

VII. THE LIVER AND BILIARY SYSTEM

*"With mirth and laughter let old wrinkles come,
and let my liver rather heat with wine than my
heart cool with mortifying groans."*

The Merchant of Venice

*"Puir auld Jimmie Purdie. His step's getting gey feeble. I'm thinkin' whusky's nae
sae guid a cure for the rheumatics after a'."*

Robert Service

A. Objectives

- What percentage of the liver's supply of O₂ comes from the hepatic artery? What is the importance of the falciform ligament? Learn the differences between the liver lobule and the functional acinus, and the components of the portal triad. Learn that the flow of blood into a liver lobule is in a direction opposite to that of bile flow. What liver cells are most vulnerable to anoxic injury? Why can pain of hepatic origin be felt at the right shoulder?
- Be able to describe the multiple functions of hepatocytes, and how loss of these functions determines the clinical syndrome of acute hepatic failure. What is hepatic encephalopathy? Why might the serum iron be elevated in acute hepatocellular injury? What is the importance of Kupffer cells?
- Give an overview of the role of the liver in drug metabolism, including the mixed function monooxygenase system of enzymes. Why might alcohol abuse contribute to the toxicity of another drug? Is ethanol regarded as a direct hepatotoxin? Describe the spectrum of alcoholic liver disease. What are the histological features of alcoholic hepatitis? What are the general characteristics of drug sensitivity reactions? Why might someone ingesting oral androgens become jaundiced?
- What are the characteristics of cirrhosis of the liver? Does the histology correlate with etiology? What is Child's classification of chronic liver disease?
- Learn about the three anatomic types of portal hypertension. What are the major complications? What factors promote the formation of ascites? How does obstruction to hepatic venous outflow contribute to the formation of ascites?
- What are the major components of bile, which is an important vehicle for excreting endogenous substances, such as cholesterol, and xenobiotics, such as drugs? How does gallbladder bile remain isotonic with plasma? Be able to distinguish between bilirubin metabolism and the enterohepatic circulation of bile salts. What are likely steps in the formation of cholesterol gallstones. How might cholesterol gallstones be prevented? What are pigment stones? How would you investigate a patient with suspected disease of the biliary tract? What theories are offered to explain acute

cholecystitis? How does the histology of chronic cholecystitis differ from the normal gallbladder? Why must stones in the common duct be treated expeditiously?

- Realize that the clinician can pinpoint, about 85% of the time, the cause of jaundice on the basis of careful history and physical exam. The liver tests are used in the context of helping to reject clinical hypotheses. What four hepatic functions do the liver tests assess? Describe the pathway of bilirubin excretion. What is conjugated bilirubin, and how does it enter the plasma? What is the half-life of factor VII? Of albumin? What is the usual elevation of serum transaminases in acute viral hepatitis? In alcoholic hepatitis? What are the pitfalls in interpreting the serum levels of alkaline phosphatase? Be able to outline results of liver tests in transfusion reaction, acute viral hepatitis, common duct stone, and chronic hepatitis.

B. Embryology

Falciform ligament

The hepatobiliary system arises during the fourth week of embryogenesis from an endodermal outgrowth of the foregut called the hepatic diverticulum. This diverticulum gives rise to the hepatic parenchyma, gallbladder, cystic duct, common bile duct, and head of the pancreas. Occasional alterations in the normal embryological development give rise to clinical disorders of the liver and biliary system (i.e., liver cysts, congenital cysts of the intrahepatic and extrahepatic bile ducts). An important embryological remnant persists as an anatomical structure of the adult liver: the falciform ligament. It is important both anatomically and clinically because (a) it divides the liver into topographical right and left lobes, (b) it carries the partly obliterated left umbilical vein, which, after birth, offers access to the portal circulation for both angiographic and hemodynamic studies, and (c) it contains small paraumbilical veins which connect the portal venous system with the systemic venous system of the anterior wall. These venous channels may enlarge in patients with portal hypertension.

C. Gross Anatomy

Topographic vs. functional anatomy

The liver is the largest organ in the human body, normally weighing about 1.5 kg (1200-1500 g) and comprises about 1/50 of the total adult body weight. The liver's functional division differs from its topographic division (Figures 1 & 2). On a visual basis (topographically), the liver is divided into a large right lobe and smaller left lobe by the falciform ligament, which connects the liver to the diaphragm and the anterior abdominal wall. The right lobe is about six times the size of the left. Functionally, however, the liver is divided according to the distribution of its main vascular and biliary channels. These are the "true" anatomic right and left lobes. This functional anatomy is important in surgical decision-making regarding resection of lobes or segments of the liver for pathologic reasons (i.e., tumors, cysts, traumatic rupture), or to use as donor "split grafts" for liver transplantation.

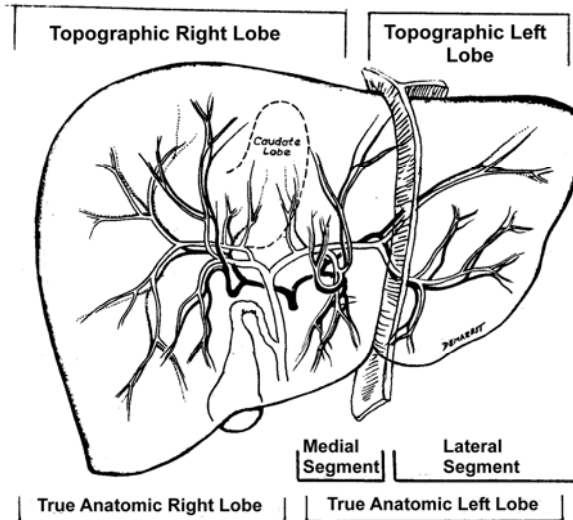


Figure 1. Distribution of bile ducts (light) and hepatic artery (dark)

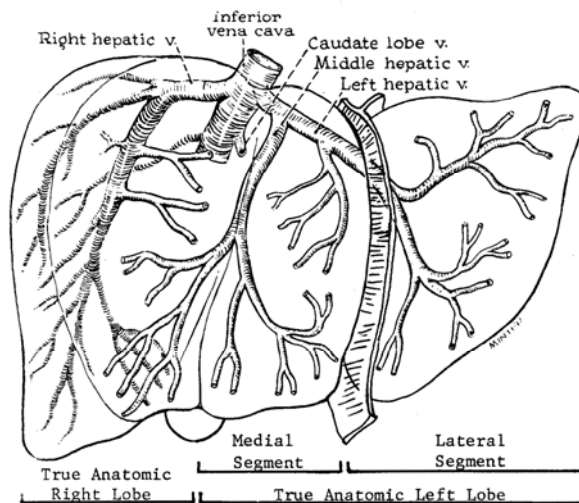


Figure 2. Venous Drainage of the Liver

Hepatic artery supplies most of oxygen despite lower flow than portal vein

The liver has a dual blood supply (Figs. 1 & 2). The hepatic artery (a branch of the celiac axis) carries well-oxygenated arterial blood to the liver. The second source is the portal vein, which carries nutrient-rich/oxygen-poor venous blood to the liver from the intestines and spleen. From 50 to 80% of the liver's oxygen supply is furnished by the hepatic artery, the remainder comes from the portal vein. Approximately 20% to 30% of hepatic blood flow is normally derived from the hepatic artery, with the portal vein contributing 70% to 80%. Nearly one-third of the cardiac output passes through the liver.

The portal vein, hepatic artery, and the common hepatic bile duct

Bile ducts perfused only by arteries

Portal triads

Opposite flow of blood in sinusoids and bile in canaliculi

Venous drainage

(accompanied by nerves and lymphatics) ascend to the hilum of the liver (Porta Hepatis) where each penetrates the liver parenchyma and bifurcates into right and left branches going to the anatomic right and left lobes, respectively. These vascular and biliary channels divide into progressively smaller branches, until they reach the "portal triads." The arterial branches first supply the bile ducts, both delivering nutrients and oxygen to the ducts and carrying metabolites from the ductal epithelium to the hepatocytes in the liver cords (the "chole-hepatic shunt"). The bile duct epithelium is susceptible to ischemia because its blood supply is entirely arterial. Each portal triad contains an artery, a portal vein, and one or two bile ducts (as well as lymph vessels and nerves). From the portal triad, blood from the artery and vein empty into the hepatic sinusoids (Fig. 3) which are endothelial-lined channels that run between the cords of hepatocytes, arranged radially from the central vein. In the sinusoids, the blood flows centrally towards the hepatic veins, whereas bile, secreted by the hepatocytes, flows in the canaliculi in the opposite direction, emptying via the Duct of Hering into the interlobular bile duct in the portal triad. The central hepatic veins merge to form progressively larger channels, that ultimately empty into the main hepatic veins that emerge at the posterior, dorsal surface of the liver and promptly enter the inferior vena cava near its point of entry into the right atrium.

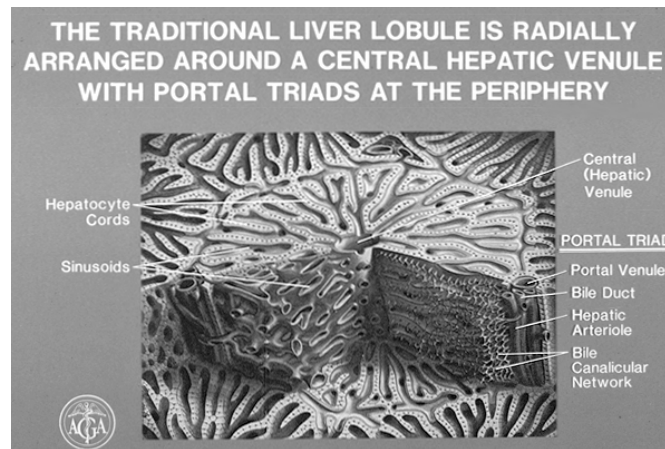


Figure 3. Anatomy of a Traditional Hepatic Lobule

Cords (plates) of hepatocytes, 1-2 cells thick, with intervening sinusoids are arranged radially around a central hepatic venule. At the periphery of the lobule are multiple portal triads, each containing a portal venule, an hepatic arteriole, and 1-2 intralobular bile ducts. Bile is secreted by the hepatocytes into an anastomosing network of bile canaliculi, then drains peripherally into ductules at the margins of the portal triads, and from there empties into the interlobular bile ducts.

©American Gastroenterological Assn., Undergraduate Teaching Project, Unit 21, Slide 2. Milner-Fenwick Timonium, MD, 1989. Modified from Levy CM, Fig. 2c in *Evaluation of Liver Function in Clinical Practice*, Eli Lilly & Co., Indianapolis, IN 1965.

<i>Hepatic lymphatics</i>	Lymph drains from the liver via superficial and deep channels. The main lymph drainage of the liver is via the deep channels. These exit the liver at the hilum and follow through nodes and trunks along the common bile duct. They merge with lymphatics from the gallbladder, stomach, and duodenum en route to the cisterna chyli and the left thoracic duct, which empties into the left subclavian vein. Channels from the upper one-third (dome) of the liver drain via the right thoracic duct into the right subclavian vein. Lymphatic channels become important in the spread of tumor as well as contributing to the formation of ascites.
<i>Hepatic Nerve supply</i>	The nerve supply of the liver is derived predominantly from the celiac plexus (sympathetic fibers), plus branches from the anterior and posterior vagal trunks (parasympathetic fibers). These nerves help regulate hepatic blood flow (such as vasoconstriction) and visceral pain. Sensory nerves extend from the diaphragm onto the superior portion of the liver. These nerves are part of the phrenic nerves (originating in dermatome segments C3-C5, which also serve cutaneous sensation on the top of the shoulders). This explains the perception of shoulder pain (referred pain) arising from certain diseases of the liver and biliary system (i.e., abscess, tumor) or following liver biopsy.
<i>Subdiaphragmatic abscess</i>	Because of the liver's position under the diaphragm, several potential subphrenic (supra- and infra-hepatic) spaces exist. Localized abscesses may develop in these spaces following intraabdominal sepsis. Knowledge of their location is important for diagnostic and therapeutic drainage procedures.

D. Microscopic Anatomy

<i>The acinus, not the lobule, is the functional unit</i>	The normal liver has a structure that is based upon the afferent and efferent blood vessels. The centrilobular veins are the smallest efferent veins and lie at the center of the classical lobule, providing a convenient landmark to describe the location of many pathological lesions. Many drug-induced injuries, for example, are most severe near the centrilobular veins. From a <i>functional</i> point of view, however, it is the "acinus," not the classical lobule that better describes the functional unit of the liver (Figure 4). Each simple acinus actually encompasses segments of several lobules, and agglomerates with adjacent acini to form more complex acini.
<i>Zone 3 hepatocytes surrounding hepatic venules are least well supplied with oxygen and nutrients.</i>	As blood flows down the sinusoids, the progressive removal of oxygen and nutrients by hepatocytes depletes the supply available to hepatocytes further along the cords. Thus, within the acini, there are zones of successively less well oxygenated liver cells (zones 1, 2, & 3), which explains why systemic hypoxia preferentially damages the zone 3 hepatocytes near the central veins. The functional capabilities of the hepatocytes (i.e., the repertoire and contents of enzymes and subcellular

organelles) also exhibit a gradient along the cords. This explains why drug-induced injuries tend to differentially affect different zones of the acini (e.g., many drugs primarily damage zone 3 hepatocytes). By contrast, due to the flow of bile toward the portal triad, the zone 1 (periportal) hepatocytes tend to suffer greater damage when bile flow is obstructed.

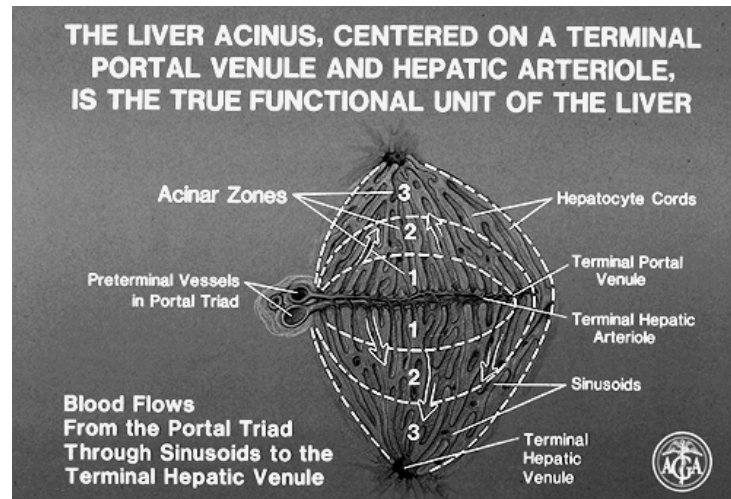


Figure 4. Anatomy of the Acinus, The Functional Unit of the Liver

The liver acinus consists of cords/plates of hepatocytes between which course the sinusoids that radiate from the terminal vascular stalk (terminal portal venule and hepatic arteriole). The sinusoids drain into several terminal hepatic venules at the periphery. The hepatocytes nearest the terminal vascular stalk (zone 1) receive the highest concentrations of nutrients and oxygen, while the most peripheral hepatocytes in zone 3, near the terminal hepatic venules, receive the lowest concentrations of these substrates. The bile canalicular network is not shown, but drains into the interacinar bile duct accompanying the terminal vascular stalks.

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Acinar and sinusoidal architecture

Within the acinus, hepatocytes are normally arranged, like a string of boxcars, in cords or plates one or two cells thick, that are separated by the sinusoids (Fig. 5). The sinusoids lack a basement membrane, but are lined by sheets of fenestrated endothelial cells. The space of Disse, which lies between the endothelial cell layer and the cords of hepatocytes, extends partially between the lateral surfaces of adjacent hepatocytes. Between hepatocytes run the bile canaliculi, fine channels that are bounded by the specialized canalicular surface of each hepatocyte membrane. The canaliculi are sealed by tight junctions that separate the canalicular bile from the hepatic lymph in the lateral extensions of the space of Disse. Each hepatocyte thus has basolateral surfaces facing the lymph in the space of Disse and canalicular surfaces facing the bile; the latter constitutes less than 10% of the total cell surface.

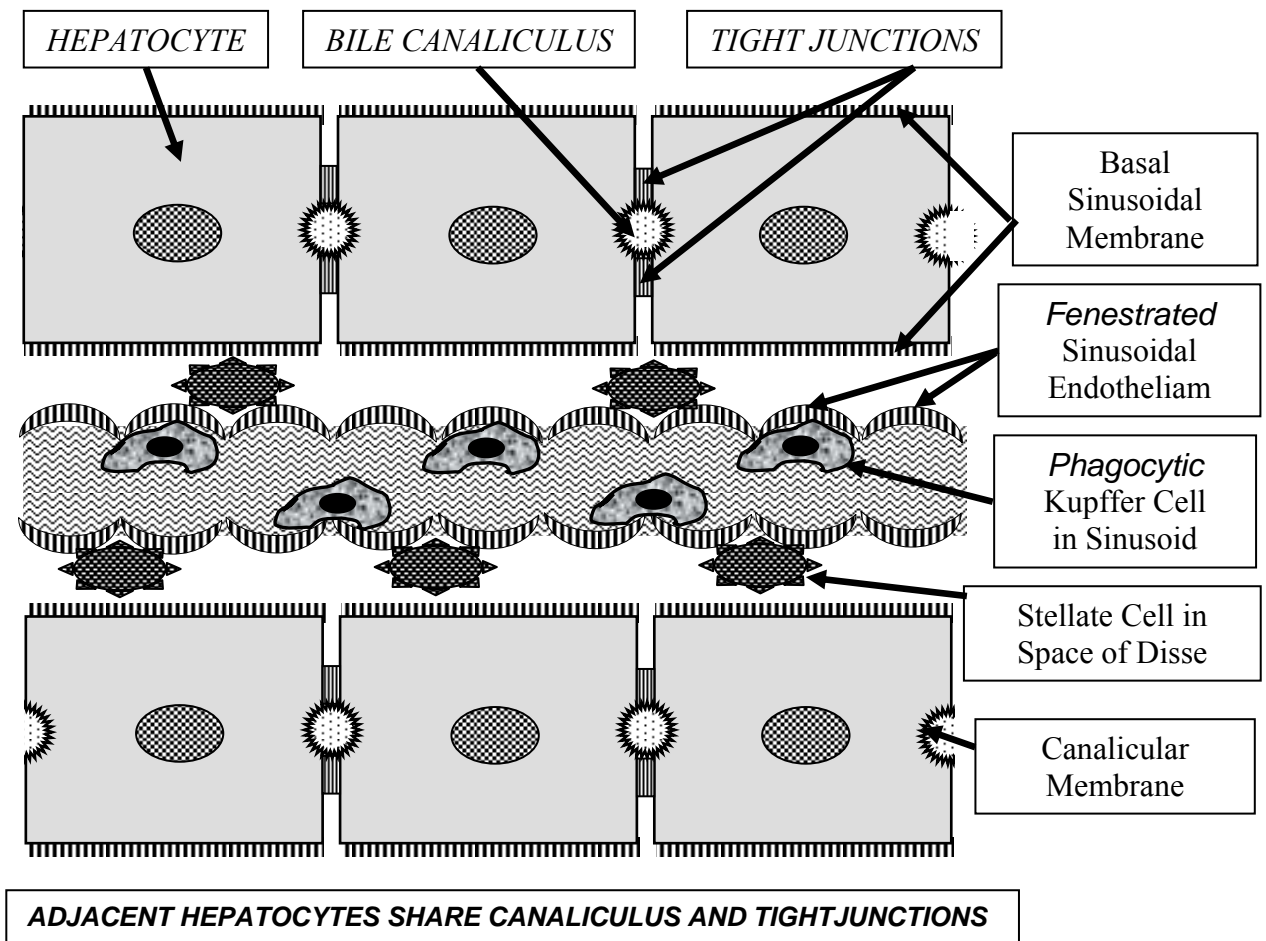


Figure 5. Microanatomic Relationships of Hepatocyte Cords and Sinusoids

A schematic view of two cords (rows) of hepatocytes straddling a sinusoid from which each cord is separated by the intervening Space of Disse. In the liver, the hepatocyte cords and sinusoids are arranged in alternating stacks, so that each of the two basal surfaces of the hepatocyte cords is related to a sinusoid (not shown). Within the cords, the adjacent hepatocytes are arranged in rows, like a series of railroad boxcars, with the apical surfaces of adjacent hepatocytes sharing a common bile canaliculus (shown in cross-section) and the tight junctions which are a barrier between the canalicular bile and the lymph in the space of Disse. The sinusoidal endothelial cells, with their large fenestrae, loose intercellular junctions and no basement membrane, allow access of proteins and their ligands to the basolateral surfaces of the hepatocytes. Phagocytic Kupffer cells overlie segments of the luminal surface of the endothelium, to clear intestinally-derived bacteria and toxins from the portal blood. Lipid-storing stellate (Ito) cells in the Space of Disse partially encircle the sinusoids and can be activated into transitional myofibroblasts that regulate sinusoidal diameter and tone and can secrete collagens to initiate fibrosis.

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Permeability of sinusoidal endothelial cells

The sinusoidal endothelial cells act as a barrier against direct contact of blood cells with hepatocytes. Unlike endothelial cells in peripheral capillaries, however, sinusoidal endothelial cells have numerous small fenestrations that provide free access of sinusoidal plasma to the basolateral surface of the hepatocyte (via the intervening space of Disse). This arrangement allows plasma proteins to come into direct contact with the numerous microvilli on the basolateral surfaces of the hepatocyte, and facilitates the efficient exchange of protein-bound molecules, between the plasma and the hepatocyte.

Other sinusoidal cells

Additionally, there are phagocytic Kupffer cells that lie on the luminal surface of the endothelium. Stellate Ito cells, which are wrapped around the endothelial sheet, regulate sinusoidal diameter.

Hepatocyte ultrastructure

Hepatocytes are polygonally-shaped cells (Fig. 6) with extensive, microvillus-rich, basolateral surfaces, across which nutrients and oxygen are taken up from the space of Disse. Hepatocytes are richly supplied with mitochondria and rough and smooth endoplasmic reticulum, to support their extensive roles in energy production, protein synthesis and metabolism/detoxification respectively. The rich smooth endoplasmic reticulum and Golgi are involved in the intracellular transport of substances to the bile canaliculi for biliary secretion.

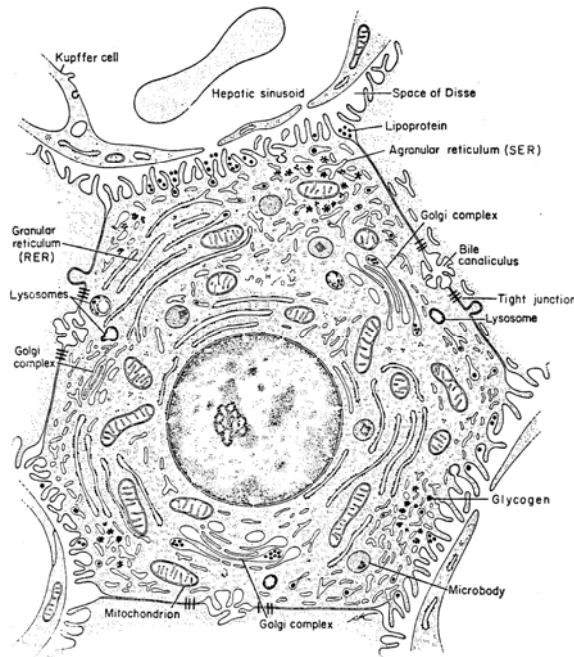


Figure 6. Ultrastructure of the Hepatocyte

From A Textbook of Histology, 9th Edition, by W. Bloom and D.W. Fawcett, W.B. Saunders Company, 1968.

Bile is secreted into canaliculi

The canaliculi, formed by the plasma membranes of adjacent hepatocytes, have specialized functions related to bile secretion. Arising from the canalicular membrane and extending into the lumen of the canaliculus are numerous microvilli, that expand the surface area available for the secretion of bile. Bile in the canalicular lumen is separated from lymph in the space of Disse by tight junctions. The canalicular tubes originate near the central vein of the liver acinus and the direction of bile flow is opposite to the flow of the blood.

Microscopic anatomy of the portal triad

The portal triad region is particularly important in the microscopic examination and evaluation of hepatobiliary disease. Since the hepatic artery, bile duct, and portal vein may be altered by hepatobiliary disease processes, their presence and normalcy must be carefully evaluated. In the normal liver, occasional mononuclear cells (lymphocytes, plasma cells, and monocytes) may be present within the portal triads. Their number increases prominently in acute and chronic inflammatory diseases of the liver.

E. Gross and Microscopic Structure of the Extrahepatic Ductal System

Common duct diameter is < 7 mm

The right and left hepatic ducts, gallbladder, cystic duct and common bile duct comprise the extrahepatic ductal system (Figure 7). Emerging from the hilum of the liver (porta hepatis), the right and left hepatic bile ducts join to form the common hepatic duct. The cystic duct arises from the common hepatic duct and connects it to the gallbladder. The continuation of the hepatic duct from the cystic duct to the papilla of Vater is the common bile duct. The diameter of the common bile duct is usually less than 7 mm.

The gallbladder is a pear-shaped hollow viscus attached in part to the under surface of the right lobe of the liver. It consists of fundus, a body, and a neck which is continuous with the cystic duct. There are remarkable variations in the size and shape of the gallbladder, and on occasion, it may be contained entirely within the substance of the liver.

Gallbladder epithelium absorbs and secretes fluids and electrolytes

Microscopically, the gallbladder wall consists of an epithelial surface, lamina propria, muscular layer and serosa (it lacks a submucosa). Its lumen is lined with columnar epithelial cells, that are separated by large intercellular spaces, sealed apically by tight junctions. These spaces, and the tightly packed microvilli on the apical surfaces (brush border), facilitate the extensive transfer of fluid and electrolytes across the gallbladder epithelium. The mucosa is thrown into folds by contractions of the muscular layer. The folds may form deep pockets, but in the normal gallbladder these pockets do not extend into the muscular layer. However, in chronic cholecystitis, these pockets extend into and beyond the muscular layer, and are called Rokitansky-Aschoff sinuses; these are of diagnostic importance to the surgical pathologist.

Stones may impact in the neck of the gallbladder

The circular muscles in the neck of the gallbladder throw the mucosa into spiral folds - the valves of Heister. These spiral valves are especially prominent at an S-shaped bend formed by the connection of the neck with the cystic duct and they are commonly visualized during contrast studies involving the gallbladder. It is in this region that gallstones become impacted in acute/chronic cholecystitis.

The lumen of the extrahepatic bile ducts is lined with tall columnar epithelium. Beneath the epithelium is a fibroelastic layer in which are variable amounts of smooth muscle fibers. Mucous glands lie in the deeper parts of this fibroelastic layer. The outer portion of the extrahepatic bile duct wall is composed of an adventitia containing nerves, lymphatics and vascular ramifications.

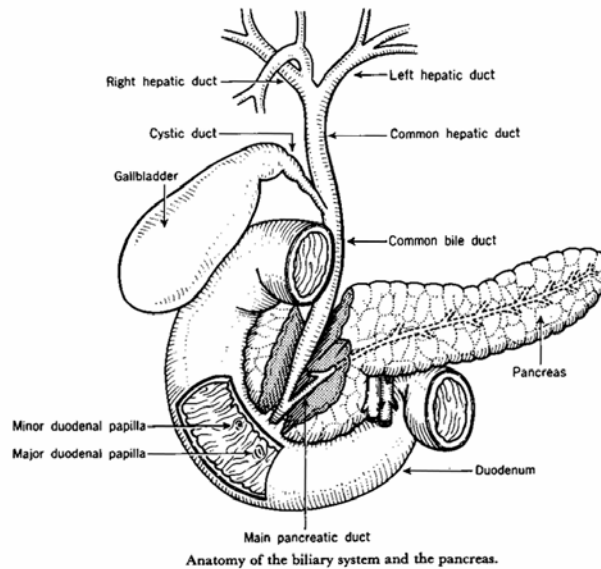


Figure 7. Anatomy of bile ducts, gallbladder, duodenum and pancreas.

From *Cholangiography and Pancreatography*, by M. Ohta, et al., Eds.
Igaku-Shoin Ltd., Tokyo, University Park Press, Baltimore, 1978.

F. Function of Components of the Liver

There are three main cell types within the liver, each performing specific functions. The largest cell mass is that of the hepatocytes. The other cell types include the sinusoidal lining cells (Kupffer cells, endothelial cells, and stellate cells [lipocytes or Ito cells]), and the bile duct epithelial cells (cholangiocytes). All have metabolic interactions with each other and the hepatobiliary system also has extensive metabolic interrelationships with the intestine and the bacterial flora contained therein (Figure 8). Thus, products of hepatic metabolism reach the intestine through the bile, and products of intestinal/bacterial metabolism reach the liver through the portal venous circulation.

1. Hepatocytes

The hepatocytes make up approximately 60-80% of the cytoplasmic mass within human liver tissue. They perform essential functions: synthesis of proteins; storage and transformation of carbohydrates; synthesis of cholesterol, bile salts and phospholipids; and detoxification, modification and excretion of exogenous and endogenous substances (many of which have important biologic activities). In addition, the hepatocyte initiates the formation and secretion of bile. Reflecting these multiple metabolic and secretory functions, the liver cells contain an extraordinarily well-developed system of organelles (e.g., mitochondria, lysosomes, peroxisomes, rough and smooth endoplasmic reticulum).

Sole site of synthesis of many proteins.

a. Protein synthesis.

The hepatocyte is the only cell in the body that manufactures albumin, fibrinogen, and the prothrombin group of clotting factors. It is the main site for the synthesis of lipoproteins, ceruloplasmin, transferrin, and glycoproteins. In addition, the hepatocyte manufactures its own structural proteins and intracellular enzymes. Synthesis of proteins, and of complexes of proteins, lipids, and carbohydrates, occurs in the rough endoplasmic reticulum. Both smooth endoplasmic reticulum and the Golgi are involved in post-translational modification and secretion of the proteins formed.

Glucose homeostasis

b. Carbohydrate metabolism.

The liver accommodates our intermittent food intake by storing carbohydrate and releasing it upon demand. Following absorption by the small intestine, galactose, mannose, fructose, and glucose are transported to the liver by the portal system. There, they are converted by enzymes in the cytosol of the hepatocyte to glucose or fructose phosphate, which may then be polymerized into glycogen, a large storage polysaccharide. Upon physiological demand mediated by various hormones, this is depolymerized and released into the blood stream as glucose. The liver is about the only tissue source of blood glucose and has the major responsibility for supporting and maintaining a consistent plasma glucose concentration. It does this by balancing the uptake of glucose and its conversion to glycogen (*glycogenesis*, stimulated by insulin) with the breakdown of glycogen to produce glucose (*glycogenolysis*, stimulated by glucagon and epinephrine). The hepatocytes may also convert amino acids and glycerol to glucose (*gluconeogenesis*).

Liver synthesizes VLDL and HDL, takes up LDL from peripheral tissues.

c. Lipid metabolism and secretion.

