are effective.

Figure 1.3 The figure depicts Burnet’s Clonal Selection Theory. Different determinants on an antigen stimulate the response of different lymphocyte clones, each bearing antibody receptors specific for one of the determinants.
E. Other facts about antibody production

- In order to elicit an immune response the substance must have a large molecular weight (or be attached to a large molecular weight species) and be foreign to the body.

- An epitope is the part of the antigen that binds specifically to the antibody. An antigen can have many different epitopes which select different B cells to produce antibodies with specificity for the particular epitope.

- The reason for the body not making antibodies to self components is thought to result from the action of T suppressor cells.
E. Hemolytic Anemias: Acquired Disorders

2. Immune Hemolytic Disorders

1) Results from antibody and/or complement binding to RBC membrane – and subsequent sequestration of “coated” RBC

2) Types
   a) Autoimmune (antibodies against self RBCs)
   b) Alloimmune (antibodies against non-self components bind to RBCs)
   c) Drug-mediated immune (antibodies bind to RBCs because of drug)
E. Hemolytic Anemias: Acquired Disorders
2. Immune Hemolytic Disorders
   a. Autoimmune

<table>
<thead>
<tr>
<th>TABLE 16-3. CLASSIFICATION OF AUTOIMMUNE HEMOLYTIC ANEMIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Warm antibody AIHA. Here the antibody is of the IgG type, does not usually fix complement, and is active at 37°C</td>
</tr>
<tr>
<td>A. Primary or idiopathic</td>
</tr>
<tr>
<td>B. Secondary to:</td>
</tr>
<tr>
<td>1. Lymphomas and leukemias</td>
</tr>
<tr>
<td>2. Other neoplastic diseases</td>
</tr>
<tr>
<td>3. Autoimmune disorders (particularly SLE)</td>
</tr>
<tr>
<td>4. Drugs</td>
</tr>
<tr>
<td>II. Cold agglutinin AIHA. Here the antibodies are IgM and are most active in vitro at 0-4°C. The antibody fixes complement at warmer temperatures, but agglutination of cells by IgM and complement only occurs in the peripheral cool parts of the body. Antibodies dissociate at 30°C or above</td>
</tr>
<tr>
<td>A. Acute (mycoplasmal infection, infectious mononucleosis)</td>
</tr>
<tr>
<td>B. Chronic</td>
</tr>
<tr>
<td>1. Idiopathic</td>
</tr>
<tr>
<td>2. Associated with lymphoma</td>
</tr>
<tr>
<td>III. Cold hemagglutinins (paroxysmal cold hemoglobinuria). In this condition, IgG antibodies bind to red cells at low temperature, fix complement and cause hemolysis when the temperature is raised to 30°C</td>
</tr>
</tbody>
</table>

Mechanism of Hemolysis in Warm Antibody Hemolytic Anemia

Cold-Reacting AHA*

1) Cold Agglutinin Disease
   - Pathologic IgM bind to RBC at cold temps
   - Complement fixed at warmer temps but cascade stopped at C3b stage
   - Hepatic macrophage take up C3b bound RBCs through specific C3b and C3bi receptors
   - Extravascular hemolysis

2) Paroxysmal Cold Hemoglobinuria
   - Pathologic IgG bind to RBC at cold temps
   - Complement fixed at cold temps
   - Lysis of RBCs occurs when higher temps are reached, activating complement cascade which proceeds to completion causing RBC lysis
   - Intravascular hemolysis

* (AHA) Autoimmune hemolytic anemia
* Both diseases worse in winter
IgM Cold Agglutinins

1) Normal Individuals
   Characteristics of cold IgM
   - low concentration
   - low threshold temperature binding (0-4 °C)

2) Pathologic IgM Cold Agglutinin Disease
   Characteristics of cold IgM
   - higher concentration than normal
   - higher temperature binding threshold (30-32 °C) than normal
Cold-Reacting AHA*

1) Cold Agglutinin Disease*
   - Pathologic IgM bind to RBC at cold temps
   - Complement fixed at warmer temps but cascade stopped at C3b stage
   - Hepatic macrophage take up C3b bound RBCs through specific C3b and C3bi receptors
   - Extravascular hemolysis

2) Paroxysmal Cold Hemoglobinuria*
   - Pathologic IgG bind to RBC at cold temps
   - Complement fixed at cold temps
   - Lysis of RBCs occurs when higher temps are reached, activating complement cascade which proceeds to completion causing RBC lysis
   - Intravascular hemolysis

*Autoimmune hemolytic anemia
* Both diseases worse in winter
E. Hemolytic Anemias: Acquired Disorders
2. Immune Hemolytic Disorders
   b. Alloimmune

Alloimmune Hemolytic Anemias

Allo – antibodies to non-self components

Allo antibodies that bind to RBCs result from:
   a) Antibodies to bacteria colonizing large intestines that cross-react with RBCs
   b) Antibodies resulting from imperfectly matched transfusion
   c) Maternal (mother) antibodies to fetal RBC antigens that enter maternal circulation that will bind to fetal RBCs in a subsequent pregnancy (Hemolytic Disease of the New Born)
Hemolytic Disease of the Newborn

1. Priming - development of mother’s Rh antigen antibody response:
   - Mother – Rh negative
   - Baby – Rh positive

2. Strong immune response of mother to second Rh pregnancy
   Due to small number fetal RBCs entering maternal blood

3. Mother’s Rh antibodies pass through placenta into fetal circulation

4. Binding of Rh antibodies to fetal RBCs

5. Extravascular hemolysis of fetal RBCs

*Rh is RBC antigen: This is most common RBC antigen causing Hemolytic Disease of Newborn. There are others.
Clinical Course of Fetus in Untreated Hemolytic Disease of the Newborn: Anemia, CHF, Edema, Death

Anemia → Congestive Heart Failure

Lack of O\textsubscript{2} to tissues overworks heart

Anemia

Extramedullary hematopoiesis (overworks liver)

Severely Damaged Liver

Decreased production of

Clinical Course of Fetus in Untreated Hemolytic Disease of the New Born (cont.)

- Intrauterine death usually
- If survives to birth, death imminent:
  - Kernicterus due to severe inability to conjugate bilirubin Why?
  - Inadequate respiration due to inability of lungs to expand Why?
  - In developing fetus mother takes on these functions so fetus can survive
Treatment of Hemolytic Disease of the Newborn

1. Preventative
   Administer anti-Rh-Ig antibody to mother after delivery of first Rh baby

2. Intrauterine transfusion and earliest possible delivery of baby

Testing in Hemolytic Disease of the Newborn

1. Test RBCs of mother and father for Rh Antigen
   At risk:
   Mother
   Father

2. Mother screened for erythrocyte Ab

3. Bilirubin testing of amniotic fluid
   - After 22 weeks (once every 1 to 3 weeks)
   - Monitors severity of fetal hemolysis to assess beginning of transfusion
E. Hemolytic Anemias: Acquired Disorders
2. Immune Hemolytic Disorders
c. Drug-Induced

Mechanisms for Drug-induced Hemolysis

1. Binding of Ab to drug coated RBC

- Example: Penicillin
Mechanisms of Drug-induced Hemolysis (cont.)

3) Methyldopa Patients
   a) Small percentage of patients develop antibodies to own RBC
   b) Mechanism of autoimmune response – drug interferes with the suppression of synthesis of red cell autoantibodies
   c) Sequestration by spleen of IgG bound RBCs
Direct Coombs Test: 1) Patient blood sample 2) reagents: anti-immunoglobulin to bind to Ab to precipitate out RBCs

Indirect Coombs Test: 1) Patient serum sample 2) reagents: 1) standard RBCs 2) Anti-immunoglobulin to bind to Ab to precipitate out RBCs
The usually concentration of serum bilirubin in chronic hemolytic anemias is 3-4 mg/dL.

Question?