The liver forms fatty acids from carbohydrates and synthesizes triglycerides and phospholipids from fatty acids and glycerol. The hepatocytes also synthesize apoproteins, which they then assemble with lipids for export as lipoproteins (VLDL, HDL). The liver receives LDL from the systemic circulation and metabolizes remnants of chylomicrons. The liver synthesizes cholesterol from acetate and then converts it to bile salts. Bile salts, synthesized only by the liver, are the major pathway for removal of cholesterol.

Bile salt synthesis occurs only in hepatocytes

ATP-dependent canalicular transporters for bile salts and lecithin

Secretion of bile salts, phospholipids and cholesterol into bile is mediated by several ATP-dependent, canalicular transporters. Bile salts are secreted into bile by the bile salt export pump (BSEP). Phospholipids (mainly phosphatidylcholine = lecithin) are secreted into bile independently by the phospholipid flippase MDR3. Secreted bile salts + lecithin form mixed micelles which strip cholesterol into bile from the outer leaflet of the canalicular plasma

Bile is a major route of cholesterol excretion

Transporters in the plasma membranes of the hepatocytes mediate basolateral uptake (OCTs, OATPs) or export (MRPs), and canalicular secretion (MRP2 and MDRs)

membrane. Bile salts, returning in the portal venous blood after reabsorption from the intestine, are taken up into the hepatocytes by the basolateral Na^+ -bile salt cotransporter (NTCP).

This biliary secretion of lipids is intimately related to the metabolism of these components and lipoproteins in the liver, and to the alterations in bile composition associated with gallstone disease.

d. Organic anion and cation transport in the hepatocyte (Fig. 9).

Both organic cations and anions are taken up into the hepatocyte by groups of transport proteins (OCTs & OATPs respectively) with overlapping specificity. None of the known OATPs import UCB (unconjugated bilirubin). Organic anions (including bilirubin and glutathione) are transported across the hepatocyte into bile, usually after being modified by covalent conjugation in the microsomes. These conjugates are secreted into bile by the ATP-dependent canalicular multispecific organic anion transporter, (cMOAT), now officially called MRP2. After uptake, some compounds may reflux back into the plasma, either by passive diffusion, by ATP-dependent export mediated by the Multidrug Resistance Associated Proteins (MRPs) and export by the newly discovered, dimeric Organic Solute Transporter ($\text{OST}_{\alpha,\beta}$); these are expressed at the basolateral membrane of the hepatocyte and show considerable overlap of substrate specificity. MRP1 exports both unconjugated and conjugated bilirubins, whereas MRP3&4 and $\text{OST}_{\alpha,\beta}$ best export conjugated bile salts. All of them have low expression in the normal liver, but are upregulated in cholestasis.

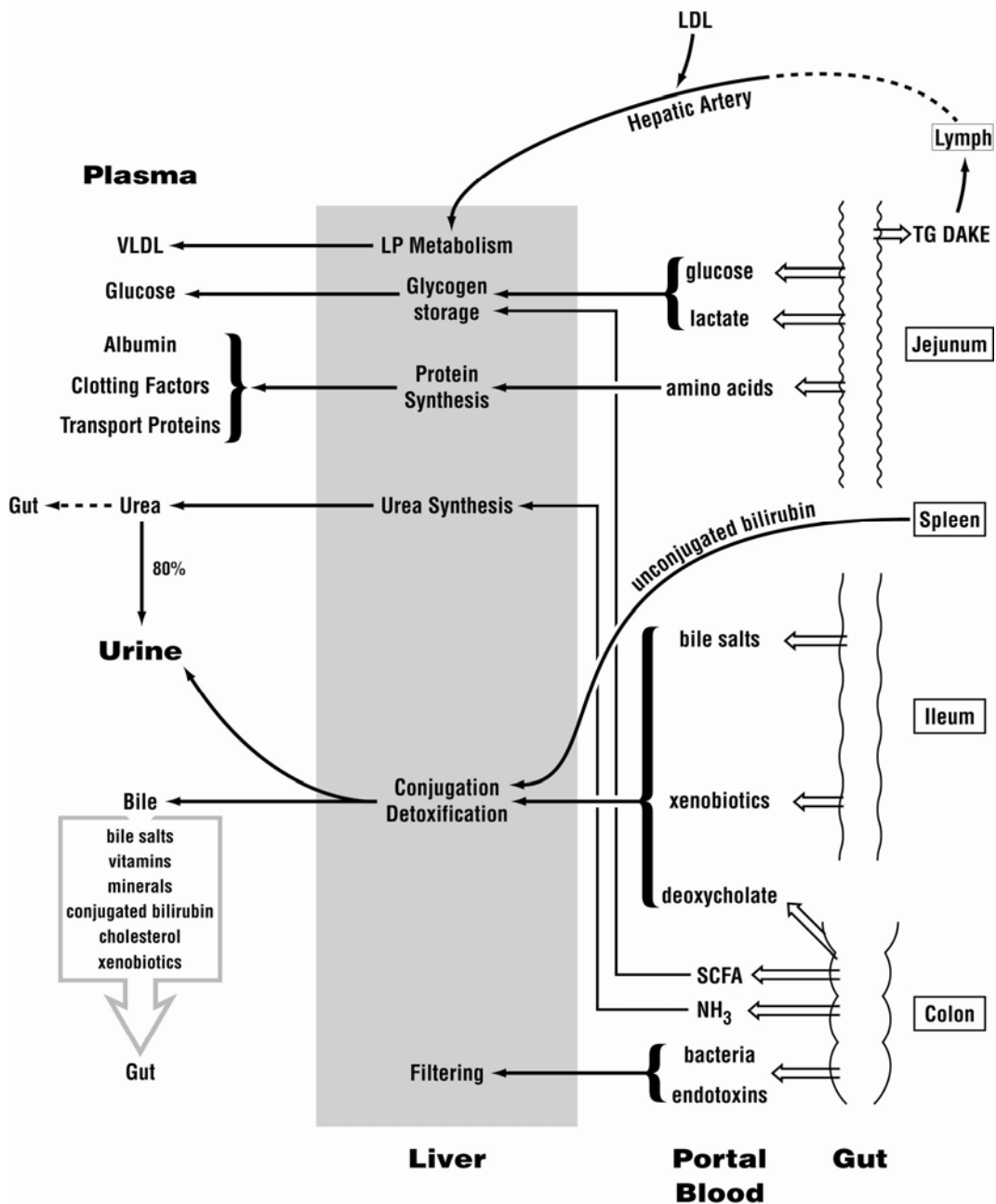


Figure 8. An overview of metabolic relationships between gut and liver.

Please note some arrows have been omitted to avoid clutter. For example, xenobiotics are absorbed from the upper small intestine, and from the colon; glucose and amino acids can be absorbed from the ileum.

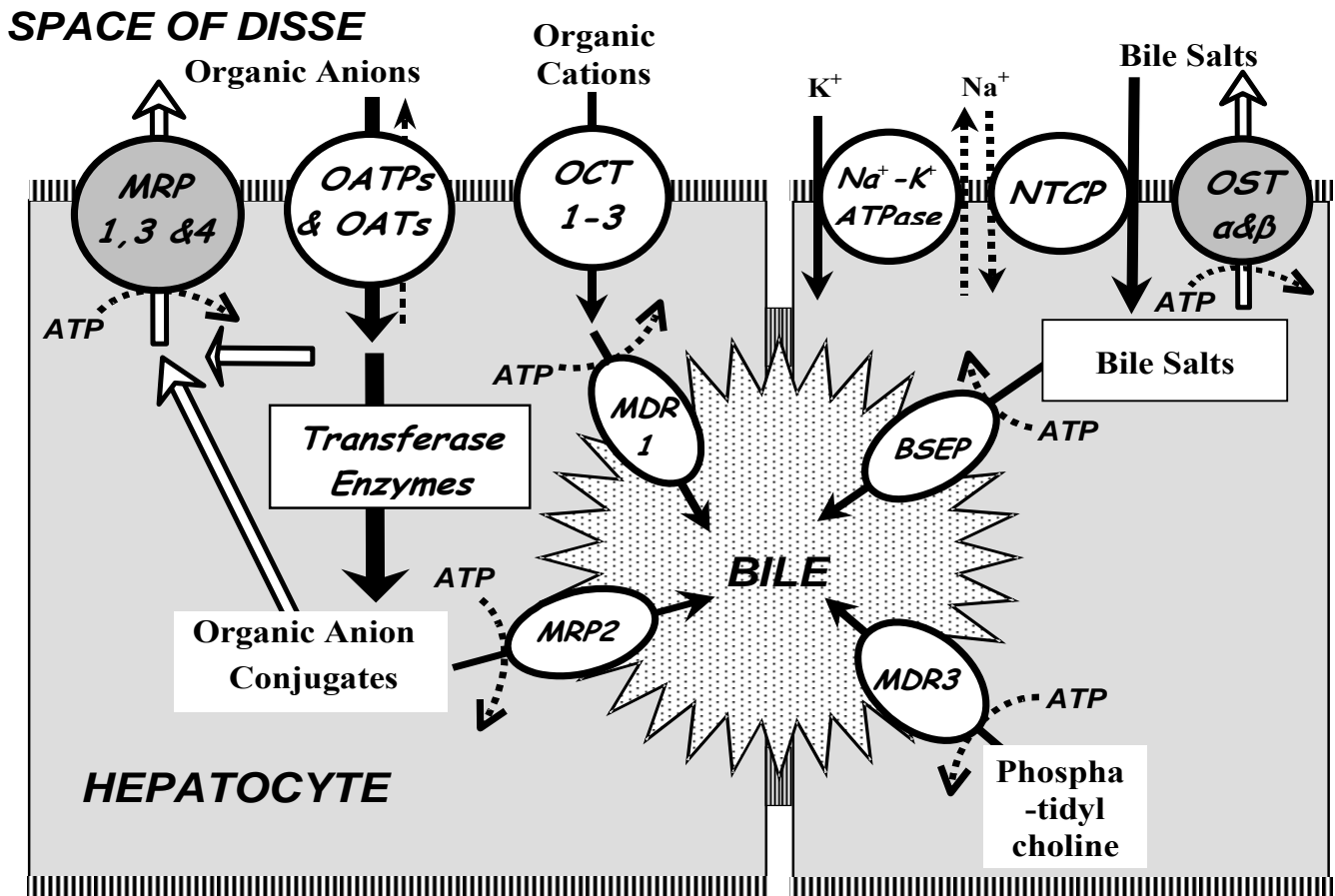


Figure 9. Transporters in Basolateral and Canalicular Membranes of Hepatocytes

A schematic of two hepatocytes and their shared bile canalculus, showing the major transporters in the basolateral and canalicular (apical) membranes. The apical transporters are all ABC (ATP-Binding Cassette) proteins that actively export their substrates into bile using energy derived from hydrolysis of ATP. As illustrated in the right-hand hepatocyte, Na⁺-coupled uptake of bile salts from the space of Disse is mediated by the basal Na⁺-Taurocholate Co-transporting Polypeptide (NTCP), driven by the plasma-to-cytosol gradient of Na⁺, created by the basal Na⁺/K⁺-ATPase. The conjugated bile salts are then secreted into bile by the canalicular Bile Salt Export Pump (BSEP). Phosphatidylcholine (lecithin) is transported to the outer leaflet of the canalicular membrane by the phospholipid flippase, MDR3, from where it is stripped into bile by secreted bile salts. As illustrated in the left-hand hepatocyte, organic anions and cations are taken up across the basal membrane by Na⁺-independent processes. Uptake of organic cations is mediated by a family of Organic Cation Transporters (OCTs). Uptake of organic anions is mediated by families of Organic Anion Transporting Polypeptides (OATPs) and Organic Anion Transporters (OATs). After conjugation, the organic anions, as well as glutathione, are then secreted into bile by MRP2, a polyspecific apical transporter at the canalicular membrane. A wide variety of amphipathic compounds (including many drugs and organic cations) are exported from the hepatocytes into bile by apical MDR1.

MRPs 1,3 & 4 and OST α,β are basolaterally located, ATP-dependent, transporters. The shading of these transporters, and the white arrows in the pathways leading to and through them, symbolize their low activity in the normal hepatocyte. With hepatocellular disease or cholestasis, they are greatly upregulated, increasing the export of organic anions back into plasma, thus limiting accumulation of toxic organic anions (e.g. bilirubin, bile salts) within the hepatocyte.

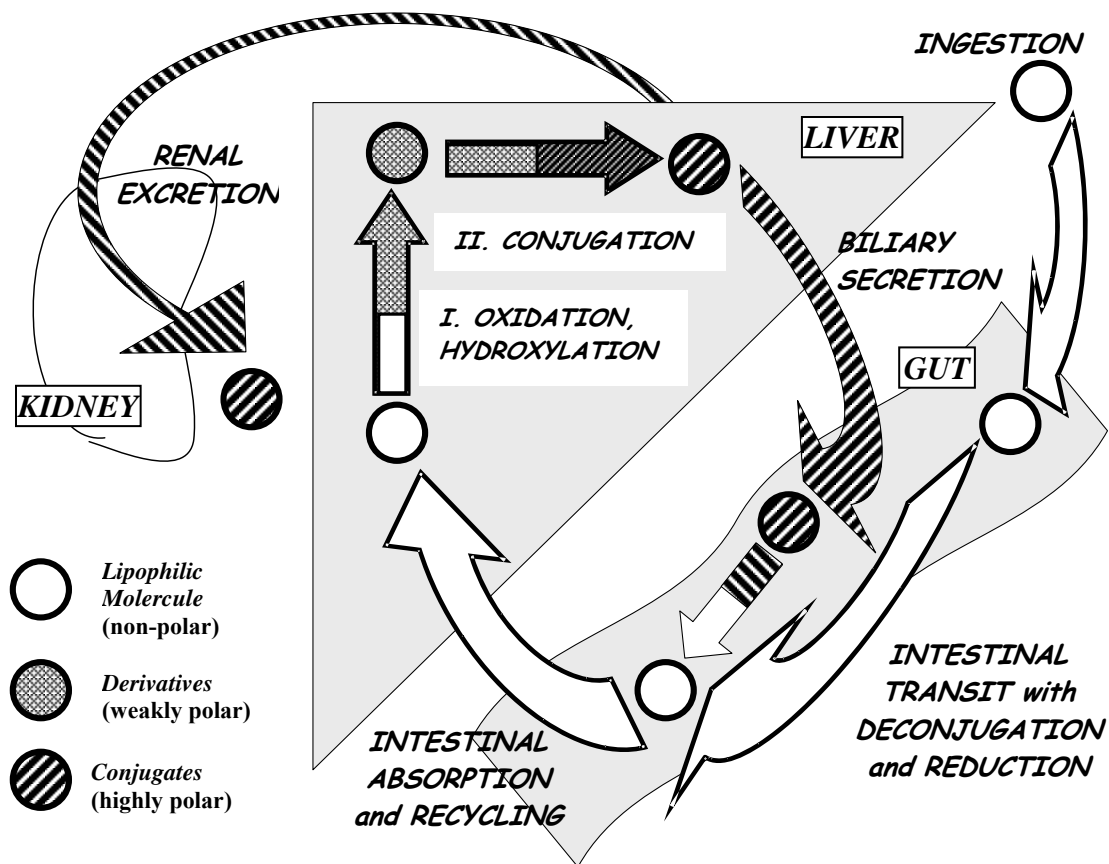


Figure 10. Hepatic Metabolism and Entero-Hepatic Cycling (EHC) of Xenobiotics

Hepatic metabolism is the other mechanism that protects the body against toxic exogenous compounds (xenobiotics), including drugs. Lipophilic xenobiotics reach the liver by passive intestinal absorption into the portal venous system after oral intake (ingestion), and also via the hepatic artery and portal vein after systemic administration (not shown). After uptake into the hepatocyte, these compounds usually undergo a two-step biotransformation that detoxifies them and allows them to be secreted into the bile. In Step I, most lipophilic compounds initially undergo modification by a wide variety of reactions, mediated primarily by the microsomal P450 and P448 cytochromes (mixed function oxygenases). These Step I reactions (mainly oxidation or hydroxylation, associated with conversion of NADP^+ to NADPH) yield weakly polar derivatives. The weakly polar groups thus introduced can then be covalently coupled to highly polar molecules (e.g. glucuronic acid, sulfate, glutathione) by Step II conjugation reactions. The resultant water-soluble conjugates are readily secreted into bile and urine by MRP2 and other transporters. Being more weakly bound to plasma proteins, the conjugates can also appear in the urine by glomerular filtration. Some xenobiotics which already possess weakly polar groups can bypass Step I metabolism and be conjugated directly.

The conjugates, being highly polar, cannot diffuse across cell membranes, and are thus no longer cytotoxic. They also cannot be passively reabsorbed from the intestine. In the lower intestine and colon, however, the enteric bacteria produce enzymes that can deconjugate and reduce the excreted conjugates back to the Phase I derivative and original xenobiotic. These relatively non-polar compounds can then be passively reabsorbed into the portal circulation and return to the liver, constituting entero-hepatic cycling (EHC).

e. Detoxification (Figure 10).

Liver metabolizes absorbed solutes carried in portal blood = “first pass metabolism”

Biotransformation of compounds by the hepatocytes involves two stages.

Intestinal metabolism of secreted conjugates may regenerate toxic intermediate.

Enterohepatic circulation of deconjugated intermediates

Impaired drug metabolism in liver diseases

The liver converts ammonia to urea via amino acid intermediates.

Hepatocytes also have the ability to metabolize and excrete a wide variety of exogenous compounds such as drugs and insecticides and endogenous compounds such as steroids and UCB. Such metabolism and excretion of miscellaneous substances is required to protect the body against ingested toxins that are absorbed from the intestine and reach the liver via the portal venous blood. This process is mediated by two types of reactions that usually occur sequentially. Stage I reactions involve metabolic transformation, such as oxidation, or hydroxylation, by the P450 cytochrome enzymes. Stage I reactions may change some compounds (e.g. vitamin D and some procarcinogens) into a biologically more active form. Stage II reactions prepare the Stage I metabolites for biliary excretion by covalently conjugating them with highly polar ligands (e.g., glucuronic acid, or glutathione). Stage II reactions convert most compounds (e.g. toxic substances and the steroid hormones) to a more water-soluble and inactive form. Individual compounds may undergo only Stage I or Stage II reactions.

As shown in Figure 10, once xenobiotics or their inactive conjugates are secreted into bile, they may be modified by intestinal bacteria, undergoing deconjugation and/or reduction. Deconjugation regenerates the oxidized, and often toxic metabolites from the phase I reactions, which may be partially reabsorbed in the intestine, undergoing an enterohepatic circulation to reach the liver, where they may contribute to hepatocellular necrosis or carcinoma. The unabsorbed metabolites that reach the colon may have oncogenic effects on the colonic epithelium, contributing to colon cancer, or on the hepatocytes, contributing to hepatocellular necrosis or carcinoma. The deconjugated metabolites may also be reduced by gut flora to the original xenobiotic, which may likewise undergo enterohepatic circulation, prolonging their half-life in the body.

Since the liver is the primary site for the metabolic inactivation of drugs, including many sedatives, steroids, ethanol, opiates, and certain antibiotics, patients with decreased hepatic function (i.e., end-stage cirrhosis) may have excessive effects from standard doses of drugs, due to impaired hepatocellular function or shunting past the liver. The liver is also the main site for the detoxification of ammonia, a noxious product of protein and amino acid catabolism. This process involves a complex of interrelated enzyme systems in the hepatocyte (the Krebs-Henseleit urea cycle), through which ammonia + citrulline eventually yield arginine, which is then hydrolyzed to form urea + ornithine. Urea is then delivered into the plasma for urinary and gastrointestinal excretion. Decreased urea synthesis, caused by (a) liver disease or (b) a congenital absence of one or more of the enzymes in the urea cycle, can result in toxic hyperammonemia with

encephalopathy.

f. Storage

Hepatocytes are important depots for storage of iron, vitamin B₁₂, and the fat-soluble vitamins D, E and K. Vitamin A is stored in the stellate cells (see below).

2. The Sinusoidal Lining Cells (Kupffer Cells, Endothelial Cells and Stellate Cells)

Sinusoidal endothelial cells have large pores (fenestrae) that permit access of plasma albumin to the Space of Disse

a. Endothelial cells

The endothelial cells that line the sinusoids have large fenestrae, which provide a graded barrier between the sinusoid and space of Disse. The size of the fenestrae determines the exchange of fluids and size of molecules that can pass from the plasma into the space of Disse and the basolateral surface of the hepatocyte.

Kupffer cells are part of the reticulo-endothelial (RE) system

b. Kupffer cells

The Kupffer cells line the sinusoids of the liver and are attached to the endothelial cells. They are derived from blood monocytes and are the largest group of fixed macrophages in the body. They actively remove macromolecules and particulate matter from the bloodstream (phagocytosis), including old cells, foreign particles, tumor cells, bacteria, yeast, viruses, and parasites. The large size of the liver and tremendous numbers of Kupffer cells makes the sinusoids a very important location for clearance of particulate matter and pathogens from the plasma. The liver's function as a filter can be appreciated when you remember that about one-third of the cardiac output flows through the liver.

Stellate cells are myofibroblasts that store Vitamin A.

c. Stellate (Ito) cells

The stellate cells, also called lipocytes or Ito cells, are smaller, and lie within the Space of Disse, encircling the sinusoidal endothelium. In the resting state, they resemble fibroblasts but their cytoplasm contains numerous droplets in which vitamin A is stored. Upon activation, stellate cells elongate to resemble myocytes and exhibit contractile function that plays a role in regulation of sinusoidal tone and resistance.

Activated stellate cells synthesize collagen.

In chronic liver disease, the stellate cells synthesize and secrete collagen into the Space of Disse, leading to "capillarization" (fibrosis) of the sinusoids. The final stage of this fibrosis is cirrhosis.

3. The Bile Duct Epithelial Cells (Cholangiocytes)

The bile duct cells form a tubular passage for the excretion of bile from the liver to the gut. It is known that, secondary to neurohumoral stimulation, these cells make significant changes in the composition of bile as it flows past, particularly in its water and electrolyte components.

G. Bile Secretion and Its Control

<i>Bile is isotonic</i>	1. Bile formation by the hepatocytes and cholangiocytes. (Figure 11). Bile is an isotonic solution containing inorganic electrolytes, bile acids, phospholipids (mainly lecithin = phosphatidylcholine), cholesterol, bile pigments (mainly bilirubin glucuronide conjugates), proteins (mainly albumin and IgA), and glycoproteins (mainly mucin) as well as detoxified xenobiotics and hormones. Bile formation is initiated by active secretion of bile salts and other organic anions from hepatocytes into the biliary canaliculi. This canalicular bile is then modified by bidirectional flux of water and electrolytes across the cholangiocytes as it passes through the ductules and intrahepatic biliary ductal system. This hepatic bile is further modified in the gallbladder.
<i>Primary canalicular bile is modified by the cholangiocytes to form "hepatic" bile</i>	
<i>Canalicular bile has bile salt-dependent and -independent components</i>	Canalicular bile has two components: a) <i>the bile-salt dependent fraction</i> , related to the active canalicular secretion of bile salts, mediated by the ATP-dependent bile salt export pump (BSEP); and b) <i>the bile-salt independent fraction</i> , related to the active canalicular secretion of other organic anions, principally glutathione, by the ATP-dependent multispecific organic anion transporter, MRP2. In both cases, the solutes generate osmotic gradients that induce both transcellular and paracellular water flow from the sinusoids. The bile-salt dependent fraction includes biliary lecithin (phosphatidylcholine) and cholesterol, since excretion of these biliary lipids is coupled to bile salt secretion (see Section N.2).
<i>Bile salts regulate the excretion of lecithin and cholesterol into bile</i>	
<i>Modification of bile by cholangiocytes</i>	In the biliary ductules and bile ducts, some bile salts are reabsorbed by an apical bile salt transporter and the bile is alkalized by secretion of bicarbonate.
<i>EHC of bile salts is major determinant of bile flow</i>	2. Regulation of bile flow. Important controls are a) the supply of bile salts, b) vagal stimuli and c) gastrointestinal hormones. The major determinant of the flow of hepatic bile is the rate at which bile salts recirculate from the intestine via the portal venous blood (the enterohepatic circulation), to be re-secreted by the hepatocytes. Vagal stimulation, cholecystokinin and gastrin all are weak stimulants of bile production. Secretin is the most potent stimulant of ductular bicarbonate secretion and ductal bile formation. Glucagon modestly increases both canalicular bile formation and ductular bicarbonate secretion. Somatostatin, by contrast, is a potent inhibitor of bile secretion at both the canalicular and ductular levels. It is important to note that all factors that increase bile secretion are increased with eating and during the early postprandial period, so that bile flow is highest when its functions in intraluminal digestion are most needed. During the interdigestive period, the entero-hepatic circulation is decreased, because little bile empties into the duodenum and intestinal motility is sluggish.
<i>Secretion of bicarbonate by cholangiocytes is stimulated by secretin</i>	
<i>Bile flow greatest with & shortly after eating</i>	

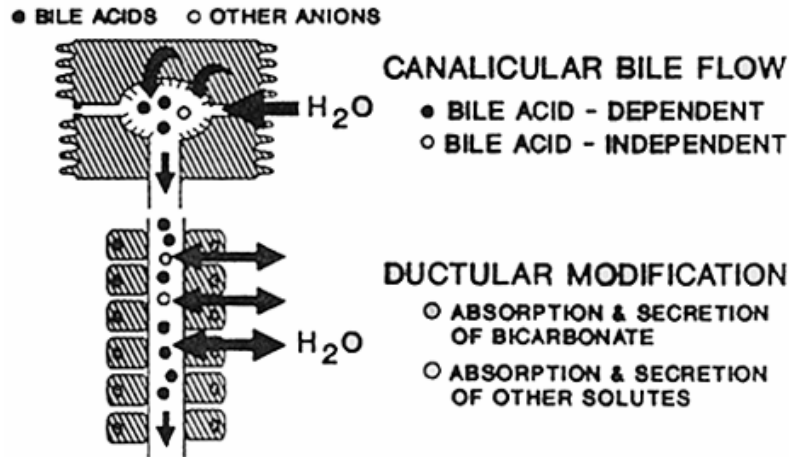


Figure 11. Hepatic Bile is Produced by Canalicular Secretion and Ductular Modification

Schematic depiction of the formation of hepatic bile. *Above:* Canalicular bile is formed in response to the active transport of primary solutes (mostly bile salts and glutathione) from the hepatocytes into the canaliculi. *Below:* Bile is modified during its passage along the biliary ductules by absorption and secretion of bicarbonate, and limited absorption of bile salts, glucose and amino acids. Water moves passively into the bile by paracellular and transcellular routes in response to the osmotic gradients generated by solute transport.

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Bile is modified in the gallbladder

3. Modification of bile by the gallbladder.

During the interdigestive phase, hepatic bile mainly enters the gallbladder, where it is concentrated, by absorption of water and electrolytes, and acidified by exchange of H^+ for Na^+ ions. (See Section N.3 and Table 4 for further details.)

H. Bile Salt Metabolism and Turnover (Figure 12)

The hepatocyte synthesizes and secretes primary bile salt conjugates that are mostly reabsorbed in the ileum and returned to the liver (EHC)

The two primary bile salts, cholic acid and chenodeoxycholic acid, are synthesized in the hepatocyte from cholesterol. They are conjugated with the amino acids glycine or taurine prior to their secretion into bile. Primary bile salt conjugates are poorly reabsorbed in the small intestine proximal to the distal ileum, but are actively reabsorbed by the ileal bile salt transporter (IBAT) and recirculated to the liver via the portal venous system (enterohepatic circulation [EHC]).

Anerobic ileocolonic bacteria deconjugate and dehydroxylate primary bile salts to form toxic secondary bile salts

The bile salts that are not absorbed pass to the distal ileum and colon, where anerobic bacteria enzymatically deconjugate and then remove the 7α -hydroxy group from the primary bile salts, yielding two more hydrophobic and toxic secondary bile salts, which also undergo EHC. One of these (deoxycholate, formed from cholate) is readily absorbed from the colon and re-excreted in a conjugated form by the liver. The other secondary bile salt (lithocholate, formed from chenodeoxycholate)

Lithocholate poorly absorbed from colon

is poorly absorbed; the small fraction absorbed is efficiently conjugated and sulphated by the human liver and then excreted in bile and lost from the body in the feces.

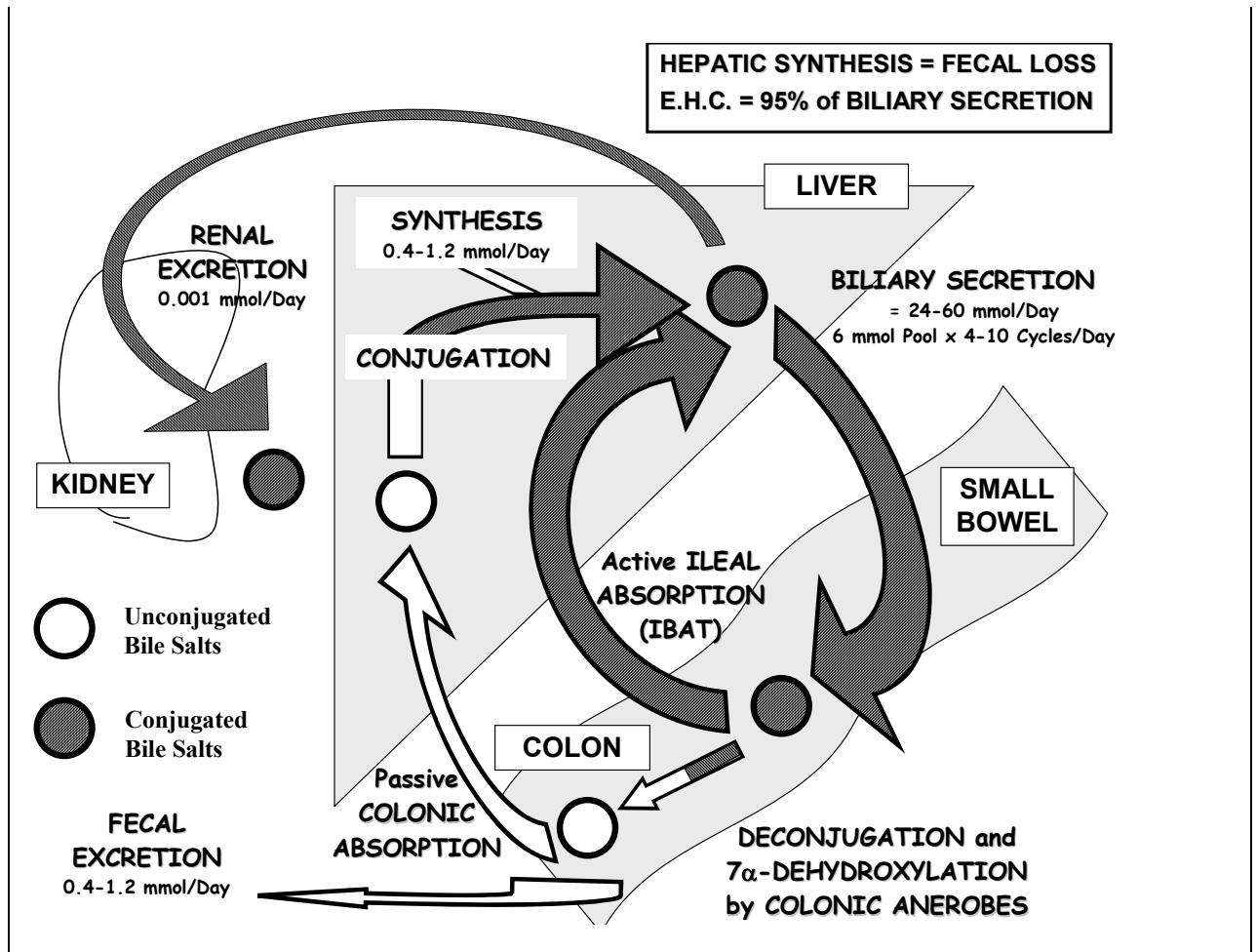


Figure 12. Hepatic Metabolism and Entero-Hepatic Cycling of Bile Salts

The hepatocyte is the only cell in which bile salts are synthesized by several multistep pathways, involving ring hydroxylation and sidechain oxidation of cholesterol. After conjugation, the bile salts are actively secreted into bile by the canalicular bile salt export pump (BSEP). In the small bowel, some passive absorption of bile salts occurs but 85-90% are removed by active absorption in the distal ileum, mediated by the ABC protein, IBAT (Intestinal Bile Acid Transporter). The remaining 10-15% of bile salts that reach the colon is in large part deconjugated and dehydroxylated by anaerobic colonic bacteria; the resulting unconjugated bile acids can be passively absorbed. These processes result in absorption into the portal circulation of 95% of the bile salts secreted into the bile, so that only 5% appear in the stool. In the steady state, this fecal excretion (0.4-1.2 mmol/day) balances hepatic synthesis of bile salts, but the vast majority of the bile salts fluxing through the liver and back into the bile (24-60 mmol/day) come from the intestine (entero-hepatic circulation, EHC). This EHC occurs principally during the 1-4 cycles that occur two hours after each meal, following emptying into the duodenum of the average 6 mmol pool of bile salts stored in the gallbladder between meals. The highly efficient (>95%) uptake of the recycled bile salts by the hepatocytes allows only a small fraction to enter the systemic circulation, where it is mostly bound to plasma proteins. Thus, the bile salt concentrations in systemic plasma are normally less than 10 μ M and less than 0.001 mmol/day appears in the urine.