What type of hyperbilirubinemia are all the hemolytic anemias?

Unconjugated Hyperbilirubinemia  
or  
Conjugated Hyperbilirubinemia
VI. Beyond Hemolytic Anemias: Other Major Causes of Hyperbilirubinemia

Steps 1 – 7 in Bilirubin Processing

Hyperbilirubinemic Conditions Resulting from Conditions Beyond the RBC Lysis (Beyond Step 1)

Table 51-2. Abnormalities that Affect Specific Steps in Bilirubin Metabolism

<table>
<thead>
<tr>
<th>Steps</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Formation of UCB from heme, mainly in the reticuloendothelial system</td>
<td>Overproduction of UCB: Hemolysis or ineffective erythropoiesis</td>
</tr>
<tr>
<td>2. Delivery of UCB in plasma, mainly via the portal vein</td>
<td>Right-sided congestive heart failure Postsystemic shunts (cirrhosis or surgery)</td>
</tr>
<tr>
<td>3 and 4. Clearance</td>
<td>Competitive inhibition of UCB uptake by drugs Gilbert's syndrome (type III) Fasting</td>
</tr>
<tr>
<td>3. Uptake of UCB from sinusoidal plasma into the hepatocyte</td>
<td>Competitive inhibition of UCB storage by drugs Gilbert's syndrome (type II) Rotor syndrome (storage disease) Fever</td>
</tr>
<tr>
<td>4. Storage of UCB in cytosol, bound to ligandin</td>
<td></td>
</tr>
</tbody>
</table>

Hyperbilirubinemic Conditions Resulting from Conditions Beyond the RBC Lysis (Beyond Step 1) (cont.)

5. Conjugation of UCB in microsomes (smooth endoplasmic reticulum) to form CB Type I: Crigler-Najjar and Gunn rat Type II: Arias' syndrome Gilbert's syndrome (all forms) Inhibition of conjugation by drugs (e.g., novobiocin) or hormones (progestogens) 

6. Secretion of CB into canicular bile Hepatocellular jaundice (alcoholic, toxic, or viral hepatitis, with or without cirrhosis) Dubin-Johnson syndrome Inhibition of CB secretion by 17a-alkyl steroids Rotor syndrome (storage disease) 

7. Flow of CB in bile down the biliary tree to the duodenum Intrahepatic cholestasis due to: estrogen, drugs, granulomas, tumors, primary biliary cirrhosis, sclerosing cholangitis, benign recurrent cholestasis, postoperative cholestasis, cholestasis of severe infections Extrahepatic cholestasis: obstruction of ampulla or extrahepatic bile ducts
VI. Beyond Hemolytic Anemias: Other Major Causes of Hyperbilirubinemia

1) Congenital disorders of decreased glucuronyl transferase leading to _____?______ bilirubin conjugation

Congenital Disorders in Glucuronyl Transferase

1. Gilberts Syndrome
   a) 50-90% decrease in UDP-glucouronyl transferase
   b) Asymptomatic – benign disorder
   c) Serum bilirubin increased (1-7 mg/dL)
   d) Higher prevalence in males (3-7%) than females (0.6 – 2%)

2. Crigler – Najjar Syndrom (Type I)
   a) UDP – glucuronyl transferase completely absent
   b) Serum bilirubin increased (>20 mg/dL)
   c) Severe jaundice and kernicterus leading to death in infancy or early childhood
   d.) Heterozygote parents or sibling have 50% decreased capacity to conjugate and excrete bilirubin
3. **Crigler – Najjar Syndrome (Type II)**
   a) UDP-glucuronyl transferase is less than 10% of normal
   b) Serum bilirubin increased: 10-19 mg/DL
   c) Benign disorder: Jaundice appears late in childhood; seldom causes kernicterus

4. **Hyperbilirubinemia in Newborn**
   a) Due to decreased levels of UDP glucuronyltransferase due to immature liver
   b) 50% of newborn population is jaundiced in first 5 days of life
   c) Increased bilirubin in serum
      - Normally up to 4-5 mg/dL
      - 10 mg/dL (small percentage)
      - >15 mg/dL (5%)
VI. Beyond Hemolytic Anemias: Other Major Causes of Hyperbilirubinemia

2) Dubin-Johnson Syndrome

Dubin-Johnson Syndrome

Congenital defect in bilirubin carrier which transports conjugated bilirubin from the hepatocyte to the bile canaliculi. This leads to conjugated hyperbilirubinemia (serum bilirubin around 10 mg/dL)
VI. Beyond Hemolytic Anemias: Other Major Causes of Hyperbilirubinemia

3) Hepatocellular and Cholestasis Diseases

- Leads to conjugated hyperbilirubinemia due to diminished* secretion of the bilirubin into the bile

  *This is an energy expending process and thus is most susceptible to injury

- Most common hepatocellular diseases are hepatitis and cirrhosis
Cholestasis

- Condition characterized by diminished or stopped flow of bile

- Classifications
  - Intrahepatic — various conditions that injure the biliary vessels in the liver
  - Extrahepatic — conditions that lead to blockage of bile ducts outside the liver include:
    - Gallstones
    - Strictures
    - Cancerous obstructions

Cholestasis leads to a conjugated hyperbilirubinemia because the conjugated bilirubin is not removed from the hepatocyte. The excess conjugated bilirubin in the hepatocyte thus leaks into the blood.

*Since conjugated bilirubin is building up in the bile because the bile is not flowing.
Simple laboratory test to distinguish conjugated from unconjugated hyperbilirubinemia disorders

![Diagram of liver cell, sinusoid, and bile duct]

Laboratory Test Differences between Hepatocellular and Cholestatic Diseases

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Cholestatic Jaundice</th>
<th>Hepatocellular Jaundice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bile acids</td>
<td>Very high</td>
<td>Modestly elevated</td>
</tr>
<tr>
<td>Steatorrhea (poor micelles)</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Serum alkaline phosphatase</td>
<td>&gt;three times normal</td>
<td>&lt;three times normal</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>Increased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Serum transaminases (SGOT* SGPT*)</td>
<td>Mildly elevated, rarely &gt;500 IU</td>
<td>Elevated, may be &gt;1000 IU</td>
</tr>
<tr>
<td>Prothrombin-time response to parenteral vitamin K</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*Serum glutamic oxaloacetic transaminase (aspartate aminotransferase).  
*Serum glutamic pyruvic transaminase (alanine aminotransferase).
Between which two steps is the dividing line between unconjugated and conjugated hyperbilirubinemia?

Hepatitis

Condition of inflammation of liver resulting in hepatocellular damage. Caused by viral or toxic agent.
Viruses Causing Hepatitis

1) Hepatitis A virus (HAV)
2) Hepatitis B virus (HBV)
3) Hepatitis D virus (HDV)
4) Hepatitis C virus (HCV)
5) Hepatitis E virus (HEV)
6) Others

Figure 1. Composition of different hepatitis viruses.
**Viruses: Composition**

Virus' components are:
- Nucleic acids (either DNA or RNA)
  - Encodes for several up to 50 proteins
- Protein coat
  - Protects nucleic acids from degradation
  - Some bind to receptors on host cells
- Replication enzymes such as polymerases (some viruses contain)
- Lipid envelope (only some viruses possess)
  - Protects nucleic acids from degradation

---

**Viral Replication**

1. Attachment of virus to host cell
   - Surface protein of virus binds to receptor on membrane of host cell
2. Entry of virus into host cell occurs by either:
   - Fusion of virus envelope to cell membrane (mechanism of many envelope viruses)
   - Endocytosis
3. Uncoating of viral nucleic acids
4. Synthesis of viral nucleic acids and proteins in host cell
5. Reassembly of newly synthesized viral components
6. Release of virus from cells (may or may not involve lysis)
7. Infection of other cells
Mechanisms of Viral Injuries to Host
(Can involve one or more of the following)