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<i>95% of secreted bile salts undergo enterohepatic circulation (EHC)</i>	Normally, more than 90% of the secreted bile salts are reabsorbed by the small intestine. Most of this intestinal reabsorption occurs in the distal ileum, mediated by the ATP-dependent intestinal bile acid transporter (IBAT) in the apical membrane of the ileal enterocytes. This permits an efficient enterohepatic recirculation of bile salts that are recycled
<i>Hepatic synthesis = fecal loss</i>	(especially following meals) about 6 to 10 times daily. The normal total body pool of bile salts (approximately 6 millimoles, mainly in the EHC), is maintained because the rate of hepatic synthesis balances the daily fecal loss of 0.4-1.2 millimoles.
<i>Regulation of bile salt synthesis</i>	Regulation of the hepatic synthesis of bile salts is determined by a) health of the hepatocytes, b) supply of precursor cholesterol, c) amount of the enterohepatic return of bile salts (feedback inhibition by dihydroxy bile salts), and d) genetic factors poorly understood.
<i>Hepatic synthesis of bile salts can increase only 4-5X</i>	The hepatic capacity to synthesize bile salts is limited. If the EHC of bile salts decreases, hepatic synthesis can increase only to 4-5X above its normal synthetic rate (from 3-5% to 20-25% of total secreted bile salts). Therefore, if interruption of the EHC increases the fecal loss of bile salts more than 4-5X, the hepatic secretion of bile salts and the enterohepatic pool of bile salts must fall. With lesser interruption of the EHC, the increased synthesis compensates for the increased loss, and the bile salt pool is maintained.

I. Bilirubin Metabolism and Jaundice

Jaundice = retention of bilirubin Jaundice (icterus), the yellow discoloration of the skin, sclerae and mucous membranes caused by retention of bilirubin and/or its conjugates, may be the first or sole clinical sign of disease. Since jaundice is not usually visible until total serum bilirubin is increased to at least 3-4X normal, elevation of this value on a blood chemistry screen is often the first indication of an abnormality in bilirubin metabolism or hepatobiliary function.

1. Structure and overall metabolism of unconjugated bilirubin (UCB)

UCB is bound to plasma albumin UCB is a yellow, tetrapyrrolic pigment that is the major product of heme catabolism. Due to internal hydrogen-bonding of all its polar groups (Fig. 13), the pK'_a values of its two carboxyl groups are both above 8.0, and its interactions with water are limited, rendering it poorly water-soluble. UCB is therefore transported in the plasma bound to albumin, which limits its renal excretion and diffusion into tissues. Though UCB can readily diffuse across all cell membranes, it is cleared mainly by hepatocytes, which convert the pigment to water-soluble conjugated bilirubins, principally the di- and mono-glucuronides. This biotransformation is essential for the biliary secretion of bilirubin, and only traces of UCB appear in the bile. Under normal conditions, conjugated bilirubins are efficiently secreted into bile, so that over 96% of the bilirubin in normal plasma is unconjugated.

Conjugation of bilirubin essential for its biliary excretion

The excreted conjugated bilirubins then travel in bile down the biliary tree to the intestine, where anaerobic bacteria hydrolyze and reduce them to a variety of products that are mostly eliminated in the feces.

Unconjugated bilirubin (UCB) is a rigid, folded, biplanar molecule with multiple internal H-bonds.

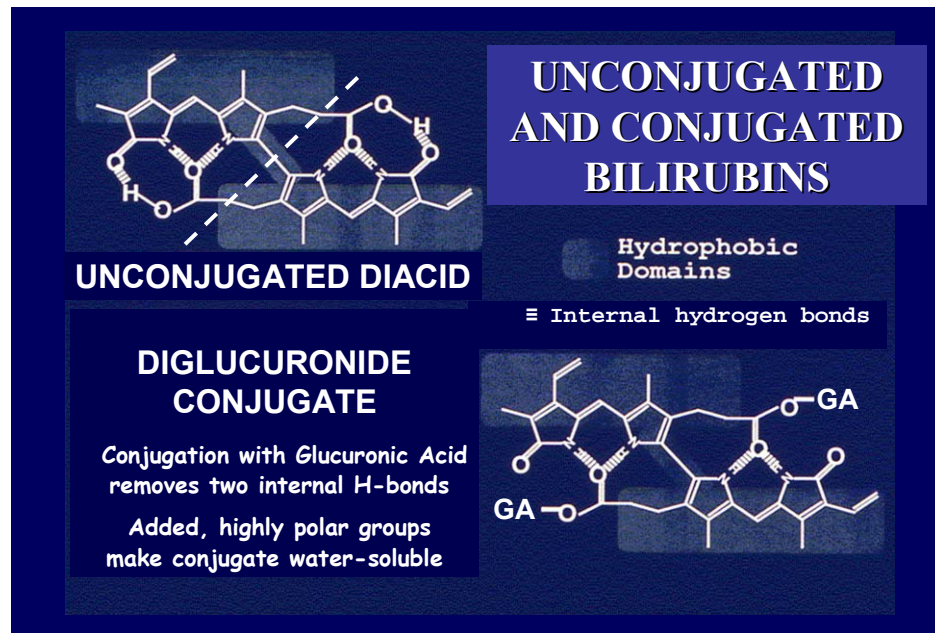


Figure 13. Structure of Unconjugated Bilirubin (UCB) Diacid

Due to H-bonds, UCB has miniscule solubility in water and high pKa values.

Conjugation of the -COOH groups of UCB with polar Glucuronic Acid groups renders the pigment water-soluble.

UCB diacid consists of two planar dipyrrolic halves connected by an -HCH-bridge, folded at a right angle along the dashed line (like a half-closed book). The -COOH group on each half interacts, via a trio of hydrogen bonds (|||), with the ring oxygens and nitrogens of the opposite dipyrrole. Shaded areas = hydrophobic domains of the molecule.

In this rigid structure, all the polar groups are tied up with each other, limiting interaction with water and the ionization of the -COOH groups. The molecule is therefore virtually insoluble in water and has remarkably high pKa values (both above 8.0). Ionization of each -COOH group removes one hydrogen bond, allowing interaction of the -COO⁻ group with water. The mono- and di-anion of UCB are therefore water soluble.

Conjugation of each -COOH group with polar Glucuronic Acid groups breaks one H-bond and renders the conjugate water-soluble.

2. Ten steps in bilirubin metabolism. (Figures 14 and 15)

The uptake, storage, conjugation, and secretion steps all involve specific carriers or enzymes whose activity may be altered selectively by competitive or noncompetitive inhibition, induction, or genetic mutation. Disorders of the liver or biliary tree often preferentially involve one or more of these steps, forming the basis for the classification and diagnosis of jaundice.

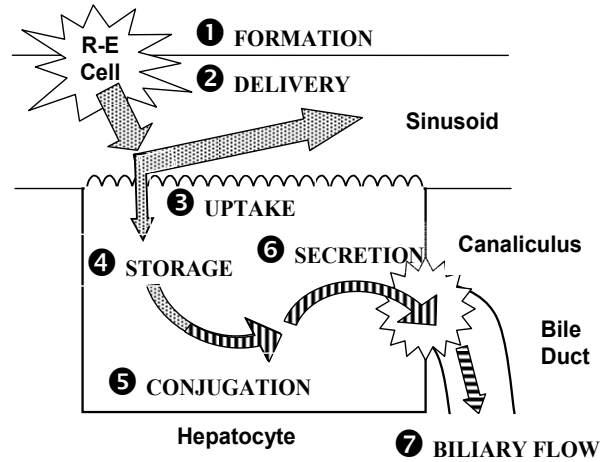


Figure 14. Steps in Normal Hepatic Bilirubin Transport

Schematic showing the seven steps from formation of UCB in the reticuloendothelial (RE) cells to flow down the biliary tree. Stippled arrows, UCB; striped arrows, conjugated bilirubins.

From Ostrow JD, Oude Elferink RPJ, Bosma PJ, Fig. 353-1, page 2149, in Stein JH, Editor, *Internal Medicine*, Mosby, St. Louis, MO, 5th Edition, 1998

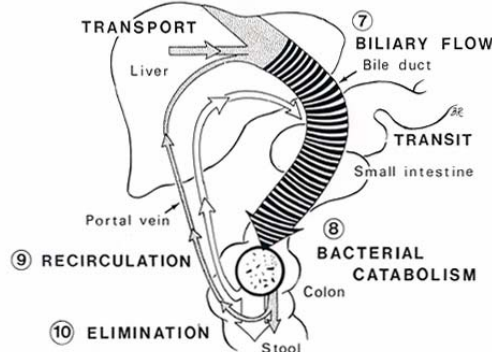


Figure 15. The Enterohepatic Circulation of Bile Pigments

Bilirubin conjugates secreted into bile are poorly reabsorbed in the intestine, but are hydrolyzed to UCB, but are hydrolyzed to UCB and then reduced to colorless urobilinogens by anaerobic bacteria in the ileum and colon. Most of the UCB and urobilinogens are eliminated in the feces, but a small proportion of each is passively reabsorbed from the colon, and returns to the liver, from where is mostly re-excreted in bile (symbols as in Figure 14; clear arrows, urobilinogens). The pigmented oxidative metabolites of UCB, the mesobilifuscins, color normal stools brown.

From Ostrow JD, Oude Elferink RPJ, Bosma PJ, Fig. 353-2, page 2149, in Stein JH, Editor, *Internal Medicine*, Mosby, St. Louis, MO, 5th Edition, 1998.

Formation of Bilirubin

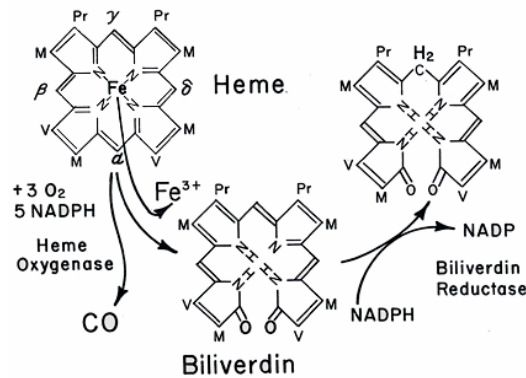


Figure 16. The Enzymatic Formation of Bilirubin from Heme.

In all cells, but mainly in the reticuloendothelial system, hemes are converted to biliverdin by the rate-limiting microsomal heme oxygenases, with equimolar release of CO and Fe^{3+} . The biliverdin is then reduced to unconjugated bilirubin by the ubiquitous cytosolic enzyme, biliverdin reductase (M, methyl; V, vinyl, PR, propionic acid side chains of the pyrrole rings).

From Fig. 1, page 2, *in* Ostrow JD, Editor, Bile Pigments and Jaundice, Molecular, Metabolic, and Medical Aspects, Marcel Dekker, New York, 1986.

UCB is formed from hemes by two successive enzymatic steps

80% of UCB is formed in spleen.

UCB in plasma is tightly bound to plasma albumin.

Bilirubin conjugates are less well bound, can appear in urine.

Only 30% of UCB in plasma is normally taken up by the hepatocytes

1) Bilirubin formation (Figure 16). All bilirubin is derived from degradation of hemes by microsomal heme oxygenases (rate-limiting), to yield equimolar amounts of Fe^{3+} , CO, and biliverdin. Biliverdin is then converted to UCB by an enzyme in the cytosol, biliverdin reductase. Seventy-five to eighty percent (75-80%) of UCB derives from hemoglobin released during destruction of senescent red blood cells in the reticuloendothelial system; the remaining 20-25% originates from tissue cytochromes (mainly in the liver) and from accelerated destruction in the marrow, spleen, and Kupffer cells of immature or defectively formed red cells (ineffective erythropoiesis).

2) Delivery in the circulation. In the plasma, UCB is tightly (99.9%) bound to plasma albumin, and therefore cannot filter at the glomerulus or appear in the urine. Conjugated bilirubins are also bound to albumin, although with much lower affinity than UCB, allowing the small unbound fraction to filter at the glomerulus. This leads to bilirubinuria in the patients with diseases that cause retention of conjugated bilirubin.

3&4) Hepatic Uptake and Storage (Clearance). In the liver, UCB and its albumin complex freely cross the sinusoidal endothelium into the space of Disse. There, uptake of unbound UCB across the basolateral membrane of the hepatocytes occurs by several Na^+ -independent, facilitated diffusion processes, whose mediating transporters have yet to be identified. Due to rate-limiting dissociation from albumin, only about 30% of total UCB is taken up on each pass through the liver. In the

cytosol, UCB is stored temporarily by binding to several organic-anion binding proteins (especially ligandin). This limits the passive reflux of UCB back into the plasma and promotes transfer of UCB to the smooth endoplasmic reticulum for conjugation.

Microsomal conjugation of bilirubin to form glucuronides

MRP-2 transports bilirubin conjugates, and other organic anions, into bile.

Bilirubin in bile is >98% conjugated; <2% is UCB

Bilirubins in bile are associated with mixed micelles.

Hydrolysis of CB and formation of urobilinogens and mesobilifuscins by intestinal bacteria.

Limited entero-hepatic circulation (EHC) of urobilinogens and UCB. Enhanced EHC of UCB in neonates.

- 5) Conjugation of Bilirubin. In the microsomes, UCB is converted to water-soluble mono- or di-conjugates by sequential covalent coupling, mainly with glucuronic acid. The sugar is first activated by enzymatic formation of UDP-glucuronic acid, and then transferred to bilirubin by a specific bilirubin-UDP glucuronosyl transferase (UGT1A1).
- 6) Secretion of Conjugated Bilirubin into the Bile. Bilirubin conjugates are secreted into the bile by an ATP-dependent, Na⁺-independent process, mediated by MRP2, the multi-specific organic anion transporter in the canalicular membrane. This process is shared by many other organic dianions but not by conjugated bile salts, which are secreted by a separate ATP-dependent transporter. The diglucuronide normally accounts for 80-85% of the bilirubin conjugates in the bile of adult humans, whereas the monoconjugate predominates in newborns. Less than 2% of the total bilirubin in normal bile is UCB, derived mainly from limited hydrolysis of secreted conjugates.
- 7) Flow of Bilirubin down the Biliary Tree. Bilirubin conjugates (and UCB), in large part associated with biliary mixed micelles, pass with the bile successively from the canaliculi, through the bile ductules and intrahepatic ducts of progressively increasing caliber, to the extrahepatic bile ducts. Between meals, bile bilirubins are mostly stored in the gallbladder, from which they are emptied into the duodenum during feeding.
- 8-10) Intestinal Catabolism, Recirculation, and Fecal Elimination of Bile Pigments (Figure 15). In the small intestine, conjugated bilirubins are poorly absorbed, but are partly hydrolyzed back to UCB by β -glucuronidases from the enterocytes and E. coli. In the distal ileum and colon, anaerobic flora mediate further catabolism of bile pigments: a) hydrolysis of conjugated bilirubin to UCB by bacterial β -glucuronidases; b) multistep hydrogenation of UCB to form colorless urobilinogens; and c) oxidation of UCB to brown-colored mesobilifuscins.
In children and adults, over 90% of the urobilinogens and unmetabolized UCB, along with the mesobilifuscins, are eliminated in the feces, so that the entero-hepatic circulation of bile pigments is limited. Metabolites that are absorbed are mostly re-excreted by the liver, but some urobilinogens are retained and appear in the urine. Infants up to 2 months of age, or patients receiving broad-spectrum antibiotics, have decreased intestinal UCB degradation, so that UCB reabsorption and enterohepatic recycling is enhanced, and serum

With severe biliary obstruction, stools can become pale yellow-white.

levels of UCB are higher.

With severe biliary obstruction, bile pigments disappear from the stool, which consequently becomes pale, yellowish-white in color (“acholic”).

J. Clinical Measurement of Conjugated and Unconjugated Bilirubin.

Total minus direct diazo reaction = rough estimate of UCB

Diazotized aromatic amines split bilirubins into two dipyrroles, each coupled with the reagent to form red-purple compounds. Due to internal hydrogen-bonding of UCB, it reacts very slowly with the reagent, so that mainly conjugated bilirubin is measured (direct reaction). Addition of accelerators that break the hydrogen bonding cause the UCB to react also, giving the total bilirubin level. The difference between total and direct bilirubin is a rough measure of UCB (indirect bilirubin).

Up to 15% of UCB may give direct diazo reaction.

Normally, the total bilirubin concentration should not exceed 1.2 mg/dL (20 μM), with less than 0.2 mg/dL (3.5 μM) reacting directly. In unconjugated hyperbilirubinemia, direct-reacting bilirubin is less than 15% of the total serum bilirubin. In conjugated hyperbilirubinemia, more than 30%, and usually more than 50%, of the total serum bilirubin is direct-reacting; there is also a significant indirect-reacting fraction, due mainly to unconjugated bilirubin formed by hydrolysis of the retained conjugates by tissue β-glucuronidases.

Bilirubinuria indicates conjugated hyperbilirubinemia

Testing the urine for bilirubin should be the initial diagnostic test in a jaundiced patient. Since only conjugated bilirubins can be excreted in the urine, the urine is normally devoid of bilirubin and detection of bilirubinuria clearly indicates that conjugated hyperbilirubinemia is present. Due to some oxidation of bilirubin, the urine in such cases may vary from dark orange to reddish brown in color. Due to the low renal threshold for conjugated bilirubin (<1 mg/dL), bilirubinuria may occur without clinically visible jaundice, and discolored urine is often noticed by patients before their skin and eyes are yellowed. Because other pigments may similarly discolor the urine, however, the presence of bilirubinuria must be confirmed chemically using a dip-stick impregnated with a diazo-reagent.

K. Other Commonly Used Tests of Hepatobiliary Dysfunction

1. Intracellular and Canalicular Enzymes

Following hepatocyte injury, intracellular and canalicular enzymes leak from hepatocytes into plasma. These enzymes vary in their degree of specificity for liver injury as compared with injury to other body organs, in their sensitivity for detecting hepatocellular injury and in the timing and degree of their elevation following hepatocellular injury.

a. Aminotransferases (Figure 17)

Aminotransferases in serum (AST and ALT) reflect hepatocyte injury

AST is also released by injured red blood cells and myocytes

Elevations of serum aminotransferases - aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - are the most useful indicators of hepatocellular injury, ranging from increased plasma membrane permeability to severe hepatocellular necrosis. As the largest organ, the liver has the highest concentrations of AST and ALT; as a result, serum levels of these enzymes are elevated to some extent in almost all hepatobiliary diseases. The highest levels are seen in severe viral hepatitis, drug- or toxin-induced hepatitis, and ischemic hepatic necrosis, usually with ALT > AST. Elevations are usually less marked in cholestasis. In alcoholic liver disease, the ALT is usually < AST, due to impaired synthesis of ALT. ALT < AST can also be seen in pregnancy-induced liver disease and fulminant hepatic failure. Although aminotransferase levels may reflect the extent of hepatic necrosis, they do not correlate with eventual outcome. ALT is present in the kidney as well as liver, whereas AST is also abundant in erythrocytes and myocytes. Thus, elevation of AST >> ALT is usual in hemolysis, myocardial infarction or skeletal muscle diseases.

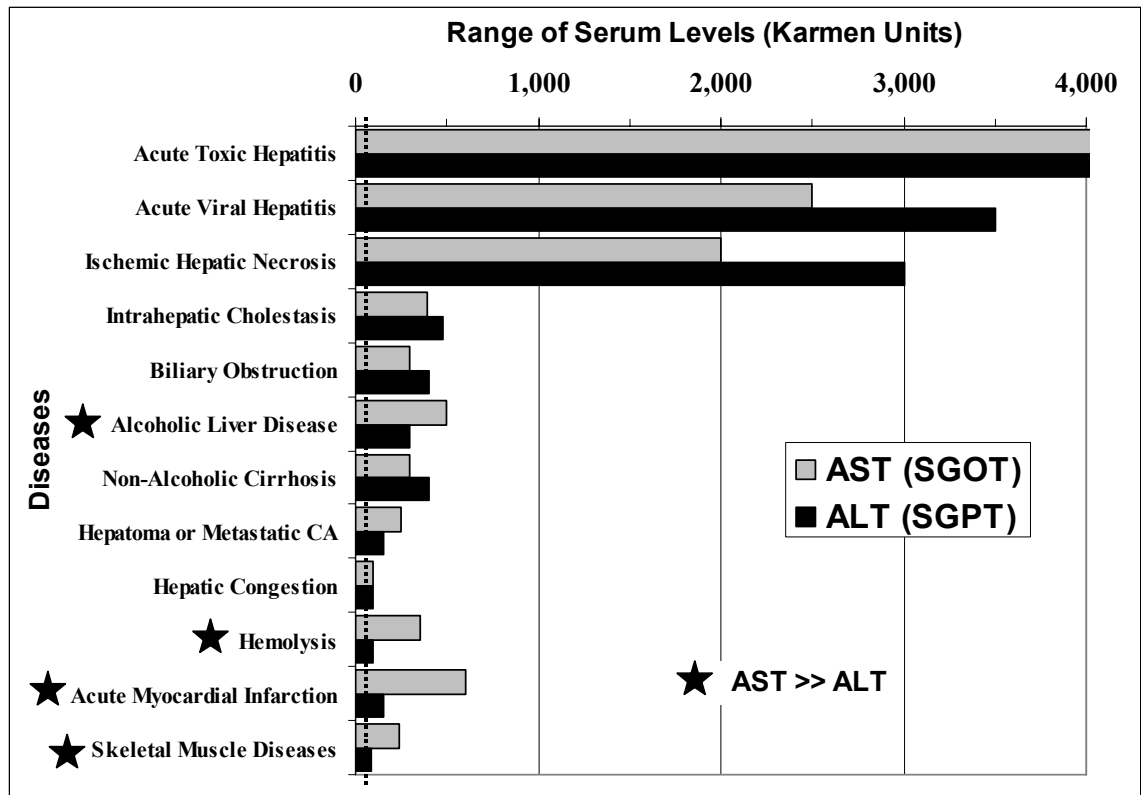


Figure 17. Serum transaminase levels in hepatobiliary diseases & myocardial infarction.

The vertical dashed line represents the upper limit of normal (40 Karmen Units)

*Based on data from: Zimmerman & West, Am. J. Gastroenterol. Oct. 1963
and Wroblewski F., Am. J. Med. Dec. 1959*

Multiple sources of plasma alkaline phosphatase

Alk. phosphatase >3X normal suggests cholestasis

b. Alkaline phosphatases

Alkaline phosphatases are isoenzymes produced in the liver, small intestine, kidney, placenta, and bone. All of these tissues contribute to the normal plasma level. These enzymes, including those synthesized in the hepatocytes and cholangiocytes, are secreted into bile. In both hepatocellular injury and biliary obstruction, plasma concentrations usually rise, but are generally much higher in cholestasis of any cause (Figure 18). This is due to both induction of alkaline phosphatase synthesis in hepatocytes by retained bile salts, as well as the diminished ability to secrete the enzyme into the bile.

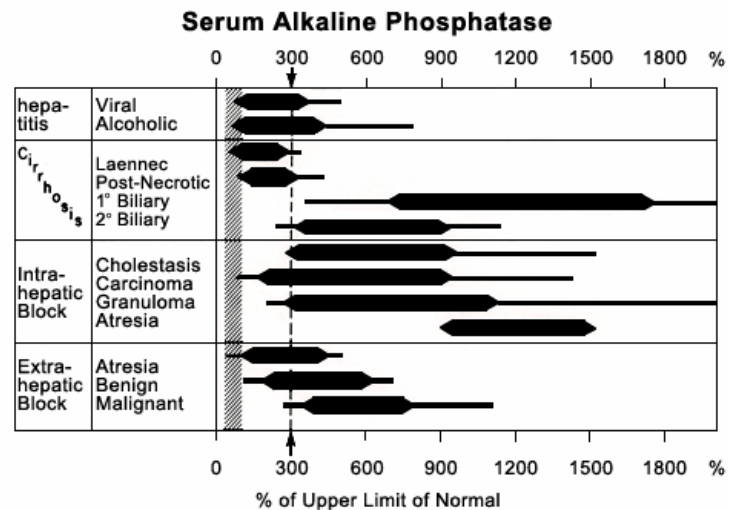


Figure 18. Serum Alkaline Phosphatase in Hepatobiliary Diseases

Ranges of serum alkaline phosphatase (horizontal axis) are expressed as a percent of the upper limit of normal. The vertical dashed line represents 3X the upper limit of normal, usually taken as the diagnostic discriminator between hepatocellular diseases (lower values) and cholestatic disorders (higher values). Note that, for each type of disease, a proportion of the values lie on the inappropriate side of the discriminatory cutoff.

© American Gastroenterological Assn., Undergraduate Teaching Project, Unit 21, Slide 105. Milner-Fenwick, Timonium, MD, 1989. Based on Zimmerman HJ, West M, Am J Gastroenterol 1963;40:387-404. Reproduced with permission.

GGT helps to pinpoint bile ducts or liver as source of increased alkaline phosphatase

c. Transpeptidases

Gamma-glutamyl transpeptidase (GGT) is localized to hepatocytes and cholangiocytes, but is a more sensitive indicator of biliary tract disease than hepatocellular disease. Its primary usefulness is limited to the exclusion of bone disease as the source of an elevated serum alkaline phosphatase level (GGT is not found in bone).

5'-Nucleotidase also helps to pinpoint bile ducts or liver as source of increased alkaline phosphatase

d. 5'-Nucleotidase

This group of enzymes catalyzes the release of inorganic phosphate from nucleotides. Although present in the cytoplasm of many organs, in the liver they are present primarily within the microsomes. It is thought that only hepatobiliary tissue can release these nucleotidases into the circulation. The plasma concentration is unaffected by bone diseases. Plasma concentrations rise in all types of hepatobiliary disease, but are highest in cholestatic conditions where the degree of elevation parallels, but is lower than, that of alkaline phosphatase. Due to this lower sensitivity, an elevation of the serum 5'-nucleotidase level is mainly useful to indicate that the liver and/or bile ducts are the source of an elevated serum alkaline phosphatase. A normal level, however, does not exclude the liver as the source of the increased alkaline phosphatase.

2. Tests Measuring Synthetic Function of Hepatocytes

The liver is the only source of many proteins and lipoproteins found in the body (including most coagulation factors and albumin). The concentration of certain plasma proteins may be taken as an indirect measure of their synthesis rate in the liver.

The short t_{1/2} (hours) of these clotting factors allows prothrombin time to increase rapidly in acute liver disease

a. Plasma prothrombin time (PT)

Only the hepatocytes synthesize coagulation factors 1 (fibrinogen), 2 (prothrombin or PT), 5, 7, 9, and 10. The PT measures blood clotting as regulated by these factors and is prolonged when factors 1, 2, 5, 7, 9, and 10 are deficient (either singly or in combination). All of these factors have very short half-lives (e.g., factor 7, 1/2 day) and turn over in the plasma very rapidly. Acute or chronic liver injury may be reflected in a prolonged PT due to failure of hepatic synthesis. Other causes of prolonged PT include congenital deficiencies of coagulation factors, circulatory congestion of the liver, consumptive coagulopathies, drugs, and vitamin K deficiency.

Malabsorption vs poor synthesis of Vitamin K

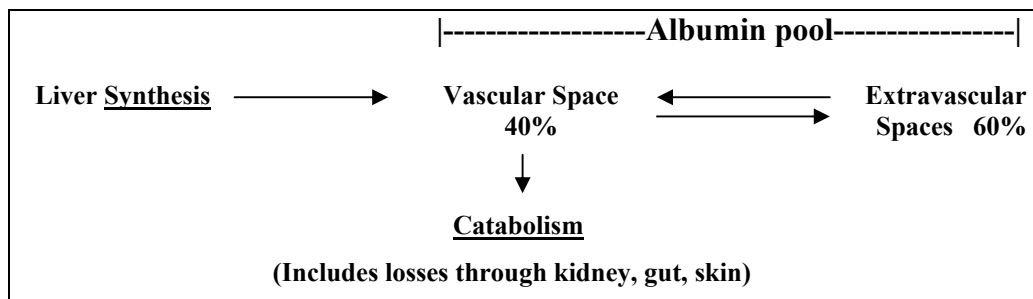
Vitamin K is essential for the synthesis of clotting factors 2, 7, 9, and 10. Being lipid-soluble, vitamin K requires the presence of bile salts in the upper intestine for efficient absorption. Thus, in chronic cholestatic conditions, impaired bile salt secretion leads to Vitamin K deficiency. By contrast, in cirrhosis, synthesis of the clotting factors is impaired, despite an adequate supply of Vitamin K.