1. Viral lysis of host cells

2. Lysis of host cells induced by host’s antibodies and complement

3. Host’s inflammation and interferon response

4. Viral infection may transform host cells to proliferate continually through viral oncogenes (cancer)

<table>
<thead>
<tr>
<th>TABLE 23A-3 Nomenclature and features of hepatitis antigens and antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatitis</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>A</strong></td>
</tr>
<tr>
<td><strong>B</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
</tr>
<tr>
<td><strong>D</strong></td>
</tr>
<tr>
<td><strong>E</strong></td>
</tr>
</tbody>
</table>
Phases of Acute Hepatitis

1) Incubation
   ➢ Asymptomatic period of viral replication
   ➢ Length of time period
      • Hep A – 28 days (range 15-45)
      • Hep B – 75 days (range 30-80)
      • Hep D – 15 - 90 days

Phases of Acute Hepatitis (cont.)

2) Preicteric
   ➢ Period in which symptoms appear prior to appearance of hyperbilirubinemia
   ➢ Symptoms
      • Initial - malaise
      • Subsequent – flu-like (fatigue, nausea, loss of appetite, fever, muscle and joint aches)
   ➢ Increased AST and ALT in blood
Phases of Acute Hepatitis (cont.)

3) Icteric
- Appearance of jaundice
- Prevalence of increased bilirubin (conjugated)
  - Hepatitis A Common in adults (not kids)
  - Hepatitis B 50% of cases
  - Hepatitis C Almost no cases
- Symptoms begin clearing

4) Convalescent
- Recovery
- Time period of symptoms prior to convalescence: few weeks to several months

Histological Changes in Liver from Hepatitis Viral Agents

1) Organ Changes
- slightly enlarged
- green color

2) Tissue Changes
  a) Diffuse cell injury causing lobular disarray
     (normal radial array of hepatocytes lost)
  b) Necrosis of isolated cells or small cluster of (Random)
  c) Marked hypertrophy and hyperplasia of Kupfer cells and other cells lining sinusoids
  d) Inflammatory infiltrate of portal tracts
     (lymphocytes and macrophages)

3) Cholestasis – may or may nor be present
Hepatitis A

Fig. 16.10. Diagram of the hepatitis A virus shown as a hexagonal body containing single stranded RNA.
Hepatitis A – Clinical Aspects

1) **Presentation**
   - Adults – fever
   - Children – mild or asymptomatic

2) **Progression**
   - Most go to full recovery (0.1% fatality rate)
   - Does not progress to chronic or carrier condition
   - Progresses to fulminant hepatitis only rarely

Heptatitis A: Mode of Transmission

Oral – Fecal (usually contaminated water or food)

- Prevalent in developing countries (poor sewage disposal)
- Others
  - Military camps
  - Male Homosexuals
Table 1. Risk factors for hepatitis A.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>% affected patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household exposure</td>
<td>24</td>
</tr>
<tr>
<td>daycare center contact</td>
<td>18</td>
</tr>
<tr>
<td>Male homosexual</td>
<td>11</td>
</tr>
<tr>
<td>Foreign travel</td>
<td>4</td>
</tr>
<tr>
<td>Parenteral drug abuse</td>
<td>2</td>
</tr>
<tr>
<td>No apparent risk factor</td>
<td>40</td>
</tr>
</tbody>
</table>

Source: Data from US Sentinel Counties Study, Centers for Disease Control and Prevention [2].