Cholestyramine, a non-absorbable, anion-binding resin binds Vitamin K as well as bile salts in the intestinal lumen. Patients receiving this resin orally may, therefore, become deficient in Vitamin K. A single 10 mg dose of parenteral Vitamin K will correct the elevated PT if poor oral intake or absorption of Vitamin K is the cause, whereas the PT does not respond to Vitamin K in hepatocellular diseases.

b. Serum (plasma) albumin

Plasma albumin is synthesized only by hepatocytes.

Albumin, quantitatively the major plasma protein synthesized by the liver, is a useful indicator of total functional hepatocyte mass. Its concentration, normally 3.5 to 6.0 g/dL, is the composite result of hepatic synthesis rate, catabolism (including external loss), and equilibration with extravascular fluids (see diagram below).



Long half-life of albumin means level falls slowly with onset of liver injury

Albumin is synthesized only by hepatocytes and has a relatively long half-life -- 14-21 days. Thus, a decreased synthetic rate will not be reflected by a decline in plasma (serum) albumin concentration for 2-3 weeks. Serum albumin levels, therefore, are often normal in acute liver disease but low levels are the best marker and index of the severity of chronic liver disease.

Increased albumin catabolism or urinary loss may contribute to a low serum albumin level

Albumin is catabolized mainly in the reticuloendothelial system (including liver). Thus, in fulminant liver failure, severe stress, sepsis, multiple organ failure, or acute alcoholic hepatitis, release of various cytokines may accelerate albumin catabolism, causing an acute decrease in the serum albumin concentration over a period of several hours to days. The normal losses across the gut wall may be increased 2-3-fold with portal hypertension or inflammatory bowel disease, and even more with protein-losing enteropathies, including sprue. The normally small losses of albumin in the urine may increase considerably with the nephrotic syndrome.

c. Plasma lipoproteins and cholesterol

Serum lipoproteins and cholesterol usually elevated in cholestasis and alcoholic liver disease, low in most hepatocellular diseases.

Most plasma lipoproteins are synthesized in the liver. LDL released from peripheral fat stores is taken up by the liver through specific receptors. In viral hepatitis, all plasma lipoproteins are usually decreased, mainly due to decreased synthesis. By contrast, in alcoholic liver disease, metabolic effects of alcohol stimulate synthesis and impair hepatic uptake of LDL, so LDL levels are often elevated, whereas HDL levels are low. In cholestasis, LDL levels increase progressively with the duration of disease, whereas HDL levels are normal early, but fall as synthesis is impaired with persistent obstruction. In hepatocellular disease, cholesterol levels are low due to impaired synthesis. In cholestasis, plasma cholesterol levels can be elevated due to retained bile salts inhibiting the conversion of cholesterol to bile salts.

3. Tests of Globulin Synthesis

a. Immunoglobulins (γ -globulins)

*Gamma globulins
high in autoimmune
hepatitis*

Most diffuse hepatic injuries induce an intrahepatic inflammatory reaction which includes infiltration by varying numbers of lymphocytes and plasma cells. The associated increase in synthesis of immunoglobulins is reflected by an increase in their plasma concentrations. In general, the immunoglobulins are increased in a non-specific manner in many different types of chronic liver disease and thus have limited diagnostic value. In certain conditions, however, some fractions are characteristically more markedly elevated (i.e., IgG in chronic autoimmune hepatitis or IgM in primary biliary cirrhosis). The normal adult range for total γ -globulins is 0.7 to 1.5 g/dL (paper electrophoresis).

b. Total globulins and albumin/globulin (A/G) Ratio

*Albumin/globulin
ratio below 1.0
usually indicates
liver disease*

The less abundant α - and β -globulins are largely lipoproteins. Changes in lipoprotein levels may thus modify the changes in total globulins that tend to be dominated by the γ -globulins. Total globulin levels in serum are normally 2.5-3.5 g/dL, and thus lower than the normal albumin level. In chronic liver disease, the Albumin/Globulin (A/G) ratio often is lower than 1.0, and an A/G ratio less than 1.0 should strongly suggest liver disease.

4. General Usefulness of "Liver Function Tests" (LFT's).

*Use "liver function
tests" selectively for
specific purposes*

Liver laboratory tests are often called "liver function tests" (LFTs), but the aminotransferases are in fact markers of hepatobiliary damage, NOT liver function. AST and/or ALT are intermittently mildly elevated in normal subjects, probably due to incidental exposures to environmental toxins, alcohol, or medications. By contrast, some other tests are poorly sensitive for assessing the degree of hepatic injury or amount of hepatic functional reserve. Although hundreds of different tests have been devised, a physician does best if he/she employs only a few for specific purposes. The clinical situations wherein LFTs are most helpful are the: a) differential diagnosis of jaundice; b) detection of hepatic dysfunction in the non-jaundiced patient; c) rough measure of the degree of hepatic dysfunction in a patient with known liver disease; and d) assessment of the progression of known hepatic illness under serial observation.

L. Hepatobiliary Defects in Bile Salt Metabolism & Secretion

1. Primary Defects in Bile Salt Synthesis

*Rare genetic
defects*

*Accumulation of
sterol precursors
may account for
many symptoms*

Genetic defects in bile salt synthesis may lead to severe deficiency of bile salts in the bile and intestine, leading, respectively, to secondary decreases in biliary phospholipid and cholesterol secretion and to fat malabsorption. The deficient conversion of cholesterol to bile salts leads to high serum cholesterol levels and accumulation in the plasma and tissues of the sterol precursor(s) proximal to the block in bile salt synthesis.

2. Cholestasis

Mechanisms of cholestasis

Stasis of bile flow may result from three processes: a) failure to secrete bile into the canaliculi, due to injury to the hepatocytes and/or the canalicular membrane, or altered function of the canalicular bile salt transporter; b) increased permeability of the canaliculi, cholangiocytes and/or their tight junctions; and c) mechanical obstruction of the biliary tree at any level. Diffuse injury to canalicular membranes causes loss of microvilli in dilated canaliculi which can be plugged by inspissated bile. In cholestasis, there is impaired secretion of all components of bile.

Impaired secretion of all bile components

Impaired secretion plus regurgitation across the biliary tract increases plasma levels of all bile components

The retained bile salts secondarily damage the hepatocytes, canaliculi, cholangiocytes and tight junctions, aggravating any primary injury to these structures. Increased intrabiliary pressure proximal to a mechanical obstruction contributes to the increased permeability of the ducts and canaliculi. This increased permeability allows all components of bile to regurgitate into the blood via the peribiliary plexus, adding to the elevated plasma levels of bile salts, lecithin, cholesterol, conjugated bilirubins and alkaline phosphatase. The retained bile salts also induce synthesis of alkaline phosphatase in the affected hepatocytes and cholangiocytes. Thus, plasma levels of this enzyme, as well as bile salts, are earliest and most strikingly elevated, often in the absence of elevated plasma bilirubin levels. Retention of other cholephiles, possibly opioids, causes pruritus (itching) that accompanies 75% of cases of cholestasis.

*Early increase in alk. phosphatase & bile salts in plasma
Itching common in cholestasis*

When cholestasis occurs, several compensatory mechanisms partially mitigate the retention of bile salts: a) bile salts retained in hepatocytes inhibit their synthesis from cholesterol; b) upregulation of MRP 1&3 in the basolateral membrane of hepatocytes facilitates export of retained organic anions into plasma; c) decreased delivery of bile to the intestine diminishes the enterohepatic circulation of bile salts; and d) sulfation of the hydroxyl groups of bile salts is up-regulated in the liver and kidney, enhancing the renal excretion of bile salts.

Compensatory mechanisms in cholestasis

Canalicular cholestasis is most often due to drugs, estrogens, and infections

a. Canalicular cholestasis results from impaired function of the export pumps for bile salts (BSEP) and other organic anions (MRP2) in the canalicular membrane. Although most often caused by steroid hormones, such as estrogens, it also may occur in acute viral, or alcoholic hepatitis, in sepsis and during total parenteral nutrition. Some degree of canalicular cholestasis is a common feature of most diffuse intrahepatic inflammations and many drug reactions, due to injury to the secretory functions of the canalicular membranes.

- i. In drug-induced cholestasis, (phenothiazines, sulfonyleureas) some degree of inflammation and hepatocellular necrosis can be present.
- ii. In steroid-induced cholestasis (normal pregnancy, or treatment with synthetic estrogens, or with 17- α -alkyl anabolic steroids), maximum hepatic secretory capacity is impaired for organic anion cholephiles in almost all subjects, but a full-blown cholestatic syndrome ensues unpredictably in a small proportion of patients.

- iii. Postoperative cholestasis. Mild to severe cholestasis may occur after long, difficult surgical procedures with multiple blood transfusions.
- iv. Other causes of canalicular cholestasis. A similar syndrome may occur during severe infections with endotoxemia and/or septicemia, total parenteral nutrition, and sickle cell anemia crises.

b. Obstructive cholestasis is subdivided according to whether the blockage is in the intrahepatic or extrahepatic bile ducts, and whether the obstruction is due to a benign process (stones, strictures) or due to cancer, most often of the biliary or pancreatic ducts. The obstruction may be focal or multifocal.

Classification of obstructive cholestasis by site of obstruction and etiology

Obstructive cholestasis can result in the same functional changes as in canalicular cholestasis. Flow of bile is retarded, and excretion of all components of bile is decreased if any portion of the biliary tree, from ductules to sphincter of Oddi is obstructed. Obstructive cholestasis is classified according to the site of damage to the biliary tree, and to the putative etiology.

- i. Obstructive intrahepatic cholestasis. Primary biliary cirrhosis, an autoimmune disease, affects the small bile ductules and intralobular ducts in the portal triads. Interlobular and larger intrahepatic bile ducts can be obstructed by multifocal lesions of the liver (granulomas, lymphomas, metastatic nodules). In sclerosing cholangitis and intrahepatic lithiasis, irregularly distributed fibro-inflammatory lesions lead to strictures and the formation of multiple, small calculi in the intrahepatic ductal system. These syndromes may cause a prolonged, marked elevation of the serum alkaline phosphatase without jaundice.
- ii. Obstructive extrahepatic cholestasis is most often caused by local lesions such as carcinoma of the head of the pancreas, or by common bile duct stones or structures. Rarer cholangiocarcinomas of the common duct or right or left hepatic ducts often cause a slowly progressive cholestasis with marked elevation of the alkaline phosphatase long before jaundice develops. Strictures or primary carcinomas of the Papilla of Vater are other less common causes.

3. Multiple Defects in Hepatocellular Diseases

- a. **Acute hepatocellular diseases (hepatitis)** may impair the basolateral uptake, synthesis, intracellular transport, and biliary secretion of bile salts as well as hepatic synthesis of cholesterol.
- b. In **chronic hepatocellular diseases (cirrhosis)**, in addition, bile salts absorbed from the intestine are partially diverted through the porto-systemic shunts into the systemic circulation, contributing to increased plasma bile salt levels.

M. Pathophysiology, Diagnosis and Treatment of Jaundice

Jaundice classified according to type of bilirubin retained and specific steps in bilirubin metabolism that are abnormal Jaundice may be classified into two broad groups according to the predominant form of bilirubin, conjugated or unconjugated, that is retained in the plasma and tissues (Table 2). As shown in Table 1, unconjugated hyperbilirubinemia is caused by abnormalities in all steps up to and including the conjugation of bilirubin (steps 1 to 5). By contrast, impairment of canalicular secretion (step 6) or biliary flow (step 7) results in retention principally of conjugated bilirubins.

Table 1. Specific steps in bilirubin metabolism and abnormalities that affect them

Steps	Abnormalities	Principal Examples
1. Formation of UCB from heme	Overproduction of UCB	Hemolysis - Ribavirin
2. Delivery of UCB in the plasma, bound to albumin	Right-sided congestive heart failure Portosystemic shunts	Cor pulmonale Cirrhosis; surgery or TIPS
3. Uptake of UCB (basolateral) into the hepatocyte	Genetic defect Competitive inhibition by drugs	Gilbert's syndrome Rifampin
4. Storage of UCB in hepatocyte cytosol, bound to ligandin	Decreased ligandin levels	Fever, hypothyroidism
5. Conjugation of UCB to form CB (by microsomal UGT1A1)	Genetic deficiency of UGT1A1	Moderate Severe Gilbert's syndrome - Indinavir Crigler-Najjar syndromes I and II
6. Secretion of CB into canalicular bile, via MRP2	Genetic deficiency of MRP2 Hepatocellular jaundice Canalicular cholestasis due to: Endotoxins Estrogens Drugs	Dubin-Johnson syndrome Hepatitis, cirrhosis Sepsis Pregnancy Sulfonylureas, cyclosporins
7. Flow of CB in bile down the biliary tree to the duodenum	Obstructive cholestasis: Intrahepatic cholestasis Extrahepatic biliary obstruction	Primary biliary cirrhosis Tumors, granulomas Strictures, gallstones Pancreatic & bile duct carcinoma
8. Intestinal transit of CB and bacterial catabolism to UCB and urobilinogens	↑ absorption of UCB – slow transit ↓ formation of urobilinogens (absence of anaerobic gut flora) ↑ hydrolysis of CB in upper gut	Ileus, fasting Neonates, antibiotic therapy Bacterial overgrowth
9. Enterohepatic recirculation	Circulatory bypass of liver Leak from biliary tree	Portosystemic shunting Acute cholecystitis
10. Elimination in the feces	↑ absorption of UCB – slow transit	Obstipation

Abbreviations: UCB: unconjugated bilirubin; CB: conjugated bilirubins; MRP2: canalicular multispecific organic anion transporter; UGT1A1: bilirubin uridine diphosphate glucuronosyl transferase-1; TIPS: transcutaneous intrahepatic portosystemic shunt

Table 2. Unconjugated versus Conjugated Hyperbilirubinemia

Finding	Unconjugated Hyperbilirubinemia	Conjugated Hyperbilirubinemia
Retained bilirubin	UCB*	CB and UCB*
Bilirubin in urine	No	Yes
Diazo reaction (serum)	<15% direct	>30% direct (usually >50%)
Usual causes	Hematologic, circulatory or functional liver disorders	Hepatocellular or biliary tract diseases
Compensatory mechanisms	Oxidation and intestinal excretion of UCB*	Renal excretion of CB*

*CB, conjugated bilirubins, UCB, unconjugated bilirubin

1. Pathophysiology of Unconjugated Hyperbilirubinemia

*Only UCB retained
No bilirubinuria*

Involves steps 1-5

Mainly functional diseases, so avoid invasive tests

a. Impaired steps in bilirubin metabolism.

Unconjugated hyperbilirubinemia, characterized by exclusive retention of UCB in the serum, without bilirubinuria (Table 2), results from insufficient hepatic clearance and/or conjugation of the load of the UCB produced each day, and is subclassified according to the step(s) in bilirubin metabolism that are deranged (Table 1). The most common etiologies are (Step 1) overproduction of unconjugated bilirubin due to disorders of red blood cells; (Step 2) impaired delivery of unconjugated bilirubin due to disturbances in hepatic circulation; and (Steps 3, 4, or 5) functional or hereditary defects in uptake, storage, or conjugation of bilirubin. Although the underlying hematological, circulatory, or functional disorders may also be serious in themselves, the primary hepatic disorders that cause pure unconjugated hyperbilirubinemia are seldom life-threatening *per se* except in some neonates, and in patients with the Crigler-Najjar syndrome. Therefore, invasive diagnostic studies of the liver, including biopsy, are rarely indicated.

1. Overproduction of bilirubin due to accelerated heme catabolism is a very common cause of unconjugated hyperbilirubinemia and often augments jaundice of other causes. Most often caused by hemolytic anemias. Diagnosis rests mainly on hematological studies.
2. Decreased delivery to the liver cells of bilirubin and other substances in plasma is a common cause of unconjugated jaundice. It is most often due to right-sided congestive heart failure and clears as the heart failure is controlled. Another major cause is portosystemic shunting, due to either cirrhosis, surgical anastomosis or TIPS, which diverts the UCB formed in the spleen past the liver directly into the systemic circulation.
- 3.& 4. Diminished clearance (uptake and storage) of UCB may be due to competitive inhibition of these processes by drugs (e.g., rifampicin). Such jaundice often resolves by 2-3 days after the drug is stopped. Hypothyroidism and febrile illnesses may impair hepatic storage capacity for UCB. Impaired uptake of UCB and other organic anions is also present in most patients with Gilbert's syndrome (see below).

Conjugation of UCB preserved in hepatobiliary diseases

Gilbert's syndrome: a combined defect in conjugation plus uptake and/or formation of UCB

Crigler-Najjar Syndromes due to severe genetic deficiencies in UGT1A1

Neonatal jaundice: impairment of most steps of UCB transport.

Treatments:
Phototherapy
Avoid fasting
Inhibit UCB formation
Trap UCB in gut
Exchange transfusion

5. Impaired conjugation of bilirubin is usually due to hereditary defects, since the activity of UGT1A1 is preserved in hepatobiliary diseases, except in end-stage cirrhosis or acute hepatic failure.

b. Hereditary autosomal recessive disorders of UCB conjugation

- i) Gilbert's syndrome is a very common chronic, mild, fluctuating unconjugated hyperbilirubinemia, due to a gene promoter abnormality, causing a 2/3 decrease in the expression and activity of UGT1A1. When combined with overproduction and/or impaired uptake of bilirubin, hyperbilirubinemia occurs, that is often augmented by fasting, stress, or viral infections.
- ii) The Crigler-Najjar syndromes are two more severe, rare, hereditary, recessive deficiencies of UGT1A1. In Type I, there is no detectable activity of UGT1A1 in the liver, jaundice is severe from the neonatal period, and bilirubin encephalopathy is the rule if the patients are untreated. In Type II, UGT1A1 activity is detectable at less than 10% of normal levels. Jaundice begins in late childhood, is less severe, and seldom causes brain damage.

2. Unconjugated hyperbilirubinemia of the newborn

Immaturity of most steps of bilirubin metabolism causes a mild, temporary, retention of UCB in virtually all neonates. Its severity is much influenced by the high rates of production and intestinal reabsorption of UCB in neonates, and the partial starvation of suboptimal breast-feeding. It is usually more marked in infants who are premature and/or have complicating hemolytic disease due to fetal-maternal Rh or ABO antigen incompatibility. Serum UCB levels >15 mg/dL may lead to deposition of unbound UCB diacid in the central nervous system, causing auditory and neurologic dysfunction (bilirubin encephalopathy), which is usually reversible but may be permanent or fatal (kernicterus).

3. Treatment of Unconjugated Hyperbilirubinemia

The mild jaundice of Gilbert's syndrome does not require treatment, and may protect against oxidant stress. Treatment is only necessary when severe jaundice may cause brain damage. The preferred treatment of neonatal jaundice is phototherapy, which converts UCB to photoisomers that can be excreted in bile and urine without conjugation. In neonates, frequent feeding speeds intestinal transit and limits the reabsorption of UCB. A newly-accepted therapy in newborns is parenteral administration of tin mesoporphyrin IX, a potent competitive inhibitor of heme oxygenase and thus of bilirubin synthesis. Oral administration of calcium phosphate traps UCB in the intestine, interrupting its enterohepatic circulation. When severely jaundiced neonates respond insufficiently to the above therapies, they are treated by exchange transfusion to physically remove UCB from the circulation. Refractory Crigler-Najjar I subjects undergo liver transplantation.

4. Pathophysiology of Conjugated Hyperbilirubinemia.

Both CB and UCB are elevated in plasma

Conjugated hyperbilirubinemia is characterized by retention principally of conjugated bilirubin in the serum. Plasma UCB concentrations are elevated also, due in part to hydrolysis of retained conjugated bilirubins by tissue β -glucuronidases, as well as by contributions from associated hemolysis and/or impairment of delivery, uptake, and storage of UCB. Conjugated hyperbilirubinemia results from impairment of the canalicular secretion or biliary flow of conjugated bilirubins. Other organic anions, which share the same MRP2 transporter as conjugated bilirubin, are also excreted poorly. The retained conjugated bilirubins regurgitate through the hepatocytes, cholangiocytes and their weakened tight junctions into the space of Disse and thence via the lymph to the plasma. The small fraction of retained conjugated bilirubins that is not bound to plasma albumin filters at the glomerulus, giving bilirubinuria; this is diagnostic of conjugated hyperbilirubinemia, and also constitutes the major alternate pathway for excretion of conjugated bilirubin and other organic anions when hepatobiliary excretion is reduced.

Bilirubinuria is diagnostic of conjugated hyperbilirubinemia.

Part of retained conjugates are δ - (delta) bilirubin which is covalently bound to albumin

With prolonged conjugated hyperbilirubinemia, up to 80% of the conjugated bilirubin can become covalently linked to serum albumin. This δ -bilirubin is not excreted by the liver or kidneys. Therefore, with remission of hepatitis or relief of biliary obstruction, direct-reacting δ -bilirubin persists in the plasma long after urinary excretion of bilirubin conjugates has ended.

Conjugated hyperbilirubinemia indicates significant hepatobiliary disease

In contrast to unconjugated hyperbilirubinemia, jaundice with bilirubinuria almost always results from significant hepatobiliary disease, and is classified further according to whether canalicular secretion (Step 6) or biliary flow (Step 7) is primarily impaired. Defects in canalicular secretion of bilirubin conjugates and other organic anions secreted by MRP2 are characteristic of the hereditary Dubin-Johnson syndrome, and are common in hepatocellular diseases. Generalized defects in canalicular secretion, or in biliary flow, produce cholestasis, in which there is also marked retention of bile salts. Dislocation of alkaline phosphatase to the basolateral membrane leads to regurgitation of this enzyme into the space of Disse and thence to plasma.

Cholestasis causes retention of bile salts, cholesterol alk. phosphatase

The retention of bile salts inhibits both hepatic synthesis of cholesterol and (more so) hepatic catabolism of cholesterol to bile salts, but increases hepatic synthesis of alkaline phosphatase. The resultant marked increases in serum cholesterol and alkaline phosphatase levels aid in the clinical distinction of cholestasis from hepatocellular diseases. Toxic effects of the retained hydrophobic bile salts may cause the secondary hepatocellular injury that develops with prolonged cholestasis.

5. Differential Diagnosis of Conjugated Hyperbilirubinemia

Hepatocellular vs. cholestatic jaundice

Once the presence of conjugated hyperbilirubinemia has been established, the next steps in diagnosis are to determine whether jaundice is hepatocellular or cholestatic and to determine the level of the block (canalicular, or ductal) to biliary flow if cholestasis is present (Table 3).

Itching virtually diagnostic of cholestatic jaundice

Itching, especially on the palms and soles, is virtually diagnostic of cholestasis but occurs in only 75% of cases. Retention of endogenous opioids is thought to cause the itching. In those who do not itch, the presence of cholestasis may be surmised from a >3X elevation of the serum alkaline phosphatase level, increased serum cholesterol level, milder elevations of serum transaminases, and response of an abnormal prothrombin time to Vitamin K. Steatorrhea may occur due to low concentrations of bile salts and mixed micelles in the intestine.

Table 3. Hepatocellular versus Cholestatic Jaundice

Clinical Finding or Laboratory Test	Hepatocellular Jaundice	Cholestatic Jaundice	
		Canalicular	Ductal
Pruritus	Uncommon	>75% of patients	>75% of patients
Steatorrhea (insufficient mixed micelles)	Uncommon	Common	Common
Serum bilirubin levels	Increased	Usually increased	Usually increased
Serum bile acid levels	Increased	Increased	Usually very high
Serum alkaline phosphatase levels	↑ Usually <3X normal	↑ Usually >3X normal*	↑ Usually >3X normal, often >10X normal [#]
Serum cholesterol levels	Decreased or normal	Increased	Increased
Serum amino-transferases (transaminases) [‡]	Elevated, may be >1000 IU	Usually <300 IU, but may be higher	Mildly elevated, seldom >300 IU
Prothrombin time and response to parenteral Vitamin K therapy	Usually increased; unresponsive to Vitamin K	Sometimes increased; responds to Vitamin K	Sometimes increased; responds to Vitamin K

*Alkaline phosphatase levels are often <3X normal in canalicular cholestasis due to estrogens, anabolic steroids, endotoxins, or postoperative state.

[#]Patients with hepatic metastases or granulomas, or chronic partial biliary obstruction, often have marked elevation of serum alkaline phosphatase with little or no increase in bilirubin levels.

[‡]Includes serum glutamic pyruvic transaminase (SGPT, alanine aminotransferase, ALT) and serum glutamic-oxalacetic transaminase (SGOT, aspartate aminotransferase, AST).

6. Overlap of Syndromes of Hepatocellular & Cholestatic Jaundice

Agents may damage canaliculi as well as hepatocytes

a) agents that typically cause hepatocellular disease (e.g., alcohol and hepatitis viruses) sometimes also affect bile canaliculi; b) some drugs that characteristically cause intrahepatic cholestasis (e.g., chlorpromazine) may also damage hepatocytes; c) prolonged cholestasis, especially extrahepatic obstruction, secondarily damages the liver cells, due to the toxic effects of retained bile salts; and d) prolonged cholestasis and hepatocellular disease often progress to cirrhosis and portal hypertension.

Chronic cholestasis damages hepatocytes.

The criteria in Table 3 are not infallible. Serum alkaline phosphatase values less than 3x normal are common in cholestasis due to infection or estrogens. Alkaline phosphatase levels, moreover, may be increased due to bone or kidney disease, or pregnancy; thus, the hepatic origin of the enzyme elevation may need verification by measurement of alkaline phosphatase isoenzymes, or determination of serum 5'-nucleotidase, which is hepato-specific though less sensitive. Serum transaminase and lactic dehydrogenase levels may be markedly elevated in patients with primary or metastatic hepatic cancer, or when cholangitis complicates biliary obstruction.

7. *Distinguishing Intra- and Extra-Hepatic Cholestasis*

All forms of cholestasis produce the same laboratory abnormalities.

Younger age, larger liver, substance abuse, and low serum cholesterol favor intrahepatic cholestasis

Older age, acholic stools, colicky pain, lack of systemic symptoms favor extrahepatic cholestasis

Non-invasive imaging followed by ERCP

a. Routine liver function tests are of no assistance in locating the site of a cholestatic lesion because all forms of cholestasis produce similar clinical, histological, and biochemical abnormalities. A careful history and physical examination, plus routine blood chemistry tests, can appropriately classify cholestatic jaundice in 80% to 90% of patients.

b. Findings that suggest a diagnosis of intrahepatic cholestasis (often associated with hepatocellular disease) include: a) age less than 40 years (viral and drug hepatitis are most common in this age group); b) liver span of more than 15 cm on percussion, especially if the liver is tender (this sign most often indicates alcoholic or nonalcoholic fatty liver disease, or malignancy); c) drug addiction or homosexuality (high risk of transmission of hepatitis); d) history of alcoholism or recent treatment with drugs or hormones, especially estrogens, known to cause jaundice; and e) a decreased serum cholesterol level with an otherwise typical cholestatic syndrome suggests viral or alcoholic hepatitis as the etiology.

c. Findings that favor a diagnosis of extrahepatic obstruction include: a) age greater than 60 years (in males in this age group, the obstruction is usually due to malignancy); b) acholic (pale) stools persisting for more than 2 weeks; c) colicky right upper quadrant pain and/or shaking chills compatible with choledocholithiasis and cholangitis; d) severe jaundice without systemic symptoms (hepatocellular diseases with severe jaundice are almost always associated with systemic symptoms); and e) a palpable gallbladder, which is most often associated with a malignant obstruction of the common bile duct.

d. Other Diagnostic Tests. When classification of cholestasis is uncertain, upper abdominal ultrasonography is advised, followed by computerized tomographic X-rays (CT scan) if the sonogram is unsatisfactory. If intrahepatic cholestasis is suspected clinically and ultrasonography reveals no dilated ducts, liver biopsy is often helpful in establishing the etiology. If extrahepatic obstruction seems most likely clinically and noninvasive imaging reveals dilated ducts, endoscopic ultrasound can be useful in identifying tumor, and ERCP can be used to visualize, and even relieve, the obstructing lesion(s).

N. Formation of Gallstones

"I got stones in my passway And my road seems dark at night
I have pains in my heart They have taken my appetite"
Robert Johnson, 1937

1. Incidence and definition of gallstones

Gallstone disease is very common Approximately 20% of North American men and 30% of women develop gallstones over a lifetime. The incidence is much higher in Native Americans. Gallstones are most common after age 40.

Gallstones consist of a 3-dimensional matrix of mucin and small polypeptides on which crystals form and grow Gallstones (biliary calculi) consist of poorly-soluble components of bile precipitated on a three-dimensional matrix of mucins and proteins. The major precipitates are cholesterol, calcium bilirubinate, and calcium salts of phosphate, carbonate and palmitate. The matrix consists of a network of large, polymeric mucin glycoproteins (the scaffolding) to which are bound small amphipathic, anionic polypeptides (the mortar). Cholesterol is deposited directly on the hydrophobic domains of mucins, whereas the calcium salts are bound to the anionic amino acid sidechains of the polypeptides. Bacteria are readily cultured from the core of almost all brown pigment stones and their RNA can be detected in cholesterol, or in black pigment stones.

2. Regulation of biliary lipid secretion

Bile salt secretion mediates the passage of lecithin and cholesterol into bile The secretion of cholesterol and phospholipids (>90% lecithin) into bile is regulated by the independent secretion of bile salts. Lecithin is translocated to the external leaflet of the canalicular membrane by a phosphatidylcholine flippase (MDR3). Bile salt micelles within the canalicular lumen leach lecithin-cholesterol vesicles from the external leaflet of the canalicular membrane. As bile flows down the biliary tree, these vesicles are gradually converted to mixed micelles, whose composition is more conducive to solubilizing cholesterol.

3. Modification of bile by the gallbladder (Table 4)

Between meals, bile is diverted into the gallbladder. Over a 24-hour period, a normal adult human produces from 400 - 1100 ml of hepatic bile. The sphincter of Oddi holds the common bile duct relatively closed at the ampulla of Vater during the interdigestive period, diverting most of the hepatic bile into the relaxed gallbladder. Within the gallbladder, the hepatic bile is converted into concentrated gallbladder bile, which is stored until ejected into the duodenum upon eating.

Bile is concentrated and acidified in the gallbladder

Cholesterol, lecithin and unconjugated bilirubin are partly reabsorbed in the gallbladder

In the gallbladder, the bile is concentrated up to 10-fold by active mucosal absorption of water and electrolytes (mainly NaCl). Isotonicity with plasma is maintained, however, because the bile salt anions and associated cations (mainly Na⁺ and Ca⁺⁺) aggregate into larger micelles, decreasing their osmotic activity. The bile is also acidified by absorption of sodium ions (Na⁺) in exchange for hydrogen ions (H⁺), while K⁺ and Ca⁺⁺ equilibrate passively with unbound K⁺ and Ca⁺⁺ in plasma. Most of the HCO₃⁻ is lost due to neutralization by H⁺ and diffusion of the CO₂ formed. Some of the phospholipid and an even greater proportion of biliary cholesterol is absorbed, so that the concentration of cholesterol relative to bile salts and phospholipid is lower in gallbladder bile than hepatic bile. A small proportion of the bilirubin glucuronides is hydrolyzed, and some of the unconjugated bilirubin thus formed is absorbed as well.

Table 4. Comparison of Normal Hepatic and Gallbladder Bile

	<u>Characteristic</u>	<u>Hepatic Bile</u>	<u>Gallbladder Bile</u>
<i>Bile is concentrated and acidified in the gallbladder</i>	Color	Golden yellow to orange	Dark brown to greenish brown
	Water	95-97%	85-90%
<i>Bile salts are the major organic solute in bile</i>	Bile salts	15-50 mM	150-400 mM
	Phospholipids	5-16 mM	40-130 mM
	Cholesterol	2-5 mM	10-30 mM
	Bilirubin (total)*	0.3-0.8 mM	1.5-7.0 mM
	Proteins	250 mg/dL	700 mg/dL
	Fatty acids	250 mg/dL	350 mg/dL
		pH	7.0-7.8
<i>Hepatic bile is alkaline, gallbladder bile is acidic</i>	HCO ₃ ⁻	20-30 mM	≤1 mM
	Ca ⁺⁺ /Ca _{Total}	0.8-1.2 mM/2-3 mM	2-3 mM/10-18 mM
	Osmolality	Similar to plasma	Similar to plasma

**Important:* Less than 2% of bilirubin in normal bile is in unconjugated form.

During eating, CCK release mediates emptying of gallbladder bile into the duodenum

Enterohepatic cycling (EHC) of bile salts enhanced during meals

Supersaturation necessary but not sufficient for gallstones to form

Inhibitors versus promoters of precipitation

Sludge

Growth on a matrix

Bile stasis, and the kinetics of stone formation

4. Emptying of gallbladder bile into the duodenum

Gallbladder bile is emptied into the duodenum during eating by a coordinated contraction of the gallbladder and relaxation of the sphincter of Oddi. This is mediated by the release of CCK (cholecystokinin) from the mucosa of the proximal small intestine, stimulated by free fatty acids and aromatic amino acids liberated during intraluminal digestion. The action of CCK on the gallbladder is enhanced by increased vagal activity during eating.

In the duodenum, the concentrated mixed bile salt-lipid micelles aid in fat digestion and inhibit the further release of CCK. The gallbladder then relaxes, sphincter tone returns and hepatic bile again flows into the gallbladder. CCK also promotes intestinal motility and thus the passage of bile salts to their major site of reabsorption in the distal ileum. CCK thus enhances the entero-hepatic circulation of bile salts during eating.

5. Requirements and stages of gallstone formation

Gallstone formation requires a) supersaturation, b) an excess of promoters over inhibitors of crystallization, c) a matrix template on which crystallization can occur (biomineralization), and d) bile stasis (retention in the gallbladder to allow time to grow).

a. Supersaturation. Gallstones can form only when bile becomes supersaturated with one or more of the components mentioned above because i) the bile secreted by the hepatocyte is already supersaturated with one of its components, and/or ii) extrahepatic bile ducts and gallbladder alter the composition and/or pH of the bile.

b. Precipitation. Supersaturated bile is necessary, but not sufficient for precipitation and/or gallstone formation to occur. Bile contains proteins and glycoproteins, some of which stabilize, and some of which promote precipitation of the supersaturated component(s). The balance between these inhibitors and promoters, as well as the degree of supersaturation, determines whether the supersaturated component(s) will precipitate. The excess of the supersaturated component may precipitate as crystals, which may aggregate loosely with mucins to form sludge; sludge particles are small and soft and normally are easily emptied from the gallbladder when it contracts.

c. Growth. If suitable matrix is present, the minerals crystallize on the matrix template in an orderly fashion, forming a miniature calculus or nidus. If the nidus is retained in the gallbladder, progressive accretion of matrix and the insoluble mineral can occur, with growth of the nidus into a gallstone. Stasis occurs when emptying of the gallbladder is impaired, or bile flow in the ducts is impeded by partial obstruction.

There are three major types of gallstones

6. Types of Gallstones

There are three major types of gallstones (Table 5): cholesterol stones, black pigment stones, and brown pigment stones, that differ in composition, structure, and pathogenesis. Cholesterol stones with a significant proportion of pigment are often called "mixed" stones. About 80% of human gallstones in the Western countries are of the cholesterol or mixed type. Although the majority of stones in Asian countries are pigment gallstones, cholesterol stones have become predominant among Asians who have switched to a more Western-style diet.

Table 5. Three Major Types of Gallstones

<u>Characteristic</u>	<u>Cholesterol</u>	<u>Black Pigment</u>	<u>Brown Pigment</u>
Color	Yellow-white with pigmented center ± rings. ± black or white shell	Black to dark brown	Yellow-brown to orange
Surface	Hard and shiny	Shiny or dull	Soft, greasy, laminated
Shape	Round or faceted	Faceted or spiky	Ovoid or irregular
Number	Single or multiple	Multiple, numerous	Single or multiple
Location	Usually gallbladder	Usually gallbladder	Usually bile ducts
Composition*	45-98% cholesterol + 2-20% Ca bilirubinates ± shell of pigment or CaCO ₃	10-50% black pigment polymer + calcium bilirubinates and phosphates ± CaCO ₃	Ca bilirubinates + polymer, 10-60%; Ca palmitate 5-20%; up to 45% cholesterol
Etiology	Increased cholesterol and/or decreased bile salt secretion into bile	Increased excretion of bilirubin and/or calcium into bile; increased bile pH	Bacterial infection, with hydrolysis of bilirubin conjugates and lecithins
Disease Associations	Multiparity, diabetes, obesity, octreotide Rx, oral contraceptive use, cholestasis of pregnancy, prolonged fasting or rapid weight loss, vagotomy	Chronic hemolysis (hemoglobinopathies, artificial heart valves), cirrhosis, Crohn's disease of ileum	Biliary strictures, primary sclerosing cholangitis, intrabiliary parasites and sutures, prior biliary surgery
Other Factors	Female gender, Native Americans, high fat diets, relatives with gallstones	Total parental nutrition, older age	Low protein diets, chronic intrahepatic cholangitis, Asians

*Note: Unmeasured residue (matrix) up to 50% in cholesterol stones, up to 75% in pigment stones

7. Cholesterol Gallstones

a. Bile supersaturation with cholesterol

Cholesterol in bile is solubilized by lecithin ± bile salts (vesicles and mixed micelles)

Cholesterol monomers are essentially insoluble in an aqueous solution. In normal bile, cholesterol is transported either complexed with lecithins in vesicles, or aggregated with phospholipids and bile acids as mixed micelles. Small, unilamellar vesicles with a relatively low proportion of cholesterol are thermodynamically stable. As more cholesterol is added,

Normal bile is supersaturated with cholesterol, but more markedly so in patients with cholesterol gallstones

the small unilamellar vesicles aggregate and fuse to yield less stable, large, multilamellar vesicles, from which cholesterol is more prone to precipitate. The total concentrations and relative proportions of cholesterol to bile salts + lecithin determine if cholesterol can be kept solubilized. Preferential transfer of lecithin from vesicles to mixed micelles increases vesicular supersaturation as the bile flows to the duodenum. Thus, bile is normally mildly supersaturated with cholesterol.

b. Causes of increased supersaturation of bile with cholesterol.

One or more factors may play a role in an individual patient.

i. Hepatic factors:

Increased hepatic cholesterol synthesis and secretion is most common mechanism

The liver can produce more saturated bile in two ways: a) by a relative decrease in synthesis and secretion of bile salts and/or phospholipids; and b) by a relative increase in synthesis and secretion of cholesterol. Impaired bile salt secretion is important in subjects with a history or family history of recurrent cholestasis of pregnancy. The second mechanism is more common, however, especially among Native Americans, diabetics and obese subjects.

Genetic factors

The composition of hepatic bile is strongly influenced by genetic factors, which may explain: a) the extremely high incidence of cholesterol gallstones (more than 50%) in certain ethnic groups (Native Americans) and absence in others (Masai of Africa); b) the well-established familial tendency to cholesterol gallstone formation; and c) the strong predominance of cholesterol gallstones in females. Genes have been identified in inbred mice that influence the lipid composition of bile, often by disturbing the feedback mechanisms that control hepatic bile acid and cholesterol synthesis.

Female preponderance

ii. Intestinal factors:

Decreased EHC of bile salts sometimes plays a role but more often leads to formation of black pigment gallstones

It has previously been mentioned that, when the enterohepatic recycling of bile salts is decreased, there is a limited capacity for the hepatic synthesis to compensate for the increased loss of bile salts. Thus, when the small intestinal reabsorption of bile salts is sufficiently (>25%) decreased, total hepatic secretion of bile salts declines and cholesterol synthesis is enhanced due to diminished inhibition by recycled bile salts. Since this enhanced cholesterol synthesis may be overbalanced by the increased consumption of hepatic cholesterol for the enhanced bile acid synthesis, supersaturation of bile with cholesterol may not develop. This scenario, which occurs with a) resections or mucosal disease of the distal ileum, or b) intraluminal sequestration of bile salts, actually more often results in formation of black pigment gallstones, rather than cholesterol gallstones (for unknown reasons).

Stasis, and altered absorptive function of the gallbladder

Laparoscopic cholecystectomy is the preferred treatment

Bilirubin conjugates and their calcium salts are soluble

UCB and its calcium salts are poorly soluble

Increase in calcium and/or bilirubinate ions can lead to pigment stone formation

iii. Cholecystic factors:

Stasis of bile in a gallbladder occurs in pregnancy, and with prolonged fasting (as when patients are fed parenterally). In the chronically infected gallbladder, enhanced deconjugation of bile salts plus increased mucosal permeability may selectively increase absorption of bile salts from gallbladder bile, increasing cholesterol supersaturation. On the other hand, the diseased gallbladder usually exhibits impaired mucosal absorption of water and electrolytes, and the consequent decrease in biliary lipid concentrations can decrease cholesterol supersaturation.

c. **Therapy of cholesterol gallstones**

Surgical removal of the gallbladder by laparoscopy or laparotomy is the definitive therapy for cholelithiasis. For patients at high risk for surgery, non-surgical alternatives exist. Oral administration of ursodeoxycholic acid enhances hepatic secretion of bile salts, reduces hepatic synthesis and intestinal absorption of cholesterol, and diminishes supersaturation of bile with cholesterol. In the 40% of patients in whom this treatment renders the bile undersaturated with cholesterol, the cholesterol gallstones will gradually dissolve. If the gallbladder is not surgically removed, however, the gallstones recur in over 50% of the patients within 5 years, especially those who initially had multiple gallstones. Long-term prophylaxis with ursodeoxycholic acid, to keep bile saturation low, and with aspirin, to limit mucin secretion, may help prevent such recurrences.

8. **Pigment Gallstones**

a. **Solubility of bilirubins and their calcium salts**

Normally, over 98% of the bilirubin in hepatic and gallbladder bile is in the conjugated form (>80% diglucuronide), which is water-soluble; its calcium salts are soluble also. The <2% of unconjugated bilirubin (UCB), formed by hydrolysis of the secreted conjugates by biliary β -glucuronidases, is, by contrast very poorly soluble in water. The calcium salts of UCB are also extremely insoluble, and may precipitate if the product of the concentrations of *unbound* calcium and UCB anions exceeds the solubility product of Ca-UCB. Normally the unbound concentrations of calcium and UCB in bile are kept low by binding to bile salts, albumin, mucins and anionic polypeptides.

b. **Factors leading to precipitation of calcium bilirubinates in bile**

Precipitation of pigments in bile and formation of pigment gallstones, can occur if there are increases in concentrations of *unbound* calcium and/or bilirubinate ions in bile. Increased ionized calcium in bile occurs mainly due to equilibration with increased plasma calcium, as in hyperparathyroidism. An increase in unbound bilirubinate anions in bile may result from an increase in pH, an increase in total UCB in bile, and/or decreased concentrations of bile salts available to solubilize UCB.

\uparrow plasma Ca^{2+}
leads to \uparrow bile
 Ca^{2+}

*Hemolysis
increases bile
bilirubin*

*Increased
hydrolysis of
bilirubin
conjugates due to
increased activity
of β -
glucuronidase*

*Polymerization of
pigment*

i) Increased ionized calcium in bile can occur only through an increase in unbound, ionized calcium in plasma (e.g. hyperparathyroidism) (free Ca^{2+} ions diffuse passively across the gallbladder and biliary tree)

ii) Increased total UCB in bile may be caused by:

- Increased secretion of bilirubin conjugates into bile with normal rates of hydrolysis to UCB, as in patients with chronic hemolysis (e.g., in sickle-cell disease and cirrhosis).
- Increased β -glucuronidase activity in bile, either released by damaged hepatocytes or cholangiocytes, or by bacteria (e.g., *E. coli*) in infected bile. In infected bile, hydrolysis of lecithins by bacterial phospholipases also occurs and the released fatty acids (mainly palmitate) precipitate as calcium soaps, giving brown stones their characteristic soft, greasy consistency.
- The increased proportion of bilirubin monoglucuronide in the bile in Gilbert's syndrome enhances the hydrolytic formation of UCB, as one less glucuronide group is removed than for diglucuronide.

Finally, by mechanisms likely initiated by oxidation of precipitated calcium bilirubinates, a 3-dimensional network polymer of pigments and matrix may be formed. The mixture of varicolored oxidative derivatives of UCB in the polymer gives the polymer its black or brown color.

O. Other Diseases of the Gallbladder and Bile Ducts

*90% of
cholecystitis is
associated with
cholelithiasis*

*Chronic
cholecystitis is
most common*

*The "non-
functioning"
gallbladder*

1. Cholecystitis

The most common disease of the gallbladder is cholecystitis - both acute and/or chronic forms. Over 90% of patients with cholecystitis have stones in the gallbladder (cholelithiasis).

a. Chronic cholecystitis.

One or more gallstones are usually present within the gallbladder lumen. The gallbladder wall is usually thickened and grey-white, due to fibrosis of the serosa and the muscular layers; the muscle fibers may be separated by collagenous connective tissue. There is usually proliferation and penetration of mucosal pouches from the luminal surface deep into the lamina propria. These Rokitansky-Aschoff sinuses, plus infiltration of inflammatory cells and loose connective tissue, thicken the mucosal layer of the gallbladder wall. Microscopically, the lamina propria and muscularis are infiltrated with scattered lymphocytes and plasma cells and occasional macrophages as well, compatible with the long-term nature of the inflammatory reaction. Scattered inflammatory cells may be present.

The fibrosis of the gallbladder wall and disruption of the muscular layer leads to decreased functional capacity of the gallbladder. The fasting volume may be reduced and the contractile response that empties the gallbladder in response to CCK is impaired. In addition, the ability of the epithelium to concentrate the bile by absorption of water and electrolytes, as well as the ability to acidify the bile, may be impaired.

Acute cholecystitis may supervene if gallbladder occluded

Multiple factors may initiate acute inflammation in the occluded gallbladder

Perforation of the gallbladder, with bile peritonitis, is the feared complication

Dilation and bile stasis proximal to bile duct obstruction

b. Acute cholecystitis

Acute inflammation of the gallbladder usually, but not always, is superimposed upon a chronic cholecystitis. Over 90% of cases involve impaction of a gallstone in the neck of the gallbladder, obstructing the cystic duct. Positive bacterial cultures of the gallbladder wall or its bile are obtained in about 75% of the cases of acute cholecystitis, although bacterial infection is felt to be a secondary event. The factors currently speculated to cause the acute inflammation of the wall of the occluded gallbladder are: a) chemical irritation caused by concentrated free bile acids remaining as water, salts, cholesterol, lecithin and pigments are preferentially absorbed from the stagnant bile; b) infection of the stagnant bile with bacteria that release endotoxins and hydrolyze lecithins to toxic lysolecithins, both of which stimulate release of inflammatory cytokines in the gallbladder mucosa; c) some cases of acute cholecystitis without stones in the gallbladder are believed to result from reflux of pancreatic juice into the biliary tree due to obstruction of the common channel at the ampulla of Vater.

In acute cholecystitis, the gallbladder is usually tense, distended and erythematous, often with splotchy, dark brown discolorations (from intramural hemorrhage). The serosal surface is dull, often covered with fibrin, and dilated vessels may be readily apparent. The bile in the lumen often contains pus as well as blood. Histologically, the typical acute inflammatory reaction is observed, with edema, polymorphonuclear leukocytic infiltration, vascular dilation; frank abscess formation and necrosis are not uncommon. If the acute inflammation subsides, the inflammatory reaction reverts to the chronic phase. Occasionally, the acutely inflamed gallbladder perforates and a localized subhepatic abscess, or localized peritonitis, develops. Sometimes, the perforation is into an adjacent loop of bowel, forming a fistulous tract. Non-steroidal anti-inflammatory agents often relieve the pain and cause the inflammation to subside. If, however, peritoneal signs, pain and fever worsen, urgent removal or drainage of the gallbladder is indicated to prevent perforation.

2. Bile Duct Obstruction

Obstruction of the extrahepatic and larger intrahepatic bile ducts (by stone, tumor or stricture) engenders bile stasis and progressive dilation of the branches of the biliary tree cephalad (proximal) to the block. The intralobular bile ducts in the portal triads dilate and reduplicate. If persistent, the reaction of adjacent tissue to retained bile salts eventually leads to a biliary (portal) cirrhosis. In the liver lobule, the bile canaliculi dilate, show inspissation of dehydrated bile, and may extravasate bile, causing localized bile necrosis of small groups of periportal liver cells ("bile infarcts"). Bile pigments accumulate prominently in hepatocytes as they become unable to secrete bilirubin conjugates into the canalicular bile.

Ischemia may cause strictures of ducts

Fibrous strictures of the common bile duct may result from surgical procedures in or near the hilum of the liver. This is thought to be due to chronic ischemia, resulting from severing the small arteries supplying the duct. The complex and fragile arrangement of its arterial supply, renders the common bile duct particularly vulnerable to this event.

Biliary obstruction predisposes to bacterial cholangitis

3. Cholangitis

Bacterial sepsis in bile commonly develops in the stagnant bile behind persistent, benign (less often malignant) obstructions of the large, extrahepatic bile ducts, especially of the common bile duct. Bacteria penetrate the adjacent bile duct walls and may ascend the blocked duct lumen and lymphatics to cause multiple peribiliary abscesses in the liver, and sometimes bacteremia. Such patients usually have a very toxic illness with fever, chills, and leukocytosis. This is an ominous situation leading to a very high mortality if the infected obstructed bile is not promptly drained endoscopically, (ERCP), radiologically, or surgically.

Acute cholangitis requires urgent treatment

P. Diseases of Hepatic Cell Injury

Massive hepatic necrosis (unusual)

As a group, these disorders are characterized by diffuse or multifocal hepatocyte damage with accompanying inflammatory reaction. They vary in degree from an injury so mild that the illness is subclinical, to massive lysis of liver cells (fulminant hepatic necrosis) with gross shrinkage of the liver. When the liver cell injury is so massive and severe that significant cellular regeneration cannot occur in time, the condition is rapidly fatal. The regenerative efficiency of the hepatocytes is so miraculous, however, that most of these conditions heal with limited residual damage, albeit with some distortion of lobular architecture.

Remarkable capacity of liver to regenerate

Spectrum of sequelae after hepatocyte injury

Between these two extremes is a spectrum of sequelae having varying degrees of a) imperfect hepatocyte restoration; b) continuing hepatic cell necrosis with reactive smoldering inflammation; and c) fibrous scarring. If these processes are sufficient and/or prolonged, the lobular architecture and vasculature become distorted, producing cirrhosis. The time schedule for the development of cirrhosis is widely variable.

Cirrhosis may develop

1. Viral Hepatitis (see section U.1 for further details)

Multiple viruses can cause hepatitis

This group of illnesses has been shown to be due to transmissible agents (viruses) residing in the lower digestive tract, in blood plasma or certain of its fractionated products, or other body secretions. With potent strains of virus, as little as 1/1,000 of a drop of infective plasma may transmit the illness. Multiple varieties exist, each having its characteristic length of incubation period, clinical and laboratory features, illness duration, and degree of severity (See Table 8, Section U).

Most patients only suffer flu-like symptoms; only a small proportion

The most common signs and symptoms, generalized malaise and fever, are usually proportionate to the severity of inflammation and liver cell necrosis. Most patients experience only a "viral-like" syndrome. In more severe cases, anorexia, nausea, and even vomiting may be seen. Jaundice,

become jaundiced

due to impairment of hepatic bilirubin transport, occurs in less than 10% of cases, and often develops as the flu-like symptoms subside. Palpable hepatomegaly may result from liver swelling secondary to inflammation and edema. Rapid stretching of the liver's capsule (Glisson's capsule) may yield tenderness to pressure on the liver and pain in the epigastric and right upper quadrant regions. In a limited number of more severe cases, the signs of acute hepatic failure develop.

High transaminases are principal lab abnormality

Laboratory detection of an acute diffuse hepatic injury is concerned chiefly with identifying a) interference with excretory efficiency of hepatic substances (e.g., bilirubin); b) exaggerated "leak" of intracellular hepatic enzymes into plasma (e.g., AST, ALT); and c) failure of hepatocytes to synthesize plasma proteins of short lifespan (especially clotting factors of the prothrombin complex).

2. Drug-induced Hepatitis and Cholestasis

a. Role of the liver in drug metabolism (Review Figure 10).

The liver is designed to detoxify lipophilic, protein-bound xenobiotics

The liver is the major organ for metabolism and excretion of drugs and toxins that are introduced into the body, especially the lipophilic xenobiotics that are carried in plasma bound to proteins and thus cannot be excreted by the kidney. In what is usually a two-step process, the liver converts most drugs to more polar products that are then available for excretion in bile and/or, due to weaker binding to plasma protein, in the urine. These processes involve a variety of cytosolic and membrane-bound enzymes; the latter are found mainly in the smooth endoplasmic reticulum. Each agent has its characteristic metabolic pathway(s) involving one or more of these enzyme systems.

b. Factors that influence drug metabolism

Heredity, age, nutrition, other drugs and liver disease affect drug metabolism

- i. Genetic polymorphisms in drug metabolizing enzyme activities.
- ii. Age: infants may show considerable immaturity in the activity of drug metabolizing enzymes. Activity declines in the elderly.
- iii. Nutrition: severe malnutrition may deplete the supply of detoxification (Stage II) cofactors, such as glutathione.
- iv. Other drugs: may either inhibit or enhance (induce) metabolism of another drug (see next section). Alcohol can do both, accounting for the adage, "alcohol and drugs do not mix." (See **d**, below)
- v. Underlying liver disease: In both acute and chronic liver disease, the impaired Phase I metabolism may prolong the disposal rate of many drugs, causing excessive therapeutic or toxic effects. In cirrhosis, orally-administered drugs often bypass the liver in porto-systemic shunts, leading to excessive plasma levels.

c. Inhibition and induction of drug metabolism

Compounds (including hormones, e.g., estrogens) that share common binding sites on transporters or enzymes may competitively inhibit

Drugs may inhibit and/or induce the monooxygenase cytochromes in the S.E.R.

Stage I reactions may produce toxic derivatives; stage II (conjugation) reactions detoxify.

Overload or impairment of conjugation can lead to toxicity.

Drug resistance due to ABC proteins that export xenobiotics from cells.

Two classes and five histological types of hepatic drug reactions

Ductules, canaliculi or hepatocytes may be primarily affected

Mixed syndromes may occur in an individual patient

each others' transport or metabolism. The inhibition may affect binding to plasma proteins, hepatocyte uptake, binding to cytosolic storage proteins, oxidation, conjugation, or biliary secretion.

Many toxins, drugs and hormones are inducers, at the transcriptional level, of the expression of drug-metabolizing enzymes, especially the cytochrome P450 and P448 monooxygenases of the smooth endoplasmic reticulum (S.E.R.).

d. Biotransformation reactions influence drug toxicity

Stage I (mainly oxidative) reactions may convert some drugs (e.g., acetaminophen) into very toxic metabolites. By contrast, the conjugated products of stage II reactions are usually too polar to diffuse across cell membranes, rendering them inactive (unless they affect enzymes bound to plasma membranes) and unabsorbable from the intestine. The supply of cofactors regulates the phase II conjugation of the toxic metabolite. When the formation of toxic metabolites overloads the capacity of the conjugating system to inactivate them, toxicity occurs. This explains why many drugs, that are not toxic in therapeutic doses, become toxic when higher doses are ingested or conjugation is impaired by depletion of cofactors.

e. ABC transporters export drugs from cells

Most cells, including the hepatocyte, have transporters that span the plasma membranes and export xenobiotics into the plasma, bile or urine, protecting cells from excessive accumulation of toxic compounds. These ABC transporters possess ATP-Binding Cassette sequences that mediate the hydrolysis of ATP, providing the energy for the export processes. These transporters are often up-regulated by the xenobiotics that they export, enhancing protection against toxicity but also diminishing therapeutic efficacy of the drug.

f. Classification of hepatic drug reactions.

Injury to hepatocytes may be classified into two major types according to the mechanism of injury: i) direct chemical reactions; and ii) drug hypersensitivity reactions. In some cases (i.e. chlorpromazine), both types of mechanisms are involved.

Individual drugs tend to produce a characteristic histological pattern of hepato-biliary damage, usually classified into one of five clinico-pathologic syndromes: a) cholestasis (e.g. estrogens, anabolic steroids); b) cholestasis with reactive inflammation (e.g., erythromycin esters, chlorpromazine); c) generalized hepatitis with liver cell necrosis of varying degrees, simulating viral hepatitis or massive acute hepatocellular necrosis (e.g., halothane, isoniazid); d) chronic active hepatitis (e.g., alpha-methyl dopa, oxyphenisatin); and e) tumors (vinyl chloride, birth control pills, and androgens). A given patient may, however, show features of several syndromes.

Direct toxic injury is predictable and dose-dependent

Cell damage due to covalent binding of toxic compound to proteins or DNA

Acetaminophen toxicity is due to its phase I metabolite, and is enhanced by chronic alcohol consumption.

*Drugs that primarily cause hepatocellular injury
Damage to canaliculi and/or ductules.*

Impaired net biliary secretion of various components of bile

Dislocation of ABC transporters from canalicular membrane

i. Direct toxic injury (chemical reactions - predictable)

Agents that are directly hepatotoxic in humans are usually injurious to hepatocytes of most mammalian species. The latent period between the exposure and onset of the reaction is brief and fairly uniform. The severity of the injury is roughly proportional to the dose, and occurs predictably in most individuals exposed to sufficient doses.

Although the damage may predominantly affect hepatocytes or cholangiocytes, some of these toxic compounds may also damage other organs (especially the kidneys). In each instance, the compound itself, or one of its stage I metabolites, interacts (usually rapidly) with one or more intracellular constituents (e.g., proteins, DNA) impairing enzyme function or cell proliferation, respectively, often resulting in cell death. The details of this sequence have been elucidated for only a few drugs, however (most notably isoniazid, acetaminophen and chlorpromazine).

Since toxicity is determined by the concentration of the reactive metabolite, the susceptibility of individuals to the drug is determined by factors that affect the balance between rates of formation of the metabolite (Stage I) and its detoxification (conjugation, Stage II). These factors were discussed in sections b, c and d, above. For example, chronic alcohol consumption increases the toxicity of acetaminophen by two mechanisms: up-regulation of CYP2E1 (the cytochrome P450's that forms the toxic intermediate) and depletion of glutathione, the cofactor for conjugation of the toxic intermediate.

Common examples of agents that primarily cause hepatocellular injury are ethanol (discussed separately in section 3), isoniazid, acetaminophen, carbon tetrachloride, chloroform, phosphorus, azathioprine, 6-mercaptopurine, and mushroom (*Amanita*) toxins.

Agents that cause cholestasis primarily affect canalicular transporters or damage the ductules, leading to obstruction and/or increased permeability of the ductules. In either case, net biliary secretion is impaired for bile salts, cholesterol, phospholipids, bile pigments, porphyrins, and exogenous agents (such as biliary contrast agents and drugs). The pattern of impaired secretion is determined mainly by which ATP-dependent, canalicular transporters are affected, most often BSEP (the bile salt export pump) and/or MRP2 (the multispecific organic anion transporter). As with other forms of cholestasis, the mechanism is dislocation of the transporters from the canalicular membrane to subapical vesicles.

Little inflammation or gross liver cell degeneration occurs, and the retained cholephiles accumulate in the hepatocyte, rather than forming "bile plugs" in the canalicular lumen. All effects are

Injury is reversible

usually reversible after discontinuing the inciting agent, although jaundice may take weeks to months to resolve.

Drugs primarily causing cholestasis

Common examples of drugs producing primarily canalicular cholestasis are: ethinyl estradiol, anabolic steroids (17 α -alkyl androgens), and the immunosuppressant, cyclosporin. Ductular damage is less common, and often caused by tropical plant toxins. As with direct hepatocellular toxicity, the degree of cholestasis is roughly dose-related, may be due to a toxic intermediate formed in phase I reactions, and the pattern tends to be characteristic of each drug. Although all humans are susceptible, individuals vary widely in their manifestations of these effects, because of the same factors that affect hepatocellular toxicity.

Injury is dose-related with individual variation

Hypersensitivity is unpredictable and independent of drug dose

ii. Drug hypersensitivity (immunogenic) reactions (unpredictable).

The principal characteristic of this group of adverse hepatic reactions is the apparent unpredictability of injury in individual subjects. Hepatic drug reactions of this type are recognized more frequently and the list of drugs implicated is sizable. The reactions are often species-specific and cannot be reproduced experimentally in laboratory mammals. There is no constant relationship between the size of the dose and the occurrence of severity of the drug reaction. The latent period between exposure to the drug and the sensitivity reaction is quite variable (sometimes as long as three or four weeks after the last drug contact), and recovery after discontinuing the drug may sometimes take many months.

Hypersensitivity reactions involve autoimmune reactions to drug-protein adducts.

In hypersensitivity reactions, the cells are damaged by immunogenic adducts of the drug or its metabolites with proteins, causing autoimmune-like damage to the liver and other organs. This accounts for the skin rashes, joint pain and inflammation, fever, and eosinophilic leukocytosis that often accompany the liver damage. Histologically, the liver shows varying degrees of injury to hepatocytes, canaliculi and/or intrahepatic bile ducts.

3. Ethanol and the Liver – Alcoholic Liver Disease

a. Ethanol metabolism

In humans, the liver is the chief site of ethanol metabolism and involves two pathways, both of which convert ethanol to acetaldehyde.

Alcoholic dehydrogenase

i. Alcohol dehydrogenase, a constitutive (non-inducible) enzyme in the cytosol of the hepatocyte and gastric epithelium that uses NAD⁺ as a cofactor.

MEOS, inducible by ethanol

ii. Microsomal ethanol-oxidizing system (MEOS), a specific microsomal cytochrome P450 in the hepatocyte that is induced by ethanol and uses NADP⁺ as a cofactor. MEOS normally

accounts for only 25% of ethanol metabolism, but becomes the predominant enzyme due to its induction with chronic alcohol ingestion. This induction is nonspecific, in that other drug-metabolizing enzymes are also enhanced. This is a major factor in the increased tolerance of the chronic alcoholic to alcohol itself as well as to certain sedative agents and other drugs metabolized by the microsomal cytochromes.

b. Mechanisms of ethanol cytotoxicity

i. Formation of toxic acetaldehyde.