---

CLONAL SELECTION THEORY

- Antigen with 3 determinants stimulate distinct lymphocytes specific for 1. ▲, 2. ▼ and 3. ●
- ▲, ▼, ● to proliferate and differentiate

Figure 1.3 The figure depicts Burnet's Clonal Selection Theory. Different determinants on an antigen stimulate the response of different lymphocyte clones, each bearing antibody receptors specific for one of the determinants.
Serology in Hepatitis A.
### Table 32-5: Viral Hepatitis Types A, B, C, D, and E: Comparison of Clinical, Epidemiological, and Immunological Features

<table>
<thead>
<tr>
<th>Features</th>
<th>Hepatitis A</th>
<th>Hepatitis B</th>
<th>Hepatitis C</th>
<th>Hepatitis D</th>
<th>Hepatitis E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>Picornavirus</td>
<td>Hepadnavirus</td>
<td>Flavivirus</td>
<td>Satellite</td>
<td>Calicivirus</td>
</tr>
<tr>
<td>Genotype</td>
<td>RNA</td>
<td>DNA</td>
<td>RNA</td>
<td>RNA</td>
<td>RNA</td>
</tr>
<tr>
<td>Incubation period</td>
<td>15–40 d</td>
<td>50–180 d</td>
<td>1–3 mo</td>
<td>21–90 d</td>
<td>2–9 wk</td>
</tr>
<tr>
<td>Usual type of onset</td>
<td>Acute</td>
<td>Insidious</td>
<td>Insidious</td>
<td>Acute</td>
<td>Acute</td>
</tr>
<tr>
<td>Prodrome</td>
<td>None</td>
<td>Arthritis, rash</td>
<td>Arthritis, rash</td>
<td>Unknown</td>
<td>None</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td>Oral (fetal)</td>
<td>Parenteral</td>
<td>Parenteral</td>
<td>Parenteral</td>
<td>Parenteral</td>
</tr>
<tr>
<td>Oral (fetal)</td>
<td>Rare</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Parenteral</td>
<td>Food- or water-borne</td>
<td>No</td>
<td>Ulitmate contact</td>
<td>No</td>
<td>Ulitmate contact</td>
</tr>
<tr>
<td>Sequelae</td>
<td>carrier</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Carrier</td>
<td>Not reported</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not reported</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>Estimated</td>
<td>mortality</td>
<td>0.1–0.2%</td>
<td>0.5–2.0% (may be higher in complicated cases)</td>
<td>1–2% (may be higher in complicated cases)</td>
</tr>
<tr>
<td>mortality</td>
<td>0.1–0.2%</td>
<td>60–65%</td>
<td>5–10%</td>
<td>3–5%</td>
<td>20% in pregnant women, 1–2% in general population</td>
</tr>
</tbody>
</table>


### Various clinical courses of Hepatitis B

- Recovery 99%
- Subclinical disease 60–65%
- Fulminant and death 1%
- Acute hepatitis 20–25%
- Chronic hepatitis 5–10%
- Chronic persistent (nonprogressive) hepatitis 2%
- Chronic active (progressive) hepatitis 3–5%
- "Healthy" carrier 5–10%
- Hepatocellular carcinoma
Chronic Hepatitis

Symptomatic, biochemical, or serological evidence of continuing inflammatory hepatic disease for more than 6 months without improvement

Types

a) **Chronic Persistent Hepatitis** (CPH)
   Benign “smoldering” infection that persists for months to years, with no hepatic impairment

b) **Chronic Active Hepatitis**
   Progressive liver damage leading to cirrhosis, hepatic failure and death

---

**Figure 19-19.** A schematic comparison of acute and chronic active hepatitis.

Histological presentation of Chronic Active Hepatitis compared to Acute Hepatitis
Fig. 16.4. Diagram of the virion of hepatitis B (HBV: Dane particle). The core contains DNA polymerase, double-stranded DNA, core antigen, and e antigen. The surface consists of HBeAg, Spheres and tubules of HBeAg are free in serum.
### Markers for Hepatitis B

1. **HBsAg**
   - Marker for acute disease, earliest Ag or Ab marker to rise
   - Also used to indicate development of carrier status if it is elevated for more than 6 months
   - "sAg" stands for surface antigen

2. **HBeAg**
   - Marker for acute disease, when present patient is infectious
   - If persists (> 20 weeks) indicates development of chronic condition
   - "eAg" stands for "e" antigen

3. **Anti-HBe**
   - Appears with loss of HBeAg
   - Indicates loss of infectivity (stays elevated for months or years)

4. **Anti-HBs**
   - Indicates convalescence (recovery) from acute disease
   - Confers immunity to patient from any future exposure
   - Immunity lasts 8-10 years (longer for some patients)
   - Presence does not necessarily mean past exposure to virus, since HBV vaccine contains HbsAg which would elicit anti-HBs response

5. **Anti-HBc (IgM)**
   - Useful for diagnosing acute disease during "window" period (period in acute disease after HbsAg drops to an undetectable level and before appearance the appearance of anti-HBs)
   - "c" stands for core antigen

6. **Anti-HBc (IgG)**
   - Indicates past exposure (vaccination does not elicit production of this antibody)
   - Does not convey immunity

7. **HBV DNA**
   - Most sensitive indicator of infection, detects virus when HbeAg is not detectable
   - Used for prognostic indicator of success of interferon treatment (patients with < 200 ng/ml HBV DNA more likely to respond to interferon treatment)
Chronic HBV Infection

Typical response with severe disease

Edward R. Ashwood, M.D., Professor of Pathology, University of Utah
ARUP Laboratories, Inc., 2003 National AACC Meeting

Chronic HBV Infection

Typical response with mild disease

Edward R. Ashwood, M.D., Professor of Pathology, University of Utah
ARUP Laboratories, Inc., 2003 National AACC Meeting
### Table 9-3: Some Common Patterns of Acute Hepatitis Serologic Markers

<table>
<thead>
<tr>
<th>Anti-HAV (IgM)</th>
<th>HBsAg</th>
<th>Anti-HBc (IgM)</th>
<th>Most Likely Diagnosis</th>
<th>Some Suggested Followup Testing and Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>? NABC, other causes</td>
<td>Test for other viral agents, toxins</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Acute HAV infection</td>
<td>Usually none</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Recent HBV infection</td>
<td>1. May want to test for anti-HBc to determine resolution, prognosis</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Acute HBV infection</td>
<td>1. Test for HBcAg and anti-HBc to determine infectivity</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Early acute HBV or chronic carrier</td>
<td>2. Repeat HBsAg test approximately monthly.</td>
</tr>
</tbody>
</table>

1. If both +, acute HBV
   - Follow testing indicated above.
2. If HBsAg+ and anti-HBc IgM+, test for anti-HBe
   - Anti-HBc+ indicates previous HBV infection and chronic carrier. Current acute infection most likely due to other causes (e.g., NABC, HDV).
Infectious agent in Hepatitis D

Two ways in which HDV infection occurs
**Laboratory Diagnosis of Hepatitis D**

1) **IgM-anti H** most reliable indicator of acute infection (appears 1 month after onset of symptoms)
2) Indicators of chronic infection
   - **HDAg** (present at low levels for months of longer)
3) **cDNA probe for HDV** – detects virus directly at early stage of symptomatic disease viremia, usually absent by time symptoms appear.
4) **IgG Anti HD** – confers protection for years

**Hepatitis C**
Clinical Outcomes of Hepatitis C

Fulminant Disease  ←  Acute Infection  →  Asymptomatic Carrier

20% 80%

Recovery  Chronic Hepatitis  Cirrhosis  Increased Risk

20% 20% (of chronic cases) 20% in pregnant women; 1–2% in general population

Hepatocellular Carcinoma

---

Blood supply risk of HCV infection decreases with the progression of laboratory testing and screening.

**Testing for HCV: Immunological Tests**

1) **Immunologic tests** – detects antibody to viral proteins
   a) EIA (Enzyme Immunoassay)
      - Initial screening test
   b) RIBA (RNA Immunoblot Assay)
      - Confirms EIA positives
Testing for HCV: Viral Testing

2) RNA Detection Techniques

a) Techniques
- Reverse Transcription-Polymerase Chain Reaction Assay (RT-PCR)
- bDNA Assay

b) Use and performance characteristics
- Earliest detection technique for infection
  RNA tests detect virus in 1-3 weeks,
  while antibodies to HCV not detected until 80-90 days
- Used for following:
  - Monitors progression of disease
  - Predicts and monitors response to α-interferon treatment
    Success in interferon treatment inversely correlated with viral load
    (i.e., concentration)

Excessive variability in present day techniques due to instability of RNA replication. This means RNA techniques must be continually updated and repertoire expanded

*Replication of RNA is not stable like DNA, which have polymerase to correct replication errors (in a years time maybe 20 or more mutations occur in HCV)*
Fig. 1. HCV genome and recombinant proteins.