Acetaldehyde is the toxic metabolite of ethanol

The first metabolite of ethanol, acetaldehyde is a major factor in ethanol hepatotoxicity, and its rate of conversion to acetate by the enzyme, aldehyde dehydrogenase, is important in determining the development of alcoholic liver disease. Some of the toxic actions of acetaldehyde are due to its ability to acetylate proteins, both inactivating the proteins and generating immunogenic adducts that trigger autoimmune liver damage.

ii. Effects on lipid and protein metabolism

Synthesis, oxidation and export of lipids is affected

The most dramatic manifestation of alcohol toxicity is the rapid accumulation of fat in the liver, caused by multiple effects of ethanol. The fatty acids that accumulate are derived mainly from the diet.

Mitochondrial damage is prominent

- Increase in acetate and ratio of NADPH/NADP⁺, secondary to ethanol oxidation, resulting in increased fatty acid synthesis.
- Mitochondrial damage, resulting in striking ultrastructural changes including swelling and distorted cristae, with an associated decrease in fatty acid oxidation.
- Impaired export of lipoproteins from hepatocytes due to impaired apoprotein synthesis and damage to the cytoskeletal elements involved in transcellular movement of lipids. The damage to cyokeratin also results in formation of the characteristic alcoholic hyaline (Mallory bodies) in liver cells.

c. Stages of alcoholic liver disease and modulating factors (Fig. 19)

Alcoholic liver disease: rising incidence and a multistage process

The incidence of alcohol-related liver disease continues to increase and is paralleled by the greater consumption of ethanol. It has been estimated that in some large urban centers, cirrhosis of the liver is at least the third most frequent cause of death between ages 25 and 65, with alcoholic and viral liver diseases as the major causes.

In humans, the excessive use of ethanol commonly results in a) fatty liver, b) alcoholic hepatitis, and/or c) fine nodular cirrhosis.

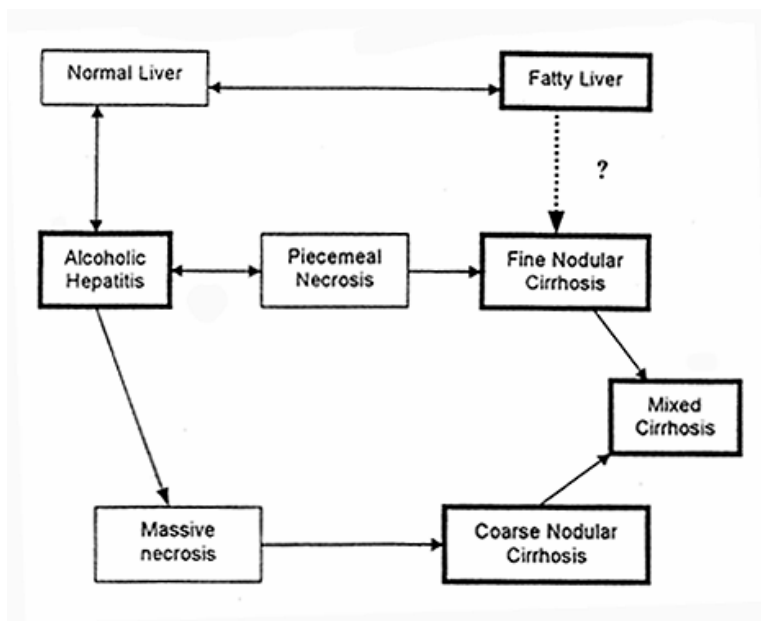


Figure 19. The Spectrum of Alcoholic Liver Disease

Fatty liver and alcoholic hepatitis may be reversible; cirrhosis is usually not reversible.

Injury worse in women than in men.

Role of malnutrition is unclear

Release of cytokines by endotoxin-activated Kupffer cells

Ethanol dose x time determines incidence of cirrhosis

The exact interrelationships among these pathological states are not clear, but they appear to represent progressive stages in the development of long-term alcoholic liver disease. Fatty liver and hepatitis are reversible with abstinence, cirrhosis less so. Although fatty liver invariably develops with excessive ethanol ingestion, alcoholic hepatitis and cirrhosis occur only in about 20% of even heavy drinkers.

The mechanisms related to the transition between these three entities and the reasons for the differences in individual susceptibility are incompletely understood. Genetic, constitutional, and dietary factors are believed to play a role. Women are more sensitive to alcohol-induced liver injury than are men. Although the association of malnutrition with chronic alcoholism is well recognized, the extent to which this malnutrition contributes to the development of alcoholic liver disease remains unclear. Experimental evidence, both in man and the baboon, demonstrates that severe liver injury can be produced by prolonged alcohol ingestion in the absence of dietary deficiencies.

Chronic alcohol ingestion causes enhanced absorption of endotoxin from the gut. This in turn activates Kupffer cells to release pro-inflammatory cytokines (e.g. TNF- α and interleukin-1 β), which may cause the leukocyte infiltration and cell necrosis that characterize the progression of hepatic steatosis to alcoholic hepatitis.

Susceptibility to alcoholic liver disease is largely determined by the amount, duration and constancy of ethanol consumption, apparently independent of the type of alcoholic beverage consumed. Above a certain threshold intake, the incidence of cirrhosis is linearly related to daily dose x time. The fat content of the liver can increase 3-fold with a

single overnight binge. More prolonged ethanol ingestion can produce clinically-significant fatty liver within several weeks. Acute alcoholic hepatitis may require several months, and well-established fine nodular cirrhosis may require more than ten years of daily consumption of 300 gm of ethanol.

d. Spectrum of alcoholic liver disease (Fig. 19)

i. Alcoholic fatty liver (steatosis).

Stages of steatosis (fatty liver)

Fat accumulates in the hepatocytes first as tiny droplets, which coalesce progressively until the entire cell may consist mostly of a single large vesicle of stored triglyceride with the nucleus and residual cytoplasm pushed to the cell margins; these cells microscopically resemble an adipose cell (macrovesicular fat). Such swollen cells may compress the sinusoids, causing reversible portal hypertension. Adjacent liver cells may rupture, merging their fat into large fatty cysts enveloped by their contiguous nuclei and other cell fragments.

Fatty liver is mostly reversible with abstinence

As its fat content increases, the liver progressively enlarges, sometimes becoming enormous (3-4 times normal size) and filling most of the abdomen. Except for the fatty cysts, the fatty vacuolization of hepatocytes is potentially reversible. In a given patient, the fat content (and size) of the liver may fluctuate for years without progressing to the more serious forms of alcoholic liver disease, and can disappear completely with abstinence.

ii. Alcoholic hepatitis.

Mallory bodies are characteristic but not diagnostic

Impressive features in alcoholic hepatitis include a) ballooning and death of hepatocytes, b) intracytoplasmic deposits of altered fibrillar cytokeratin ("alcoholic hyaline" or "Mallory bodies"), that are typical of, but not unique to, alcoholic liver disease, c) infiltration of leukocytes (PMNs), and d) reactive proliferation of fibrous tissue. Varying degrees of fatty vacuolization of the hepatocyte occur also.

Damage is mainly centrilobular (acinar zone 3)

The accumulation of fat and destruction of liver cells are spotty, but occur initially and most severely in perivenous zone 3 (centrilobular). The Mallory bodies stain intensely with eosin, but differ from the round eosinophilic bodies seen in acute viral hepatitis. They are frequently perinuclear, and appear first as tiny droplets that later coalesce into ropy strands and amorphous clumps.

Polymorphs are the characteristic inflammatory cells

Inflammatory cells both infiltrate the portal triads and collect within the lobule around the debris of dying hepatocytes. In contrast to acute viral hepatitis, the invading leukocytes are characteristically polymorphonuclear, but some lymphocytes and macrophages may appear also. As the acute injury subsides, the necrosis and Mallory bodies disappear, but an infiltrate of lymphocytes lingers in the portal triads.

Fibrosis is due to condensation of reticulin plus new collagen synthesis by Stellate Cells

iii. Fibrosis and cirrhosis; the role of stellate cells

As small groups of hepatocytes die off, there is a local condensation of their supporting reticulin fibers, plus synthesis of new collagen by activated, proliferating Stellate Cells in the space of Disse. The new collagen fibrils form a barrier to diffusion of large molecules (including plasma albumin and its ligands) to the surface of the hepatocytes ("capillarization of the sinusoids"). Strands of new collagen later insinuate themselves between hepatocytes, and the irregular fibrous fingers gradually isolate groups of liver cells into small nodules without relevance to lobular architecture. The residual hepatocytes attempt to generate new liver cells, forming micronodules.

Cirrhosis is the end stage if high alcohol intake continues

iv) Patterns of cirrhosis

Cirrhosis is the final step in the spectrum of alcoholic liver disease. With continued alcoholism, progression of the foregoing processes eventually leads to diffuse cirrhosis of very small nodular type. Over time, parenchymal remodeling can transform micronodular cirrhosis into macronodular cirrhosis, indistinguishable from cirrhosis due to other causes.

Patterns of necrosis determine patterns of scarring

Occasionally, other patterns of the fibrous scarring are observed: a) septal scars may form, often connecting perivenous (centrilobular) zones of adjacent acini; and b) if extensive, massive necrosis of hepatocytes occurs regionally, the supporting framework collapses and the residual collagenous structures from multiple adjacent lobules condense, resulting in broad scars. Large nodules of residual hepatic tissue may remain between such thick post-necrotic scars. A mixed small and large nodular cirrhosis is the result.

4. Nonalcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease represents a spectrum of liver disease which includes benign steatosis (fatty liver), nonalcoholic steatohepatitis (NASH), isolated portal fibrosis, and cirrhosis with resultant liver failure. It resembles alcohol-induced liver disease, but occurs in the absence of significant alcohol intake. Whether steatohepatitis is alcohol-related or not is often difficult to determine – there is no uniform agreement as to what “significant” alcohol intake is. Most agree, however, that steatohepatitis which occurs in the setting of alcohol consumption of over 20-40 g/day in men and 20 g/day in women constitutes alcoholic steatohepatitis and not NAFLD. NAFLD is defined as fat accumulation in the liver exceeding 5% to 10% by weight. This is generally estimated as the percentage of fat-filled hepatocytes seen by light microscopy. Recently, a grading and staging

system for NAFLD/NASH has been proposed (Table 6).

NAFLD is probably the most common cause of liver disease in many countries, including the United States. Obese patients have a 60% prevalence of Grade 1 NAFLD, a 20% prevalence of grade 3 NAFLD/NASH, and 2-3% have cirrhosis. 75% of type 2 diabetics have some form of fatty liver disease. About 70% of patients with “cryptogenic” hepatitis are now believed to have NAFLD. Gender distribution is equal and even persons of normal body weight develop NAFLD. Certain drugs (i.e., methotrexate, amiodarone, tamoxifen) and surgical procedures (i.e., intestinal bypass for weight loss) have also been associated with NAFLD/NASH. Clinical predictors of more advanced disease remain to be adequately elucidated but have traditionally included female gender, age >40 to 50 years, obesity, diabetes, and dyslipidemia (particularly hypertriglyceridemia).

TABLE 6. Proposed Grading/Staging of NAFLD/NASH

Grade 1, mild	Benign NAFLD (simple steatosis)
<i>Steatosis:</i>	<i>Mainly macrovesicular, ranges from <33% to 66% of lobules</i>
<i>Ballooning:</i>	<i>Occasionally observed; zone 3 hepatocytes</i>
<i>Lobular Inflammation:</i>	<i>Scattered and mild acute (polymorphs) and chronic inflammation (mononuclear cells)</i>
<i>Portal Inflammation:</i>	<i>None or mild</i>
Grade 2, moderate	NAFLD (steatosis with lobular inflammation)
<i>Steatosis:</i>	<i>Any degree, usually mixed macrovesicular and microvesicular</i>
<i>Ballooning:</i>	<i>Present in zone 3</i>
<i>Lobular Inflammation:</i>	<i>Polymorphs may be noted associated with ballooned hepatocytes, and/or pericellular fibrosis; ± mild chronic inflammation</i>
<i>Portal Inflammation:</i>	<i>None; mild to moderate</i>
Grade 3, severe	Florid Steatohepatitis – NASH
<i>Steatosis:</i>	<i>Usually >66% (zone 3 or panacinar); commonly mixed steatosis</i>
<i>Ballooning:</i>	<i>Predominantly zone 3; marked</i>
<i>Lobular Inflammation:</i>	<i>Scattered acute and chronic inflammation; polymorphs may be concentrated in zone 3; areas of perisinusoidal fibrosis</i>
<i>Portal Inflammation:</i>	<i>Mild or moderate; not predominant or marked</i>
Staging:	Stage 1: <i>zone 3 perivenular, perisinusoidal, or pericellular fibrosis, focal or extensive</i>
	Stage 2: <i>same as stage 1, plus focal or extensive portal fibrosis</i>
	Stage 3: <i>bridging fibrosis, focal or extensive</i>
	Stage 4: <i>cirrhosis with or without residual perisinusoidal fibrosis</i>

The pathophysiology of NAFLD/NASH is poorly understood. NAFLD/NASH is often associated with metabolic syndromes (obesity, diabetes, hyperlipidemia, polycystic ovarian disease, peroxisomal diseases, mitochondrialopathies, Wilson’s disease, abetalipoproteinemia), many of which have either abnormal fat

metabolism and/or mitochondrial injury/dysfunction. NAFLD/NASH is also commonly associated with insulin resistance. While these mechanisms probably play a fundamental role in development in NAFLD/NASH, it is not yet known whether they are causal.

NAFLD/NASH may be suspected based upon imaging studies (ultrasound, CT scan) suggesting hepatic steatosis or when liver enzymes elevations are found and there is no other identifiable reason for liver disease. Liver biopsy remains the gold standard for diagnosis and is extremely helpful in defining the stage of disease. Exercise and diet remain the cornerstone of therapy. Avoidance of alcohol may be prudent. Recent randomized controlled studies suggest no benefit to Ursodeoxycholic acid. Use of antioxidants is being evaluated. Medications directed at insulin resistance (i.e., thiazolidinediones) have shown promise and are being evaluated in larger trials.

Lack of longitudinal studies hinders our understanding of the prognosis of NASH. The 5- and 10-year survival is estimated at 67% and 59% respectively. Based on few numbers of patients, it is estimated that the risk of class 3 NAFLD/NASH for developing increased fibrosis over 5 years is 25% and for developing cirrhosis is 15%. Classes 1 and 2 may have a more benign course, although there are reports that these patients are also at risk for progression. There is also a significantly increased risk for the development of hepatocellular carcinoma in the subset of patients who progress to cirrhosis. Many patients with advanced disease are poor liver transplant candidates because of comorbid medical conditions, however, successful transplant can prolong life. As our understanding of the pathophysiology of NAFLD improves, broader treatment options should be available.

Q. Chronic Liver Diseases

The clinical, biochemical, and morphological classification of chronic inflammatory diseases of the liver (continuing hepatitis) is presently undergoing extensive study, thus a clear simple organization is not yet available. The discussion in this section will focus upon the end stage of this spectrum -- cirrhosis of the liver.

*Cirrhosis = fibrosis
+ distortion of
lobular architecture*

*May be progressive
or reversible*

*Etiology correlates
with histology about
two-thirds of the time*

*Clinical features of
cirrhosis are due to
effects of
hepatocellular
insufficiency and
portal hypertension*

1. Cirrhosis of the Liver

Cirrhosis of the liver is defined pathologically as widely distributed, irregular hepatic fibrosis with distortion of the lobular architecture and vasculature, resulting from persistent inflammation and/or parenchymal cell necrosis, combined with nodular regeneration. Cirrhosis always has three basic features: a) destruction of liver cells; b) replacement of groups of lobules, entire lobules, or parts of lobules with fibrous tissue; and c) nodular regeneration of the residual hepatic parenchyma.

Cirrhosis is usually progressive; at a tempo which may be variable and intermittent. In the earlier stages, this is due to persistent or repeated damage to liver cells from the causative agent, but resorption of fibrous tissue and regeneration of hepatocytes may repair the damage and reverse the process if the offending agent is treated or removed.

By contrast, in late stages of cirrhosis, the secondary distortion of the hepatic circulation may lead to chronic ischemia, with increasing fibrosis, continued cell loss and eventually liver failure.

Although the morphological appearance of cirrhosis depends to a large measure upon the inciting agent or event, the various etiologic syndromes of cirrhosis correlate well with morphologic patterns in roughly only two-thirds of cases. In the remainder, the cause of the disorder matches the evolving fibrous-nodular design poorly or not at all, and strikingly different patterns of fibrosis and nodularity may sometimes be seen in different regions of the same liver. The three main morphologic types of cirrhosis are: a) coarse nodular cirrhosis (also known as macronodular or post-necrotic), b) fine nodular cirrhosis (also known as micronodular), and c) mixed cirrhosis.

2. Clinical Consequences of Cirrhosis

Clinically, the features of cirrhosis and chronic end stage liver disease result mainly from hepatocellular insufficiency and portal hypertension.

- a. Diminished hepatocytic synthetic capacity** leads to hypoalbuminemia, deficiency of clotting factors, and (usually) hypocholesterolemia (except with chronic cholestasis).
- b. Impaired estrogen metabolism** causes palmar erythema (red palms), spider angiomas, amenorrhea in females, complicated by alcohol-induced testicular atrophy, and feminization in males.
- c. Impaired detoxification/excretory function**, combined with shunting of blood around the liver, causes jaundice, encephalopathy, and excessive responses to administered drugs.
- d. Altered metabolism of vasoactive substances** leads to splanchnic vasodilatation, activation of the renin-angiotensin-catecholamine system, sodium retention, ascites and edema, and functional renal failure (hepatorenal syndromes).

- e. **Portal hypertension** and the compensatory development of porto-systemic collateral circulation cause esophageal varices (often with GI hemorrhage), splenomegaly with pancytopenia, and ascites (hypoalbuminemia contributes).
- f. **Bacterial overgrowth**, with translocation of bacteria and toxins through the congested bowel wall into the portal system, leads to systemic infections and spontaneous bacterial peritonitis (infected ascites).

3. Prognosis in Chronic Liver Disease

The prognosis of patients with cirrhosis is difficult to predict, but generally depends upon the number and severity of complications, best assessed by the Child-Turcotte-Pugh criteria ("Child's Classification"). Its validity has been well demonstrated and it gives a good indication of prognosis. This classification is composed of clinical and laboratory measures which are available to any physician (Table 7). The score for a "normal" person would be 5. Cirrhotic patients with scores of 5 or 6 (Child's A) have an excellent prognosis (a ten-year survival of 80%-90%). By contrast, those patients with scores greater than 10 (Child's C) have a very poor prognosis and often succumb within 6 to 12 months. Child's B cirrhotics (score 7-10) have an intermediate survival. The most common causes of death from cirrhosis are hepatic failure, gastrointestinal hemorrhage, hepatocellular carcinoma, infections, and renal failure.

Child-Turcotte-Pugh classifications of severity of cirrhosis

Major causes of death from cirrhosis

Table 7. Child-Turcotte-Pugh (CTP) Scoring System to Assess Severity of Liver Disease

	<u>Classification (Score) and Points Scored for Increasing Severity</u>		
Clinical and Biochemical Measurements	Class A (5-6) 1 point each	Class B (7-9) 2 points each	Class C (≥ 10) 3 points each
Encephalopathy	None	Stages 1 and 2	Stages 3 and 4
Ascites	Absent	Slight or controlled by diuretics	At least moderate despite diuretic treatment
Bilirubin, mg/dL (μM)*	<2.0 (<34)	2.0-3.0 (34-51)	>3.0 (>51)
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
Prothrombin time (PT) (secs. prolonged) or (INR)	<4 <1.7	4-6 1.7-2.3	>6 >2.3
Clinical and Biochemical Measurements	Class A (5-6) 1 point each	Class B (7-9) 2 points each	Class C (≥ 10) 3 points each
Bilirubin, mg/dL (μM)	<4 (<68)	4-10 (68-171)	>10 (171)

** For cholestatic liver diseases, e.g. primary biliary cirrhosis, the values for bilirubin below are to be substituted for the values above.*

A major criticism of the CTP scoring system is that items such as encephalopathy and ascites are qualitative, too dependent on history and physical examination.

The Model for End-Stage Liver Disease (MELD) is a numerical scale, ranging from 6 (less ill) to 40 (gravely ill), that is used for adult liver transplant candidates. It gives each individual a “score” (number) based on how urgently he or she needs a liver transplant within the next three months. The number is calculated by a formula using three routine lab test results:

- bilirubin, which measures how effectively the liver excretes bile;
- INR (prothrombin time), which measures the liver’s ability to make blood clotting factors; and
- creatinine, which measures kidney function. (Impaired kidney function is often associated with severe liver disease.)

A patient’s score may go up or down over time depending on the status of his or her liver disease. Many patients will have their MELD score assessed a number of times while they are on the waiting list. This will help ensure that donated livers go to the patients in greatest need at that moment. Candidates under the age of 18 are placed in categories according to the Pediatric End-Stage Liver Disease (PELD) scoring system. PELD is similar to MELD but uses some different criteria to recognize the specific growth and development needs of children. PELD scores may also range higher or lower than the range of MELD scores. The measures used are as follows:

- bilirubin, which measures how effectively the liver excretes bile;
- INR (prothrombin time), which measures the liver’s ability to make blood clotting factors;
- albumin, which measures the liver’s ability to maintain nutrition;
- growth failure; and
- whether the child is less than one year old.

R. Portal Hypertension

1. Pathogenesis of Portal Hypertension (Figure 20)

↑ *Intrahepatic
vascular
resistance* + ↑
splanchnic inflow

Increased pressure in the portal venous system (portal hypertension) results whenever there is: a) an increased resistance to the flow of blood through the liver, and/or b) a large increase in the amount of blood trying to enter the liver from the splanchnic circulation. Both factors play an important role in the setting of cirrhosis, which is the most common cause of portal hypertension in the developed countries. In cirrhosis, intralobular collaterals connecting the high pressure hepatic arterioles to the portal

venules contribute also to the elevated portal pressure.

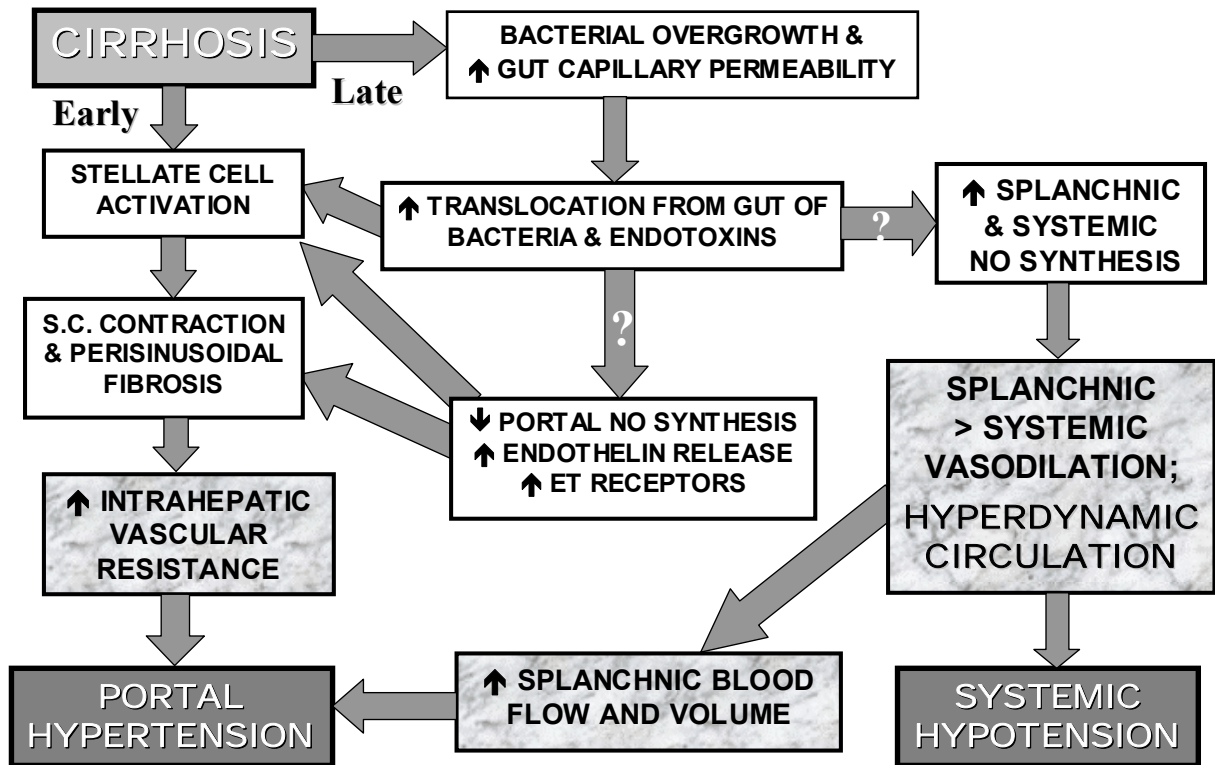


Figure 20. Pathogenesis of Portal Hypertension in Cirrhosis

Flow diagram of the sequence of changes in the portal and splanchnic circulations that lead to the portal hypertension and systemic hypotension in cirrhosis.

The two major pathways leading to portal hypertension are the increased intrahepatic vascular resistance (left) and the increased inflow from the dilated, hyperdynamic splanchnic circulation (right). In addition, the responsiveness of the splanchnic and portal vessels to vasoconstrictive agents is abnormally diminished in portal hypertension (not shown)

a. Portal blood flow and pressure, measurement & regulation

Portal blood flow is normally 1.0-1.2 L/min. Pressures within the portal venous system can be measured by several different methods: a) wedging a catheter into an hepatic vein via the jugular or femoral veins; b) catheterizing a left intrahepatic branch of the portal vein via the umbilical vein; c) transhepatic portal vein catheterization via a thin needle; or d) inserting a needle into the splenic pulp. The most commonly used method is the hepatic wedge technique. The difference between the "wedged" hepatic (= sinusoidal) pressure (WHVP), and inferior vena caval (IVC) pressure is the portal (sinusoidal) venous pressure gradient. Normal portal venous pressure gradients are roughly 4-9 mm Hg, slightly above resting inferior vena caval pressure.

Normal portal pressure gradient = 4-9 mm Hg

Gradients in cirrhosis are >10 mm Hg

Gradients of 10-15 mm Hg or higher are seen in cirrhosis; if > 12 mm Hg, they are usually associated with the development of dilated porto-systemic collateral veins (such as esophageal varices), through which portal blood flow is shunted around the liver.

Large increase in portal pressure and flow after eating in patients with cirrhosis

The system is quite dynamic so there are fluctuations of both pressure and flow with changes in respiration, temperature, body position, straining at stool, coughing, and especially eating. During and for several hours after a meal, portal flow increases by 60 –130%. Normally, this increased blood flow is compensated by vasodilation of the portal veins and a decrease in hepatic arterial flow, so that portal pressure rises at most 1-2 mm Hg. In cirrhosis, the rigid hepatic venous vasculature cannot dilate, and portal pressure may rise by 5-6 mm Hg after eating.

Three sites of increased resistance to portal blood flow.

b. Causes of increased resistance to portal blood flow

The anatomic location and etiology of the increased resistance to hepatic blood flow determines the clinical problems that result and their prognoses. The anatomic types are labeled presinusoidal, intrasinusoidal, and postsinusoidal, according to the normal direction of blood flow through the liver.

Portal venous obstruction (presinusoidal)

i) Increased presinusoidal resistance is usually secondary to blockage of the main portal vein or its downstream tributaries. Worldwide, it is most often due to annular fibrosis of the intrahepatic portal venules secondary to granuloma formation stimulated by eggs released by *Schistosoma mansoni* residing in the portal veins. This disease is common in Africa and Latin America, and in immigrants from these regions to the USA. If there is no other associated liver disease, the major manifestation is recurrent bleeding from esophageal varices, without ascites or encephalopathy. In the USA, thrombosis is the more common etiology, usually related to therapy with sex hormones or to hypercoagulable states, but often idiopathic.

Sinusoidal and portal venule narrowing in cirrhosis (intrasinusoidal)

ii) Increased intrasinusoidal resistance is one of the two major factors in portal hypertension due to cirrhosis. It is due to sinusoidal narrowing from three causes: subendothelial deposition of collagen in the space of Disse; distortion from regenerating nodules; and constriction due to impaired synthesis of the vasodilator, nitric oxide (NO) and increased release of the vasoconstrictor, endothelin, by the sinusoidal endothelium. The stimulus underlying these changes may be impaired hepatic removal and increased absorption of toxins produced by the ileo-colonic bacterial flora. Compression of sinusoids and small hepatic venules by primary or metastatic tumors is another cause.

Obstruction of the hepatic vein or its tributaries (postsinusoidal)

- iii) Increased postsinusoidal resistance occurs in three conditions:
- Veno-occlusive disease – multifocal obstruction of the small hepatic venules, often due to irradiation or chemotherapy for malignancies, or to ingested plant toxins (herbal remedies).
 - Budd-Chiari syndrome – obstruction of the main hepatic veins, or of the inferior vena cava at or above the liver, secondary to thrombosis, endophlebitis, congenital webs, or hepatocellular or renal cancer growing intravascularly.
 - Severe right-sided congestive heart failure, which raises right atrial pressure and impedes venous return.

Normal arteriolar vasoconstriction is balanced by vaso-dilator influences.

In cirrhosis, vasodilators (NO) ↑ and sensitivity to vaso-constrictors ↓.

Hyperdynamic circulation results.

c. Causes of increased flow of splanchnic blood into portal system

Normal splanchnic vascular resistance and flow are maintained by a balance between tonic constriction of the splanchnic arterioles, mediated by norepinephrine, vasopressin and angiotensin II, countered by the vasodilator effects of nitric oxide (NO) and glucagon. In cirrhosis, splanchnic (and systemic) arterial resistance falls and flow increases dramatically, the so-called **hyperdynamic circulation**. A combination of factors contribute: (i) increased synthesis of NO from arginine by the vascular endothelium, due to enhanced catalytic activity of endothelial nitric oxide synthetase (eNOS). (ii) secondary increases in systemic glucagon concentrations, due to shunting past the liver in porto-systemic collaterals; (iii) decreased sensitivity of the arterioles to the compensatory increase in release of vasoconstrictors; (iv) increased cardiac output. The stimulus for (i) and (iii) may be increased absorption and impaired removal of bacterial toxins produced in the intestinal lumen.

2. Consequences of Portal Hypertension (Figure 21)

a. Splenomegaly and pancytopenia.

Since there are no valves in the portal system, increases of portal vein pressure are transmitted immediately to all venous tributaries, and the spleen, intestines and stomach become congested. The engorged spleen more effectively destroys blood cellular elements, contributing to anemia, leukopenia and thrombocytopenia.

Splenomegaly and pancytopenia

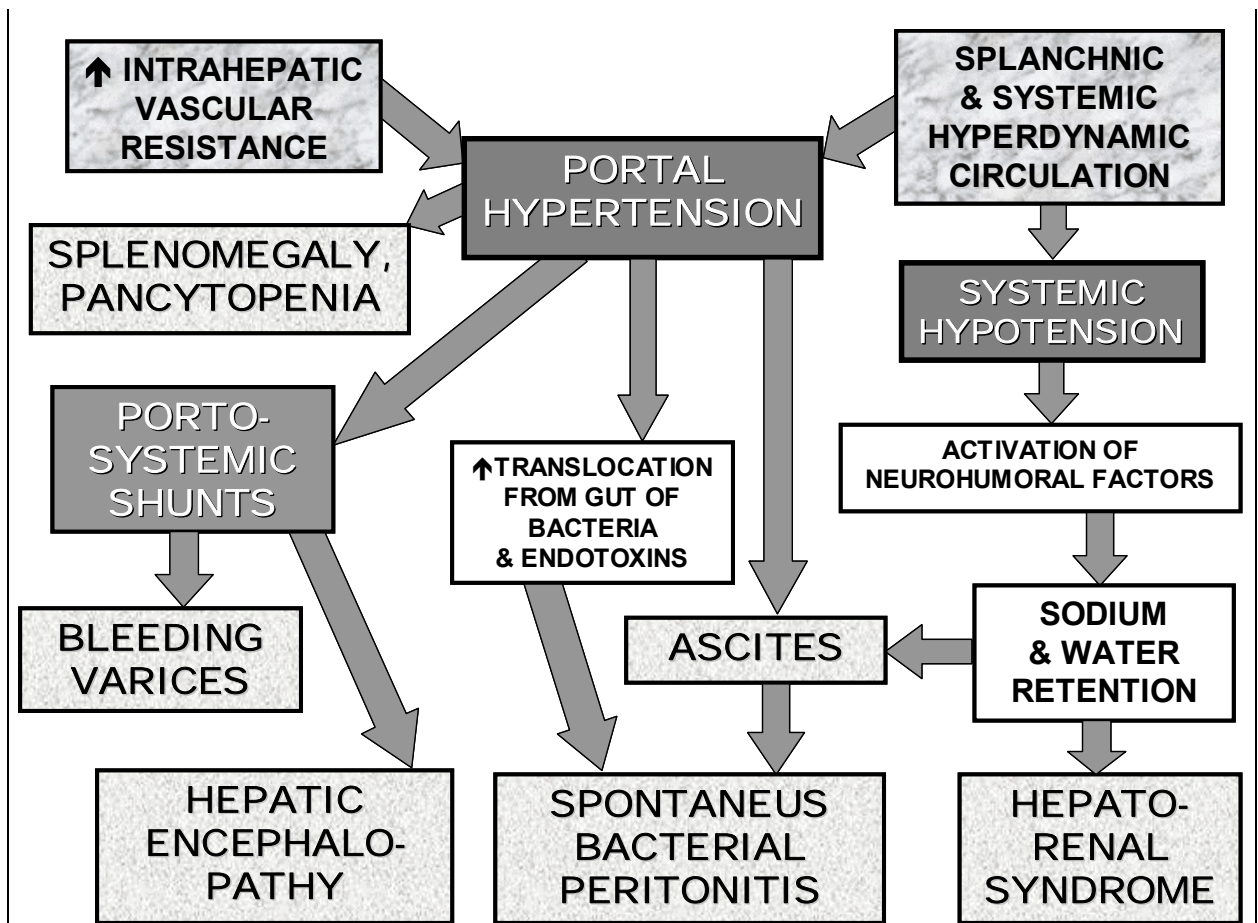


Figure 21. Consequences of Circulatory Changes in Cirrhosis

Flow diagram of the complications caused by portal hypertension and systemic hypotension in cirrhosis. Portal hypertension causes **splenic congestion and enlargement with pancytopenia**, as well as development of **porto-systemic shunts**. Large collateral veins in the esophagus (**varices**) may rupture, causing massive **GI bleeding**. Shunting around the liver of nitrogenous compounds absorbed from the gut causes **hepatic encephalopathy**. The sodium and water retention and systemic hypotension contribute to the **hepato-renal syndrome**, and, combined with high portal pressure cause accumulation of fluid in the peritoneal space (**ascites**). Bacteria and endotoxins, translocated from the gut due to portal hypertension, may circulate into the ascitic fluid, causing **spontaneous bacterial peritonitis**.

b. Porto-systemic collaterals.

Portal venous collaterals to systemic veins bypass the liver cells

Prolonged portal hypertension causes development of a collateral venous circulation, which partially decompresses the portal venous system. Portal blood flowing through these collaterals, including porto-central anastomoses within the liver acini, bypasses the liver, decreasing access of substances absorbed from the intestine to removal by the liver. Normally, 100% of the portal venous blood flow can be recovered from the hepatic veins, but in cirrhosis, as little as 13% may be recovered. The collaterals are quite variable, but the most common and clinically important is esophageal varices:

Bleeding from esophageal or gastric varices is a major complication of portal hypertension

Absorbed toxins bypassing the liver impair brain function

Functional renal failure in cirrhosis

Ascites due to high portal pressure + low plasma albumin

Translocated gut bacteria may infect ascitic fluid

- i) Gastroesophageal varices. Dilated, tortuous veins (varices) develop in the submucosa of the lower esophagus and gastric fundus. This system is fed by the coronary and short gastric veins (connecting from the spleen) and empties into the azygous system in the thorax. Sudden increases in portal pressure that exceed the wall tension of these fragile vessels causes their rupture into the esophageal or gastric lumen, resulting in sudden, often massive, upper gastrointestinal hemorrhage. In the stomach, smaller submucosal arterio-venous communications may likewise bleed (portal hypertensive gastropathy). Bleeding varices may be occluded by endoscopic ligation or sclerosant solutions.
- ii) Rectal varices: The superior hemorrhoidal branches of the mesenteric veins connect with the middle and inferior hemorrhoidal veins, which drain into the inferior vena cava. Rectal varices often occur and may bleed, and are more frequent after esophageal varices have been endoscopically obliterated.
- iii) Other porto-systemic collaterals:
 - *Retroperitoneal* veins constitute a major decompressive route which connects the portal and caval circuits at multiple sites, wherever gastrointestinal organs lie retroperitoneally.
 - *Periumbilical* veins run in the falciform ligament to the umbilicus where they connect to superficial abdominal veins that may be clearly visible on the abdominal wall (caput medusae).
 - *Diaphragmatic-periesophageal veins*: Many small branches in and around the diaphragm connect the portal and systemic circuits.
- c. Hepatic encephalopathy**

In the presence of porto-systemic collaterals, ammonia and other nitrogenous compounds that are naturally absorbed from the intestine, partially bypass the liver and reach the brain, causing impaired neurologic function. For details, see section T.
- d. Hepato-renal syndrome**

Functional renal failure due to extreme vasoconstriction of renal afferent arterioles in cirrhotic patients with severe fluid retention.
- e. Ascites and edema.**

Elevated portal venous pressure and a low plasma oncotic pressure due to hypo-albuminemia are key determinants of the accumulation of fluid in the peritoneal cavity (ascites). Compression of the vena cava by the nodular, cirrhotic liver contributes to edema of the legs. For details, see section S.
- f. Spontaneous bacterial peritonitis (SBP)**

The increased portal pressure increases the permeability of the intestinal capillaries. Combined with bacterial overgrowth in the small intestine, this leads to increased translocation of bacteria and their toxins from the gut into the circulation, via which the bacteria may be carried to and infect the ascitic fluid. Except for fever, SBP is often asymptomatic, and detected only by an increased number of polymorphonuclear leukocytes in the peritoneal fluid.

Reverse both the intrahepatic vasoconstriction and splanchnic vasodilatation.

3. Treatment of Portal Hypertension

- a. Decrease intrahepatic vascular resistance -**
 - i. NO precursors (e.g. isosorbide mononitrate)
 - ii. Block angiotensin II & endothelin receptors (Losartan, Bosentan)
- b. Constrict splanchnic arterioles to decrease portal venous inflow**
 - i. β -adrenergic antagonists (e.g. propranolol) – also \downarrow heart rate
 - ii. Splanchnic-selective vasopressin analogue (Terlipressin)
- c. Inhibit splanchnic vasodilatation.**
 - i. Decrease NO synthesis mediated by eNOS
 - Inhibit eNOS (N-nitro-L-arginine)
 - \downarrow endotoxin formation in gut (Norfloxacin antibiotic)
 - ii. Inhibit CCK, VIP & glucagon receptors (somatostatin analogue)
- g. Decrease intravascular volume – Sodium restriction, diuretics**
- h. Decompress portal system by diverting blood to systemic circulation.** This also helps restore depleted central blood volume.
 - i. Surgical splenorenal or porto-caval shunt.
 - ii. Transjugular intrahepatic porto-systemic shunt (TIPS)

S. Ascites, Edema and Renal Failure in Liver Disease

Renal sodium and water retention occurs even before ascites formation

Sodium and water retention occur in virtually all cases of cirrhosis, even before fluid accumulation is detectable clinically within the peritoneal space (ascites) and (less often) peripheral tissues (edema). In this pre-ascitic phase, the major factor is increased renal release of renin, activating angiotensin II, causing increased proximal tubular reabsorption of sodium. The unknown stimulus for this increased renin release originates in the liver. As cirrhosis progresses, multiple other factors, whose relative importance varies among patients, determine whether fluid retention develops and where it accumulates.

1. Factors promoting renal sodium and water retention (Figure 22)

Primary event is splanchnic vaso-dilatation

- a. Splanchnic vasodilation** leads to pooling of blood in splanchnic vessels, with diversion of blood volume from peripheral circulation. Systemic (including renal) circulation is underfilled, pressure falls.

Release of renin and catecholamines

- b. Decrease in renal arterial and glomerular pressure and flow** causes release of renin; decreased peripheral arterial pressure causes release of catecholamines. The catecholamines cause an increase in cardiac rate and output, aspects of the hyperdynamic circulation.

Increased release of angiotensin and aldosterone

- c. Renin activates angiotensin II**, which causes increased adrenal formation and release of aldosterone. Aldosterone mediates increased distal tubular reabsorption of sodium in exchange for potassium.

Decreased
glomerular blood
flow and filtration

Increased Na⁺
reabsorption in
distal tubules

- d. **Vasoconstriction of preglomerular arterioles** occurs, with shunting of blood from the glomeruli (renal cortex) to the tubules (renal medulla), with further decrease in glomerular blood flow.
- e. **Glomerular filtration of sodium decreases**, with decreased delivery of Na⁺ to the distal tubules. Distal tubules continue to avidly reabsorb Na⁺ in exchange for K⁺.

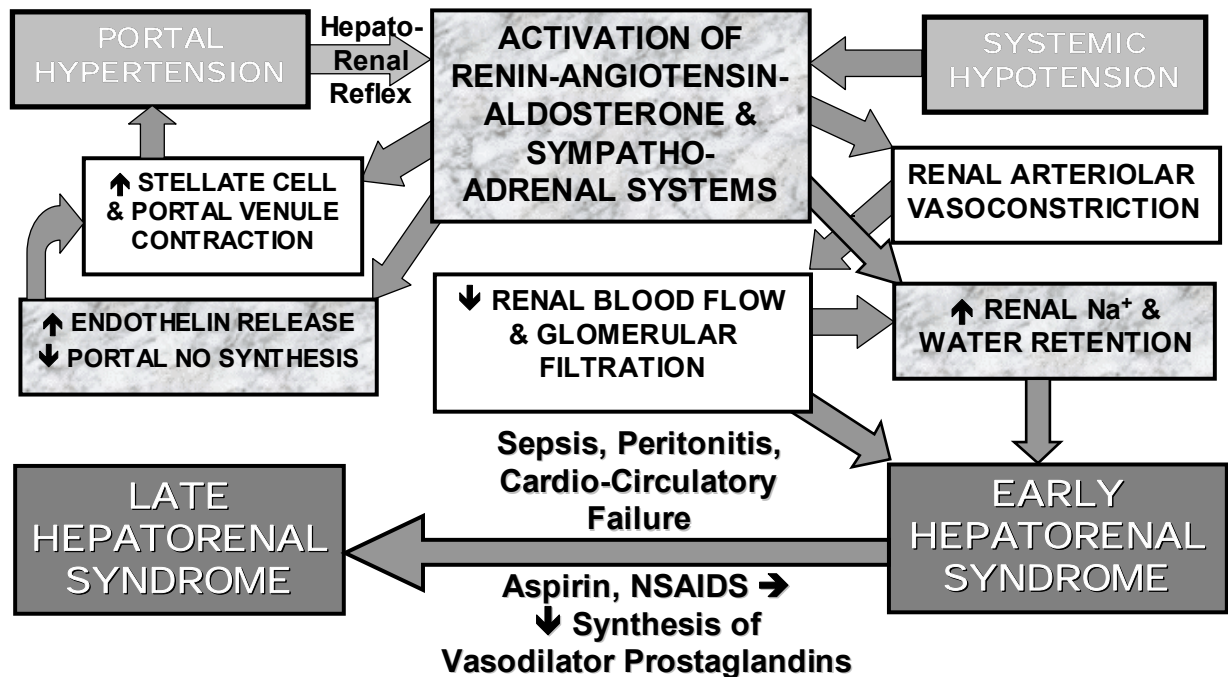


Figure 22. Pathogenesis of Sodium Retention and Renal Complications of Cirrhosis

Flow diagram of the sequence of changes in renal and neurohumoral function that lead to the major clinical complications of cirrhosis. These complications are highlighted by the shaded boxes.

The renal sodium and water retention and late renal failure are primarily secondary complications of the activation of the renin-angiotensin-aldosterone system and sympathetic nervous system in response to the systemic hypotension generated by the shift of fluid from the systemic to splanchnic vascular beds (“splanchnic steal”).

Conversion of the reversible early hepatorenal syndrome to the usually fatal late hepatorenal syndrome is usually due to superimposed cardiocirculatory failure, often triggered by infection (such as spontaneous bacterial peritonitis) and the resultant release of cytokines. In some cases, the late hepatorenal syndrome is triggered by inhibition of renal synthesis of vasodilating prostaglandins resulting from treatment with aspirin and non-steroidal anti-inflammatory drugs (not shown).

Not shown also is the very early increase in renal sodium and water retention, before there is increased portal pressure, triggered by yet unidentified neuro-humoral stimuli that increase intrarenal release of renin.

2. Renal Failure in Cirrhosis – The Hepatorenal Syndrome (Fig. 22)

Hypovolemia causes release of vasoconstrictors

Two stages of hepatorenal syndrome

Late syndrome often precipitated by NSAID Rx.

Low plasma albumin decreases plasma oncotic pressure

Increased portal and sinusoidal pressure

Increased lymph flow and leakage from liver surface

Limited capacity of peritoneum to reabsorb fluid

- a. **Central hypovolemia** secondary to splanchnic pooling of blood leads to activation of the renin-angiotensin II-aldosterone system and increased release of catecholamines and antidiuretic hormone (ADH).
- b. **Early hepato-renal syndrome** results from the consequent intense renal arteriolar vasoconstriction, with decreased glomerular filtration and increased Na⁺ and H₂O retention.
- c. **Late hepato-renal syndrome** ensues when more extreme renal vasoconstriction, often combined with cardiac failure, leads to a rapidly progressive decrease in glomerular perfusion. This may be triggered by ischemic activation of other intrarenal vasoconstrictors (endothelin, adenosine) and/or by failure of the compensatory intrarenal synthesis of the vasodilators, NO and prostaglandins. This often irreversible syndrome is frequently precipitated by treatment with prostaglandin synthesis inhibitors (aspirin, NSAIDs), as well as by sepsis or hemorrhage.

3. Factors Determining the Degree and Site of Fluid Accumulation

a. Decreased plasma oncotic pressure

This results from a combination of reduced hepatic albumin synthesis and dilution of albumin by the body's expanded fluid pools. The decreased oncotic pressure increases filtration of fluid across all capillary beds, including the hepatic sinusoids. This is the principal mechanism of the peripheral edema often seen in cirrhosis.

b. Portal hypertension

This is considered an essential element to the formation of ascites in cirrhosis. As the pressure in the portal venous system and sinusoids increases, the tendency to form ascites increases also.

c. Increased formation of hepatic lymph

The increased intrasinusoidal pressure, combined with decreased oncotic pressure, causes up to a 20-fold increase in formation of hepatic lymph. This exceeds the flow capacity within the thoracic and right hepatic lymphatic ducts, and lymph leaks from the surface of the liver into the peritoneal space, producing ascites.

d. Resorption of fluid and protein across the peritoneal membrane

i. Peritoneal permeability.

Normally, the poorly permeable peritoneal membrane can absorb no more than 1 liter per day of fluid leaked into the peritoneal space. If there is inflammation and/or infection of the peritoneum, as in spontaneous bacterial peritonitis, capillary permeability increases and fluid, protein and leukocytes leak into the peritoneal cavity.

Variations in intra-peritoneal pressure

- ii. Intraperitoneal Pressure.
As the peritoneal cavity fills with fluid, the ascites becomes tense, and intra-abdominal pressure rises, promoting reabsorption of fluid. When ascites is removed therapeutically by abdominal puncture (paracentesis), the acute drop in intra-abdominal pressure may favor rapid reaccumulation of the ascites.

4. Treatment of Ascites, Edema and Hepatorenal Syndrome

Decrease portal pressure and plasma volume

a. Ascites and edema:

- i. Improve hepatic function by treating underlying liver disease
- ii. Pharmacologically decrease portal pressure (see section **R.3**).
- iii. Directly remove fluid from peritoneal cavity (paracentesis)
- iv. Decrease plasma volume (low Na⁺ intake, diuretics, aldactone)
- v. Increase plasma oncotic pressure (infuse albumin or polymers)
- vi. Mechanically decrease portal pressure (see section **R.3**).

Expand plasma volume and give splanchnic vasoconstrictor

b. Hepatorenal syndrome:

Early hepatorenal syndrome often responds to correction of precipitating factors, plus plasma volume expansion with albumin to normalize central venous pressure. Addition of terlipressin or norepinephrine, to selectively constrict dilated splanchnic vessels, reportedly reverses the majority of cases of late hepatorenal syndrome. The others respond to successful liver transplantation.

Renal failure is more serious than ascites

Note: Since treatments of ascites and hepatorenal syndrome may be contradictory, and the hepatorenal syndrome is much more serious, always treat renal failure rather than ascites.

T. Hepatic Encephalopathy (HE)

1. Definition of Hepatic Encephalopathy.

Acute vs. chronic HE

A disturbance of the central nervous system (CNS) secondary to hepatic insufficiency and/or porto-systemic shunts. HE can mimic virtually any neurological condition. It can occur in both acute (fulminant) hepatic failure as well as in chronic liver disease (cirrhosis), and it is potentially reversible if liver dysfunction can be improved.

2. Etiology of Hepatic Encephalopathy

CNS toxicity of gut-derived nitrogenous metabolites

- a. Many neurotoxins** may contribute to the disturbance in CNS function. Paramount importance, however, is given to nitrogenous substances, derived from metabolism of proteins in the intestine. These reach the systemic circulation due to porto-systemic shunts, or decreased hepatic function. These nitrogenous substances, especially ammonia and glutamine, alter neurotransmission in the CNS.

Ammonia is the prime toxin.

Multiple sources of ammonia

Decreased hepatic ammonia removal in liver diseases

Ammonia leads to astrocyte swelling & ↓ glutamate, a neuro-excitor

Role of other toxins and drugs that are CNS depressants

Asterixis (flapping tremor): intermittent lapse of posture of the outstretched hands

b. Ammonia is accepted as the prime factor in the pathogenesis of HE. The concentration of NH_3 is highest in portal venous blood; portal NH_3 is derived from the urease activity of colonic bacteria, and the deamination of glutamine in small bowel mucosa. Other major sources of blood ammonia are metabolism of urea and glutamine by the kidney and of glutamine by skeletal muscles.

Normally, the liver extracts about 80% of portal-venous NH_3 (first pass metabolism), and converts it to urea. In liver disease, arterial NH_3 levels can increase, due to both decreased metabolism by hepatocytes and increased porto-systemic shunting around the liver. If renal failure is present also, the increased levels of urea in the intestine promote increased formation of NH_3 .

Entry of NH_3 into astrocytes leads to increased formation of the osmolyte, glutamine, causing astrocyte swelling and brain edema. Depletion of neuro-excitatory glutamate depresses brain function.

c. Other putative gut-derived neurotoxins include: short-chain fatty acids, mercaptans, benzodiazepine-like substances.

d. Many sedatives and tranquilizers are metabolized more slowly in liver disease and may add to CNS depression. Avoid their use!

3. Clinical Stages of Hepatic Encephalopathy

- 0.** (subclinical). No grossly evident changes in personality or behavior, except by special tests of CNS function; no asterixis
- 1.** Shortened attention span. Impaired handwriting and ability to perform simple arithmetic. Impaired sleep and memory. Altered mood. May have asterixis.
- 2.** Lethargy and/or apathy. Disorientation. Inappropriate behavior. Slurred speech. Obvious asterixis.
- 3.** Gross disorientation. Semi-stuporous or stuporous. Asterixis may be difficult to elicit.
- 4a.** Frank coma. (Seizures may occur in fulminant liver failure)
- 4b.** Fatal herniation of swollen cerebrum through foramen magnum.

4. Treatment of Hepatic Encephalopathy

- a. Improve liver and kidney function** (withdraw drugs, toxins, alcohol; treat infections, shock, dehydration; correct acid-base balance)
- b. Decrease nitrogenous sources** in intestine (control GI bleeding, laxatives to clear bowel of blood and bacteria, low-protein diet)
- c. Decrease formation and absorption of ammonia** in the gut (neomycin to diminish intestinal bacteria; lactulose to increase fecal nitrogen excretion)

- d. Stop treatment with sedatives and tranquilizers
- e. Benzodiazepine antagonists may mitigate chronic encephalopathy

U. Overview of Some Other Common Hepatic Diseases

(This section is for general interest; you will not be tested on the details.)

1. Viral Hepatitis

Table 8. FIVE FORMS OF VIRAL HEPATITIS IN HUMANS

	<i>Hepatitis Virus</i>				
	A	B	C	D	E
Virus	HAV	HBV	HCV	HDV	HEV
Family	Picornavirus	Hepadnavirus	Flavivirus	Deltavirus	Calcivirus
Genus	Hepatovirus	Ortho Hepadna	Hepacivirus	Deltavirus	Unnamed
Size	27 nm	42 nm	30 - 60 nm	40 nm	32 nm
Genome*	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA
Length	7.5 kb	3.2 kb	9.4 kb	1.7 kb	7.5 kb
Acute mortality	0.2-0.4%	1.0-1.2%	0.2%	2-20%	0.2% #
Chronicity	None	2-7%	80-90%	2-70%	None
Transmission	Fecal-oral	Parenteral Sexual	Parenteral ?Sexual (rare)	Parenteral Sexual	Fecal-oral
Antigens	HAVAg	HBsAg HBcAg HBeAg	HCVAg	HDVAg	HEVAg
Antibodies	Anti-HAV	Anti-HBs Anti-HBc Anti-HBe	Anti-HCV	Anti-HDV	Anti-HEV
Viral Markers	HAV RNA	HBV DNA DNA polymerase	HCV RNA	HDV RNA	Virus-like particles

* ss = Single stranded; ds = Double-stranded # Mortality up to 20% in pregnant women

a. Hepatitis A (HAV):

HAV is an important cause of acute liver disease worldwide. It is caused by the hepatitis A virus, a 27 nm single-stranded RNA. The severity of the hepatitis caused by HAV is age related: among children, most infections are asymptomatic, but most adults become symptomatic and often jaundiced. The case fatality rate among reported cases is 0.2% to 0.4% due to fulminant hepatic failure.

This form of hepatitis is transmitted primarily by person-to-person contact, generally through fecal contamination and oral ingestion. Recently, cases of hepatitis A acquired via percutaneous transmission among intravenous drug users have been reported with increasing frequency. Common-source epidemics from contaminated food and

water occur (i.e., restaurants), but are not common. They are, however, widely publicized. Hepatitis A remains a frequent infection among older children and young adults. On occasion, it may cause confusion as an intercurrent infection in a patient with another form of chronic liver disease (i.e., alcohol-induced, chronic hepatitis B). It may also cause worsening of chronic liver disease. Serologic testing for anti-HAV IgM provides rapid and accurate identification of this form of acute hepatitis (Figure 23). Acute hepatitis A does not develop a chronic carrier state or progress to chronic hepatitis/cirrhosis. There is no specific treatment for acute hepatitis A. Passive immunization for exposed patients exists and may prevent development of the disease. A vaccine for hepatitis A is effective in preventing this important cause of acute liver disease.

b. Hepatitis B (HBV):

HBV is a 42-nm double-stranded DNA virus with a long incubation period and generally insidious onset. The reported case fatality rate is 1%, due to fulminant hepatic failure.

The virus may cause chronic infection. The likelihood of becoming chronically infected is inversely proportional with the age at which the infection occurs (i.e., 90% of infants, 6-10% of adults). This carrier state is central to the persistence and epidemiology of HBV.

HBV can lead to chronic hepatitis and carcinoma

Worldwide, HBV infection is a major cause of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma. The frequency of HBV infection and the patterns of its transmission vary markedly in different parts of the world. The disease is of low endemicity in the United States, occurring primarily in adulthood with only 0.2% to 0.9% of the population chronically infected. In China, Southeast Asia, and Africa, however, most HBV infection is acquired at birth, and 8% to 15% of the population is chronically infected.

Several serologic tests for HBV exist and interpretation may be complex. A typical serological pattern is illustrated in Figure 23. The risk of acquiring HBV from a blood transfusion has been almost completely eliminated with the advent of serological testing. Currently, IV drug abuse and sexual transmission remain the most common routes for acquiring HBV. Active and passive immunization is available for HBV, and the US Public Health Service has established new guidelines for an immunization program which should eventually lead to the control of HBV in this country. Potential treatment regimens continue to be explored to prevent the chronic sequelae of HBV. Interferon-alpha cures about 30% - 40% of patients.

The nucleoside analogues, lamivudine, adefovir, and entecavir have been FDA-approved and are as effective as interferon, with fewer side effects, but viral resistance can develop. Further drugs are in clinical trials.

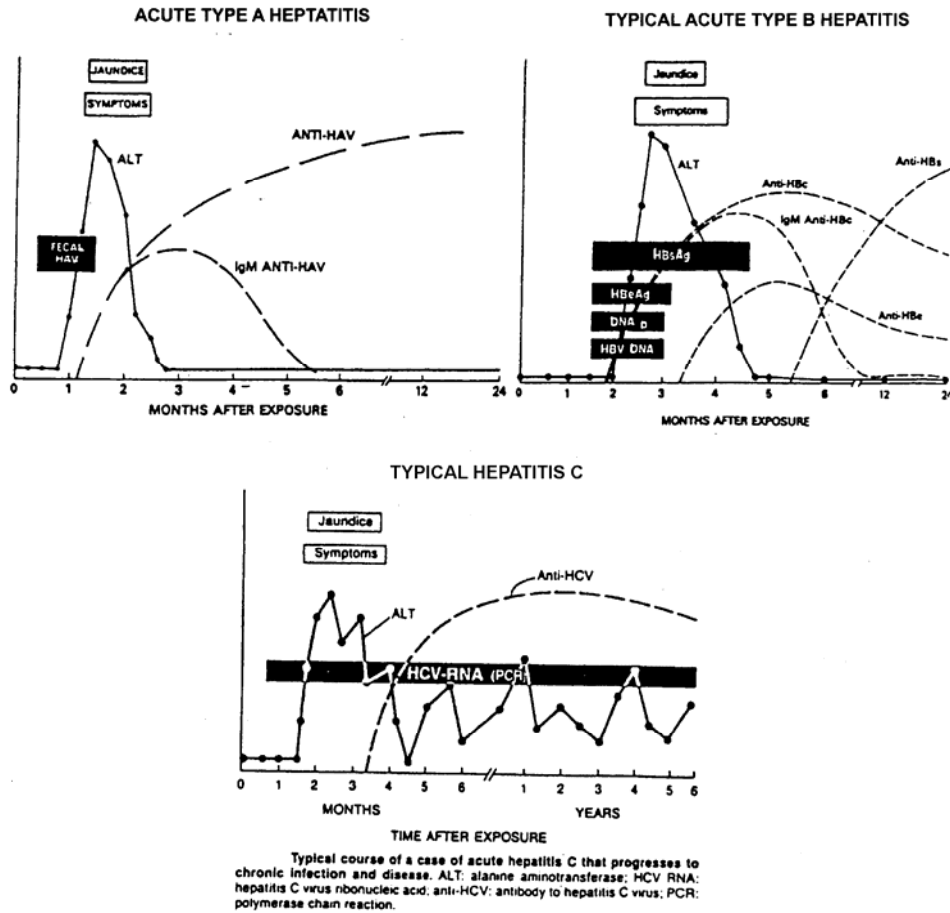


Figure 23. Serological responses to Types A, B, and C Hepatitis

c. Hepatitis C (HCV):

Hepatitis C virus (HCV) is an RNA virus of approximately 30-60 nm. The prevalence of HCV antibodies in the United States population is about 1.8%; however, it is higher in certain high-risk groups (i.e., IV drug users and hemophiliacs, 60% - 90%; dialysis patients, 28% - 30%). Nearly 4 million Americans carry antibody and 3 million are chronically infected with HCV. The predominant route of transmission is parenteral (IV drugs, transfusions, etc.) but transmission has been associated with other risk factors (rarely sexual) and postulated for others: sniffing cocaine, body/ear piercing, tattooing. As many as 10% of patients may have no identifiable risk factor. HCV shares epidemiological characteristics with those of HBV and multiple episodes of viral hepatitis may be observed in individuals at risk (i.e., HBV and HCV).

Chronic hepatitis is common after infection with HCV

HCV accounts for approximately 20% of all cases of acute hepatitis in the US, and nearly for all cases (>90%) of non-A, non-B post-transfusion hepatitis. Eighty-five percent of patients develop chronic hepatitis. Chronic hepatitis is often silently progressive, with symptoms developing only after 10-20 years of disease.

Approximately 10% - 40% of chronically infected patients will progress to cirrhosis after 18-20 years of infection, and of these, about 20% will develop hepatocellular carcinoma. HCV causes significant morbidity and mortality, and is the leading indication for liver transplantation in the United States today.

*Risk of hepatitis C infection from blood transfusion
~1:100,000 units*

A serologic test (anti-HCV) provides identification of exposure to this virus and allows diagnosis of this important and common form of chronic viral hepatitis (Figure 23). Chronic HCV is more common (1.8%) than chronic HBV infection (0.1% of normal volunteer blood donors). Our ability to identify the virus in blood products has markedly improved the safety of the United States blood supply. Treatment is designed at virus eradication with prevention of sequelae of chronic infection. While treatment has been difficult, newer regimens (i.e. long-acting pegylated interferon and ribavirin) offer response rates up to 70%-80% in selected patients. There is currently no active or passive immunization for HCV.