A. Detection of antibodies to HCV

a. Antigens used for immunoassays

Genomic organization of precursor polypeptide

Recombinant antigens used for immunoassays

Specific immunoassays
EIA Detection of HCV Abs

1. Preparation of well

One or more HCV antigens are adsorbed to the surface of the well

2. Serum sample added

A positive HCV sample will contain antibodies (Ab) to the immobilized HCV antigens and will bind to the them on the well

3. Washing step

All unbound components of sample (not bound to HCV antigens) are washed out of well (washes away non-HCV Abs)

4. Addition of enzyme-labeled anti-IgG Ab (G*)

Add enzyme-labeled anti-IgG which binds to Ab

5. Wash off unbound labeled Ab

6. Add substrate to enzyme

7. Incubate

Substrate is converted to product by enzyme on antibody

8. Measure [product] by spectrophotometry

[Product] is proportional to amount of labeled Ab present which is proportional to amount of HCV Ab bound to antigen on the surface of the well
### EIA Testing of HCV

<table>
<thead>
<tr>
<th>Generation</th>
<th>Antigens Immobilized</th>
<th>Initial Time of Detection of HCV Antibodies</th>
<th>Other Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA-1</td>
<td>c100</td>
<td>12-26 weeks</td>
<td>Missed 15-30% cases</td>
</tr>
<tr>
<td>ELISA-2</td>
<td>c22, c33, c100</td>
<td>shortened ELISA-1 by 10-20 days</td>
<td></td>
</tr>
<tr>
<td>ELISA-3</td>
<td>c200, c22, NS5</td>
<td>shortened ELISA-2 by 10 days</td>
<td></td>
</tr>
</tbody>
</table>

EIA testing for HCV antibodies has high number of false positives so need to do a confirmation test. Positive predictive value for EIA is 25-65% compared to 70-75% for the combined EIA and RIBA testing strategy.

---

### RIBA Testing of HCV

1. Diagram of 4 recombinant HCV antigens and controls on strip

   Fig. 2. Antigens and controls for the RIBA HCV 3.0 strip immunoblot assay.

2. Results

   - Positive: 2 or more antigens with antibody bound
   - Negative: no antigens with antibody bound
   - Indeterminant: 1 antigen with antibody bound

3. Characteristics

   - Percent indeterminants: RIBA-2: 6.7%, RIBA-3: 0.5%
   - RIBA-3 uses synthetic peptides for c100 and c22 (c33 and NS5 are recombinant). Use of synthetic peptides leads to less non-specific adsorption
### Frequency of Acute Hepatitis Going to Chronic Hepatitis

<table>
<thead>
<tr>
<th>Virus</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV</td>
<td>0%</td>
</tr>
<tr>
<td>HBV</td>
<td>5% (1/3 CPH, 2/3 CAH)</td>
</tr>
<tr>
<td>HDV</td>
<td>0% Co-infection 10-40% Superimposed HDV on HBV carrier</td>
</tr>
<tr>
<td>HCV</td>
<td>80%</td>
</tr>
</tbody>
</table>

### Incidence of Carrier Status

<table>
<thead>
<tr>
<th>Virus</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV</td>
<td>0%</td>
</tr>
<tr>
<td>HBV</td>
<td>0.1 – 1%</td>
</tr>
<tr>
<td>HDV</td>
<td>Unclear</td>
</tr>
<tr>
<td>HCV</td>
<td>2-3%</td>
</tr>
</tbody>
</table>