Therapy of hepatitis C is difficult but improving

d. Hepatitis D (HDV)

HDV (formerly Delta virus) is a fairly recently identified and characterized hepatitis virus. It appears to be a small circular RNA virus, however, it is defective and requires the presence of HBV for its replication. This form of hepatitis affects only patients who are chronically infected with HBV (e.g., HBsAg positive). HDV is relatively rare in the United States, but is most commonly found among IV drug abusers, hemophiliacs, homosexuals, and patients from areas of the world where HDV infection is endemic (southern Europe, Middle East, south India, the Balkans, the Amazon basin).

In general, HDV is more severe than HBV alone. HDV may be acquired at the same time as HBV (co-infection) or it can occur spontaneously in a known HBV carrier (superinfection). Both forms may be associated with a severe or fulminant type of hepatitis; however, the spontaneous form of HDV appears to more commonly accelerate the progression of chronic HBV to cirrhosis. Potential treatment regimens for HDV follow those described for HBV (e.g. interferon, lamivudine, adefovir, , or entecavir). There is no active or passive immunization available for HDV.

e. Hepatitis E (HEV):

HEV has been identified as the agent accounting for sporadic and major epidemics of viral hepatitis in under-developed countries (India, Pakistan, Mexico, Central Asia, Southeast Asia, North Africa) and travelers returning from these areas. This virus is serologically distinct from acute hepatitis A. HEV is a 32-34 nm single-stranded RNA virus.

HEV has fecal-oral transmission

Clinically, HEV resembles hepatitis A. It generally affects young adults and has a self-limited course. The mortality is very high (about 20% - 25%) in women in the last trimester of pregnancy. Chronic infection does not occur. Serological testing is available to identify HEV, but it is not yet routinely available. No passive or active immunization for HEV exists.

f. Potential Human Hepatitis Viruses:

With the advances in molecular biologic techniques, the nomenclature of the human hepatitis viruses will be expanded. Several potential human hepatitis viruses have been cloned and serologic assays are being developed.

g. Histopathology, Acute Hepatic Injury:

All forms of viral hepatitis have similar pathology. The initial lesion is an acute inflammation of the entire liver. The inflammatory infiltrate generally begins in the portal triads and consists of mononuclear cells (lymphocytes and monocytes). Occasional plasma cells and eosinophils may be seen. Neutrophils are usually absent. Random hepatocytes, singly or in clusters, undergo degeneration (necrosis). As the cellular necrosis evolves, the hepatocytes become smaller, deeply eosinophilic with pyknotic nuclei. When the pyknotic nucleus is extruded, the remaining cell is called an eosinophilic, acidophilic, or apoptotic body, a common histologic feature of acute viral hepatitis. Some hepatocytes become swollen and pale staining and are referred to as undergoing "ballooning" or "feathery" degeneration. Biliary canaliculi occasionally show inspissation of bile stained material. The reticulin framework generally remains well preserved and provides the scaffolding when the liver cells regenerate. In severe inflammation, the sinusoidal spaces are congested, and may give the impression of stromal hemorrhage (which may actually occur if inflammation is severe enough to destroy the reticulin framework). During recovery, inflammatory cells gradually disappear and Kupffer cells scavenge dead cells. Following inflammation, there is a marked stimulus for hepatocyte regeneration. Frequent mitoses are seen.

h. Variations in the clinical course of acute viral hepatitis (Fig. 24):

i. Normal convalescence:

Following hepatitis, the entire process may gradually heal. Liver cells no longer degenerate, bile canalicular plugging disappears, and the inflammatory collection diminishes in amount (with mononuclear cells remaining the longest within the portal triad zones). The liver returns to its original size, and hepatic biochemical functions become normal. This is by far the most frequent course in Hepatitis A, B, and E. Recovery occurs in only about 15%-20% of patients with Hepatitis C (Figure 24)

Complete recovery usual in Hepatitis A, B and E, but not C.

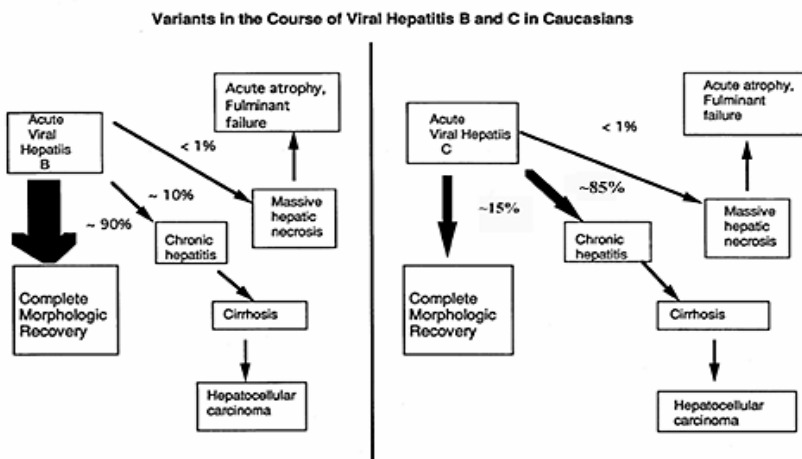


Figure 24. Variations in the Course of Viral Hepatitis B and C in Caucasians

ii. Acute, fulminant, massive necrosis:

Massive loss of hepatocytes with collapse of residual structures

Fortunately, fulminant, frequently lethal, variant is relatively rare. Most of the hepatocytes undergo acute necrosis without regeneration. Sinusoids dilate and intralobular hemorrhage may occur. All residual structures, including their associated inflammatory cell collections collapse together. Occasional random islands of fairly normal hepatocytes may remain which are likely to include multinuclear giant hepatocytes. Although initially swollen, the liver can shrink to a very small size (often less than 1/3 normal) over a few weeks. The cut surface appears red and hemorrhagic.

iii. Submassive necrosis:

Severe massive or submassive necrosis may eventually result in permanent focal fibrosis. If the liver cell loss extends between adjacent central veins and portal triads, the corresponding collapse of the reticular structure of the liver leads to "bridging". This may have a more serious pathogenic course, and if sufficiently extensive may progress to cirrhosis.

iv. Chronic hepatitis:

Chronic Hepatitis common with HCV, but not a sequela of Hepatitis A or E

Occasionally, the acute hepatitis subsides but never totally heals and may even show intermittent acute relapses. Scattered focal liver cell death continues to occur, especially in the periportal region. Consequently, the border between the portal triads and lobular hepatocytes (terminal plate) becomes irregular and inflammatory cells and fibroblasts creep into the hepatocyte cords along the sinusoids (interface hepatitis or piecemeal necrosis). Regeneration is irregular and liver cell plates become two to three cells thick. Mononuclear inflammatory cells (small lymphocytes, plasma cells) abundantly infiltrate the portal triads and extend into

the periportal region. An increase in the number of fibroblasts (derived from stellate cells) occurs in the portal areas. Tongues of fibrous tissue reach out from portal triads to penetrate among the hepatocytes and may bridge between portal triads leading to progressive cirrhosis.

Classify by virus and degrees of inflammation and fibrosis/cirrhosis

i. Classification of chronic hepatitis

The current nomenclature for chronic hepatitis entails the etiology (i.e., HBV, HCV) and the severity which is arbitrarily graded mild, moderate, or severe. Liver biopsies from patients with chronic viral hepatitis are scored on a scale of 0-4 for histologic inflammatory activity (grade) and fibrosis (stage) of the underlying chronic hepatitis ± cirrhosis.

j. Therapy of chronic viral hepatitis

i. Medical treatment of chronic Hepatitis B.

IM or SubQ alfa-interferon for chronic HBV

It is now possible to eliminate HBV replication and ameliorate the underlying liver disease in approximately 20% - 40% of Caucasian patients using alfa-interferon therapy. The pattern of clinical, biochemical, and serologic response to therapy is complex, most likely reflecting the heterogeneity of the pathogenesis of the liver disease in this setting. Alfa-interferon provided the first promise for an effective therapy of chronic HBV. Side effects limit its use and the sustained response rate is only approximately 20% - 40%. Clinical trials to date have examined alfa-interferon treatment of relatively mild to moderately severe chronic HBV liver disease. The role of alfa-interferon in patients with decompensated cirrhosis (i.e., ascites, edema, varices, encephalopathy) - the group that needs treatment most - has not been defined. These patients may actually worsen with therapy. It is currently recommended that these patients should be treated only in the context of controlled clinical trials or in medical centers with expertise in antiviral therapy. The nucleoside analogs, lamivudine, adefovir, and entecavir, have been FDA-approved and play an important role in the treatment of chronic hepatitis B. Importantly, they are oral agents, with limited side effects, and appear to be efficacious in both Caucasian and Asian patients. Other antiviral agents are currently being evaluated in clinical trials. The different genotypes of HBV have different susceptibilities to therapy.

Antiviral agents offer new promise

ii. Medical treatment of chronic hepatitis C.

Low long-term response with interferon alone

Combination therapy with pegylated interferon + Ribavirin.

Alfa-interferon therapy has been extensively examined in chronic HCV infection. The results of many randomized controlled trials clearly demonstrate both safety and efficacy. Current treatment with long-acting (pegylated) interferon, allows once weekly dosing. Its use, in combination with Ribavirin, is the standard of care. This regimen will eradicate the virus in about 40%-50% of patients with HCV genotype 1 and 75%-85% of those infected with genotypes 2 or 3.

Liver transplantation, the court of last resort.

Limited by expense and shortage of donor livers.

Hepatocellular vs cholangiocarcinoma

Risk factors

iii. Liver transplantation has proven to be a clinically appropriate therapeutic modality for selected patients with irreversible fulminant or chronic liver disease. In the relatively uncommon setting of fulminant liver failure, where mortality without treatment is up to 90%, liver transplantation can be life saving. Patients with chronic liver disease (i.e., viral, Wilson's disease, alcohol, etc.) not only have improved long-term survival, but also improved quality of life following liver transplantation. Chronic viral hepatitis is the leading indication for liver transplantation worldwide. The results of liver transplantation have been best in children in whom long-term survival of greater than 5 years may be as high as 85%-90%. In adults, the 5-year survival rates range from 80% to 90%, and are best with predominantly cholestatic liver disease (i.e., primary biliary cirrhosis). The results from liver transplantation are even more impressive when they are compared with the alternative of medical therapy for end stage chronic liver disease: medical therapy yields 1-year survival rates of only 0-30%. Although the results of liver transplantation are very good, a number of problems remain. These are predominantly related to the complications from immunosuppressive therapy in the post-transplant setting (i.e., infection, hypertension, and diabetes). Techniques for better organ preservation are continually evolving. Donor organ availability remains a major problem, and is the major reason why patients die waiting for an organ. The cost of liver transplantation is substantial: the price ranges from \$100,000 to \$250,000 or more in the United States (depending upon complications). The cost of medications after transplantation can run several thousand dollars a month.

2. Neoplasms of the Hepatobiliary Tract

a. Primary malignant liver tumors

Primary neoplasms of the hepatobiliary tract are derived either from hepatocytes (hepatocellular carcinoma, HCC, or "hepatoma") or from the bile duct epithelium (cholangiocarcinoma). Both of these tumor types tend to invade locally. Distant metastases are uncommon, especially for cholangiocarcinoma. Hepatocellular carcinoma is one of the most common malignant tumors in some parts of the world, especially southeast Asia. In adults, HCC occurs frequently in association with cirrhosis of the liver. Major risk factors for HCC include metabolic liver diseases (hemochromatosis), environmental factors (aflatoxin), alcohol, hepatitis C virus infection (after cirrhosis has developed), and chronic hepatitis B viral infection (even before the development of cirrhosis).

i. Hepatocellular carcinoma.

Hepatocellular carcinoma (HCC) appears as irregular cords of cells, which resemble hepatocytes and form plates several cells thick. The included biliary canaliculi often contain bile. HCC is often multifocal and commonly invades and grows within the

channels of the hepatic veins. HCC may extend into and occlude not only the hepatic venous outflow tract, but also the inferior vena cava. Tumor emboli may be delivered into the lungs, where metastases grow readily. HCC usually arises in cirrhotic livers and is initially manifested by worsening of the signs and symptoms of the cirrhosis, associated with a disproportionate increase in serum alkaline phosphatase. Later complaints are generally associated with rapid increase in tumor size, liver size, and abdominal girth. The swollen liver may cause right upper quadrant pain. Unless the tumor is small and localized to a single lobe of the liver, surgical removal is usually difficult and unrewarding.

Serum alpha-fetoprotein is a marker of HCC

Malignant hepatocytes may synthesize a variety of proteins (alpha-fetoprotein, erythropoietin, parathyroid-like hormone) not normally produced by the adult liver. Alpha-fetoprotein is a normal product of embryonic hepatocytes, but, in early infancy, it ceases to be produced in significant quantities. Rapidly regenerating hepatocytes (following acute liver injury), regenerating nodules in cirrhosis, and hepatoma cells may synthesize generous amounts of this protein. In adults with HCC, a high plasma level of alpha-fetoprotein can be demonstrated in 50% - 80% of patients. This, associated with a large liver mass and high alkaline phosphatase, is virtually diagnostic of HCC. The secreted erythropoietin may cause erythrocytosis and the parathyroid-like hormone may cause hypercalcemia.

ii. Cholangiocarcinoma

Risk factors

Cholangiocarcinoma shows a geographic variation as well, although not as much as HCC. Cholangiocarcinoma occurs more commonly in older individuals (average age at presentation ~ 60 years), and is rare before age 40. Cholangiocarcinoma coexists with cirrhosis less commonly than does HCC. Major risk factors include long-standing ulcerative colitis, Crohn's disease, primary sclerosing cholangitis, developmental abnormalities of the bile ducts, intrahepatic cholelithiasis, and chronic liver fluke infestation. There is no known relationship with chronic hepatitis B or C infection.

Biliary duct and gallbladder carcinoma

The tumors arise equally from the gallbladder and the biliary ducts. There is an increased incidence arising in gallbladders which contain stones. For the biliary ducts, the chief sites of origin are: a) the papilla of Vater; b) the junction of the cystic and common hepatic ducts; and c) the bifurcation of the two main intrahepatic ducts. Cholangiocarcinomas are adenocarcinomas that typically grow in tubular elements (resembling bile ducts) embedded in abundant fibrous stroma. The cells vary from tall columnar to short cuboidal and usually grow in great disarray, but may show a glandular organization. Sometimes, when the sclerosis is intense, it is difficult to identify the few neoplastic cells.

Fibrous stroma is characteristic

Early rise in serum alkaline phosphatase

Cholangiocarcinomas grow slowly, and are initially manifested by a prolonged, progressive, marked elevation of the serum alkaline phosphatase, without symptoms. Pruritus and jaundice do not appear until the obstruction of the bile flow is more marked.

Do not produce alpha-fetoprotein

Above the main bile duct obstruction, dilation of the intra- and extrahepatic biliary radicles occurs. Jaundice occurs more commonly than in HCC, but cholangiocarcinomas do not produce alpha-fetoprotein.

Cholangiocarcinomas of the papilla are sometimes associated with intermittent common bile duct obstruction because of fluctuations of tissue edema around the tumor and its periodic sloughing of necrotic portions into the gut lumen. Metastases occur by lymphatic spread. Surgical removal of the tumor is the treatment of choice if technically feasible, but often the tumor is too large by the time it is detected. The results of radiation therapy and cancer chemotherapy are poor, so they are usually treated palliatively by endoscopic stent placement.

Metastases to liver more common than primary tumors

b. Secondary (metastatic) tumors of the liver

The incidence of secondary tumors of the liver far exceeds the incidence of primary hepatobiliary neoplasms. The large blood flow to the liver, including its exclusive portal venous drainage from the gastrointestinal organs, is the most likely explanation for this. Breast, lung, and particularly colon, pancreas, and stomach, are the most frequent sources of hepatic metastases.

Usually multiple

Metastatic tumors in the liver usually form multiple (less often solitary) well circumscribed nodules of varied size, differing in color, texture, and firmness from the surrounding hepatic parenchyma. These nodules tend to displace and compress the parenchyma. On microscopic examination, tongues of tumor tissue are frequently seen infiltrating into the surrounding hepatic tissue, where they elicit a reaction of acute and chronic inflammatory cells. With expansive growth, the large nodules may compromise the blood supply to themselves or the surrounding parenchyma; local infarction results. A newly elevated serum alkaline phosphatase is often the only clue to the presence of metastases. They are generally easily visualized on abdominal ultrasonography.

An elevated alkaline phosphatase is the earliest abnormal laboratory finding

Leukemic and lymphomatous infiltrates may be deposited along sinusoidal channels, especially in the regions of the portal triads. Such metastases are widely infiltrative rather than circumscribed and nodular.

3. Infection of the Liver

Multiple routes for bacteria to the liver

a. Bacterial infection in the liver may arrive by hepatic arterial, portal venous, or biliary routes. The abundant blood supply received by the liver makes it a frequent site of the localization of bacterial growth in systemic sepsis. In systemic bacteremias, arterial dissemination may lead to localized single or multiple liver abscesses of varying size. As

with other multifocal, space-occupying lesions in the liver, the most common laboratory abnormality is an elevated alkaline phosphatase. Severe sepsis in areas of the abdomen drained by the portal venous system may allow portal dissemination of bacteria to the liver with secondary hepatic abscesses and, rarely, septic portal thrombophlebitis.

*Hepatic granulomas:
tuberculosis, fungi*

*Elevated alkaline
phosphatase typical*

*Sarcoidosis
Amebiasis*

b. Granulomas. The liver is a common site for growth of tubercle bacilli and fungi. In these conditions, small inflammatory granulomas are randomly scattered throughout the organ, often without particular relationship to lobular zones. Alkaline phosphatase elevation is again characteristic and often the only abnormal liver function test.

Sarcoidosis produces a similar clinical and pathological picture.

Amebiasis, due to infection with parasitic ameba (*E. histolytica*) may produce focal liver abscesses of varying size and numbers. Single very large abscesses involving the right lobe of the liver are frequent.

*Factors determining
hepatic blood flow*

*Causes of increased
splanchnic arterial
resistance*

*Ischemic necrosis
most affects zone 3
(centrilobular)
hepatocytes*

4. Disorders of Hepatic Circulation (other than portal hypertension).

Liver blood flow is dependent upon a) cardiac output, b) the tone and patency of the splanchnic vasculature, c) sinusoidal tone and patency, and d) the hepatic venous return via the inferior vena cava to the right atrium.

Portal venous inflow to the liver is reduced whenever splanchnic venous dilation, and/or vasoconstriction of the splanchnic arterioles, occurs. Such significant increases in splanchnic arterial resistance occur in response to: elevation of body temperature, sudden assumption of upright posture, peripheral arterial hypotension, and the direct actions of vasoconstrictive drugs (such as vasopressin and ergot derivatives).

Diffuse ischemic liver necrosis most often results when portal venous inflow is severely decreased, most commonly due to shock in association with massive hemorrhage, complications of surgery, or severe acute myocardial damage. The centrilobular (zone 3) hepatocytes are most severely affected. Although jaundice is often present, milder degrees of this syndrome are manifested by transient, isolated, striking elevations of serum AST and ALT levels.

When the ischemia results from impaired hepatic venous outflow combined with hypoxia, the centrilobular zone exhibits hemorrhage as well as necrosis. Causes of this syndrome include severe cor pulmonale (right-sided heart failure secondary to chronic lung disease) and acute hepatic venous occlusions (acute Budd-Chiari syndrome).

a. Arterial insufficiency

*Arterial blood
supply generally
crucial to liver
survival*

Anoxic liver cell death occurs rapidly in the absence of arterial blood supply. The cirrhotic patient is more sensitive to arterial insufficiency because of the already distorted vascular anatomy. The hepatic arterial system is protected by a variety of available collateral sources, including branches of the phrenic, right gastric,

gastroduodenal arteries. The sufficiency of these extrahepatic collateral vessels varies widely among individuals. In some, it is possible even to ligate the main hepatic artery without fatal necrosis. Collaterals between intrahepatic arterial segments are only spottily available. Extensive anoxic death of hepatocytes engenders a fulminant toxic illness with high fever, high white blood cell count, and profound metabolic hepatic failure with encephalopathy and jaundice. With less severe or more distal arterial occlusion, the infarction may be localized to specific, wedge-shaped segments of the liver, and only jaundice and elevation of serum transaminases may be seen.

b. Venous congestion

Congestion of the liver may likewise be uniformly diffuse or localized to specific segments when there is occlusion of hepatic vein branches. The latter set of conditions may evolve from neoplastic occlusion (especially with hepatoma), local thrombosis, or endophlebitis (veno-occlusive disease). Generalized hepatic congestion results from interference with venous return to the heart from the entire liver and may be due to obstruction of the main hepatic vein, obstruction of the inferior vena cava between liver and heart, or right ventricular failure due to pulmonary hypertension or tricuspid valve insufficiency). Acute, complete hepatic venous obstruction (acute Budd-Chiari syndrome) leads to hepatic cell necrosis with proportionate hepatic metabolic failure. Congestion and edema of the liver rapidly causes organ swelling with stretching of Glisson's capsule, often causing right upper abdominal pain and tenderness. Partial, long-standing interference of hepatic venous outflow (e.g., due to hepatoma or renal cell carcinoma) yields sinusoidal dilation, hepatic cell atrophy and eventual stellate fibrosis, all mainly centrilobular. Only very rarely does this scarring extend to connect the central vein areas of adjacent lobules ("bridging") and evolve into congestive ("cardiac") fibrosis of the liver. Liver lymphatics become extremely engorged and carry massively increased volumes of plasma-rich fluid. The engorged subcapsular hepatic lymphatics weep fluid directly from the liver surface into the peritoneal cavity in increased amounts, contributing to ascitic fluid collections.

May be localized in large vessels or diffuse throughout hepatic veins.

May be secondary to right-sided heart failure.

Acute vs. Chronic Budd-Chiari syndrome.

V. Key Points

1. The functional unit of the liver is the acinus, the array of cells radiating from a portal triad that contains the terminal bile ductule(s), portal vein and hepatic artery.
2. Portal blood pressure is primarily determined by the vascular radii of vessels in the liver and flow of blood into the portal venous system. Portal hypertension ensues when hepatic vessels are constricted and/or inflow from the splanchnic circulation increases.
3. Commonly used liver tests are not quantitative tests of liver function; they are a reflection of interrelated physiological and pathological events, but are useful in determining the diagnosis, course, severity and prognosis of hepatobiliary diseases.
4. The liver has a central role in the metabolism of nutrients and xenobiotics absorbed from the gut; such biotransformation of toxic compounds neutralizes their activity and allows their excretion in bile. The liver synthesizes essential proteins, clotting factors, and lipoproteins, which circulate in the blood, and bile acids, which are key detergents in the bile and intestine.
5. The Kupffer cells shield the body from exposure to foreign antigens, endotoxins and bacteria absorbed from the gut.
6. Over 95% of the bile salts secreted in bile are reabsorbed from the small intestine, where they are modified and returned to the liver for re-excretion into bile (enterohepatic circulation).
7. About 80% of the bilirubin produced each day is derived in the spleen from the hemoglobin in senescent red blood cells. Mechanisms that protect against the toxicity of unconjugated bilirubin include binding to plasma albumin, and rapid hepatic uptake and conjugation, which allows its excretion by hepatocytes into bile. Bilirubin conjugates are water-soluble, and not absorbed by enterocytes in the gut, but their excretion in the urine limits their accumulation in the body when hepatocellular or cholestatic jaundice occurs. Unconjugated bilirubin also accumulates in the plasma due to associated hemolysis and to hydrolysis of retained conjugates.
8. In cholestasis, conjugated bile salts and alkaline phosphatase regurgitate into the lymph through permeabilized bile canaliculi and biliary ductules as well as into plasma by reflux across the sinusoidal membrane of the hepatocytes.

W. Exercises

Case I

A 22-year old male University student developed malaise, anorexia, nausea, and right upper abdominal discomfort followed by dark urine, pale stools, and jaundice. He had no pruritus or shaking chills. Six weeks prior to his illness, he had been reunited with his girlfriend who had recently developed jaundice while on holiday in Egypt. He drank four or five beers a day, but he denied abusing intravenous drugs or having receptive anal intercourse.

On examination, he was markedly jaundiced, although he had no cutaneous stigmata of chronic liver disease (e.g., spider angiomas). His liver was enlarged to 4 cm below his right costal margin (17 cm total span), and his spleen was palpable 2 cm below the left costal margin. There was no evidence of ascites or hepatic encephalopathy. His bilirubin was 10 mg/dL, alkaline phosphatase three times normal, AST 30 times normal, serum albumin normal, and prothrombin time 11 seconds prolonged.

- a. Would you expect bilirubin to be present in his urine? Why?
- b. What is the most likely diagnosis, and why?
- c. What blood tests may help to establish the precise cause of his hepatic dysfunction?

Case II

A 32-year-old female nurse developed malaise, fatigue, anorexia, nausea, and intermittent fever of 38 degrees centigrade. Two days later, she complained of right upper quadrant pain, jaundice, pale stools, dark urine, and pruritus. One week previously, she had been diagnosed as having bronchitis. Five days before admission, she was given a course of erythromycin with resolution of her wheezes and coughs. She had received this antibiotic once previously without any adverse reactions. There was no other past history. She denied blood transfusions, sexual activity or contact with jaundiced patients.

On examination: Fever and jaundice were confirmed, but there were no stigmata of chronic liver disease. She was tender in the right upper quadrant, but the liver was not palpable. The bilirubin was 5 mg/dL, alkaline phosphatase twice normal, AST 4 times normal, serum albumin normal. Her total white cell count was elevated with atypical lymphocytes on the blood smear.

- a. What diagnoses would you consider, and why?
- b. How would you proceed to establish the diagnosis?

Case III

A 43-year old female housewife had a prolonged, increasingly severe episode of right epigastric and right upper quadrant discomfort, with fever, severe nausea, and some vomiting. After four days, she sought medical attention. 20 years ago, after her first child, she began to have periodic, late evening short (1-4 hours) attacks of sudden onset of mid-or-right epigastric pain, sometimes fluctuating in intensity, often very severe, with vomiting, but without fever. Sometimes the morning after, she had mild right upper quadrant tenderness. She was told that she had gallstones, based on an oral cholecystogram.

During the past eight years, she also experienced attacks which were of somewhat different character. Although starting in the same manner, the pain was more constant, more intense, more prolonged, and accompanied by rather severe penetrating pain in the back just below the right scapula. There was marked deep tenderness over the entire right upper quadrant of the abdomen, and also soreness and sensitivity of the thoracic wall posteriorly just below the right scapula. The upper abdominal tenderness lingered much longer (1-4 days) after each of these attacks.

On examination, she was uncomfortable and sweating. Oral temperature was 38.5, pulse 100. There was marked tenderness to slight pressure over the entire right upper abdomen, with splinting. Icterus was noted in the eyes. Her gallbladder failed to fill during a radionuclide (PIPIDA) scan. The abdominal pain became much worse, extending higher anteriorly (over the lower chest) and also over the top of the right shoulder, with some exaggeration on deep breathing.

- a. How would you proceed, and why?

Case IV

A 60-year old apple farmer presented to his rural physician with jaundice. He denied abusing alcohol, but stated that he had sprayed his orchard for many years with insecticides. He had not had any contact with jaundiced people or blood transfusions. The physical exam indicated a firm, but smooth liver edge, which was palpable 2 cm below the right costal margin. The tip of the spleen was just palpable. Serum chemistries revealed an alkaline phosphatase four times normal with ALT of 1½ times normal. The prothrombin time was 3 seconds prolonged, but it was corrected by giving subcutaneous vitamin K. The patient was told that he had a viral hepatitis and was followed for another month while his jaundice deepened. He began to experience itching of the skin and was referred to the University Hospital. An ultrasound examination showed a normal common bile duct, but dilated intrahepatic ducts.

- a. What is your diagnostic impression?
- b. What investigations would you order?

Case V

A 46-year old single woman fell down the stairs and fractured three ribs. Three weeks later, she developed nausea, anorexia, right upper quadrant pain, jaundice, dark urine, and pale stools, but no pruritus or chills. She denied taking drugs and claimed to drink alcohol on social occasions only.

On examination, she was obese and had a fever of 38° C with a tachycardia of 100 beats per minute. There was marked jaundice, tenderness in the right upper quadrant, and a positive Murphy's sign. The liver was palpable 6 cm below the right costal margin. There was no splenomegaly or ascites.

Serum bilirubin was 8 mg/dl, alkaline phosphatase three times normal, AST 80 IU per liter, ALT 50 IU per liter, serum albumin 2.9 g/dl. Her white cell count was elevated at 16,000. Her red cells were described as larger than normal in diameter. Her platelet count was reduced. The prothrombin time was prolonged by 7 seconds. An ultrasound scan identified an enlarged liver with a gallstone in the gallbladder, but a normal biliary tree.

- a. What is the most likely diagnosis, and why?

Case VI

A 53-year old man was admitted with hematemesis and melena. He had not experienced abdominal pain or indigestion, and he had taken no drugs. He drank two to three pints of beer per week and four years previously had undergone a cholecystectomy with removal of calculi from the common bile duct. During this operation, the bile duct was accidentally cut and postoperatively he developed septicemia and peritonitis secondary to the leakage of bile.

On examination, he was pale, but there was no jaundice or stigmata of chronic liver disease. His liver was not palpable, but the spleen was detected 4 cm below the left costal margin. No ascites or hepatic encephalopathy were present. He was anemic, and the platelet count was half normal. The remainder of the blood examination, including liver tests, were normal. Upper gastrointestinal endoscopy confirmed bleeding esophageal varices.

- a. What is your explanation for his portal hypertension?
- b. What investigations would you perform?

Case VII

A 50-year old "bon-vivant" who abused alcohol for thirty years presents with bright red hematemesis. After two unsuccessful attempts to staunch esophageal variceal bleeding with sclerotherapy, a transvenous intrahepatic portosystemic shunt (TIPS) was performed. Post-TIPS, he became more encephalopathic and died.

- a. How would you account for his encephalopathy?
- b. Predict the morphological appearance of his liver.

Case VIII

A 60-year-old housewife complains of nagging right upper quadrant discomfort of one month's duration, but otherwise claims good health. The only remarkable finding on exam is an enlarged liver (total span of 17 cm) whose lower edge is 3+ firm. The liver function tests: normal albumin; normal prothrombin time; AST twice normal; alkaline phosphatase four-times normal.

- a. What is your diagnostic suspicion, and how would you proceed?

Extra Question

You are challenged to distinguish between the abrupt onset of jaundice caused by hemolysis versus extrahepatic obstruction.

Which of these diagnostic tests would help to distinguish between these two entities?

- a. The urinary bilirubin
- b. The serum bilirubin (total and direct)
- c. The color of the stools
- d. The serum alkaline phosphatase
- e. Serum AST
- f. Serum ALT

Extra Question

A patient undergoes a prolonged surgery to correct injuries sustained during an automobile accident. Jaundice is obvious on the second postoperative day. The conjugated hyperbilirubinemia rises to 30 mg/dL.

- a. What mechanisms would you invoke to explain the conjugated hyperbilirubinemia?
- b. Would you expect an increase also in unconjugated bilirubin in the plasma? Why?