BILE FORMATION

The Secretion of Canalicular Bile Is Active and Isotonic

Table 45-3. COMPOSITION OF BILE

PARAMETER	HEPATIC BILE	GALLBLADDER BILE
pH	7.5	6.0
Na^+ (mM)	141-165	220
K^+ (mM)	2.7-6.7	14
Ca^{2+} (mM)	1.2-3.2	15
Cl^- (mM)	77-117	31
HCO_3^- (mM)	12-55	19
Total phosphorus (g/liter)	0.15	1.4
Bile acids (g/liter)	3-45	32
Total fatty acids (g/liter)	2.7	24
Bilirubin (g/liter)	1-2	3
Phospholipids (g/liter)	1.4-8.1	34
Cholesterol (g/liter)	1-3.2	6.3
Proteins (g/liter)	2-20	4.5

Data from Boyer JL: Mechanisms of bile secretion and hepatic transport. In Andreoli TE, Hoffman JF, Fanestil DD, Schultz SG (eds): Physiology of Membrane Disorders. New York, Plenum, 1986.

The formation of bile occurs in three discrete steps. First, the hepatocytes actively secrete bile into the bile canaliculi. Second, intrahepatic and extrahepatic bile ducts not only transport this bile but also secrete into it a watery, HCO_3^--rich fluid. These first two steps may produce approximately 900 ml/day of so-called hepatic bile (Table 45-3). This production of hepatic bile is termed **choleresis**. Third, between meals, about half the hepatic bile-perhaps 450 ml/day-is diverted to the gallbladder, which stores the bile and isosmotically removes salts and water. The result is that the gallbladder concentrates the key remaining solutes in bile fluid-bile salts, bilirubin, cholesterol, and lecithin-by 10- to 20-fold. The 500 ml/day of bile that reaches the duodenum through the ampulla of Vater is thus a mixture of relatively "dilute" hepatic bile and "concentrated" gallbladder bile.

The first step in bile formation cannot be ultrafiltration because the hydrostatic pressure in the canaliculi is significantly higher than the sinusoidal perfusion pressure. This situation is in marked contrast to glomerular filtration by the kidney (see Chapter 33), which relies predominantly on passive hydrostatic forces for producing the fluid in Bowman's space. Instead, bile formation is an *active process*. It is sensitive to changes in temperature and to metabolic inhibitors. Bile formation by hepatocytes requires the active, energy-dependent secretion of inorganic and organic solutes into the canalicular lumen, followed by the passive movement of water. This movement of water through the tight junctions between hepatocytes carries with it other solutes by the process of solvent drag (p. 469). Canalicular bile is an isosmotic fluid, thus indicating that the intercellular junctions allow the passage of water and small ions. Further down the biliary tree (i.e., ducts and gallbladder), where the pore size of paracellular junctions is significantly smaller, solvent drag is not as important. Organic solutes do not readily enter bile distal to the canaliculi.

Major Organic Molecules in Bile Include Bile Acids, Cholesterol and Phospholipids

Bile has two important functions: (1) bile provides the sole excretory route for many solutes that are not excreted by the kidney, and (2) secreted bile salts and acids are required for normal lipid digestion (p. 963) and absorption (p. 965).

Both hepatic bile and gallbladder bile are complex secretions that are isosmotic with plasma (~300 mosmole/kg) and consist of water, inorganic electrolytes, and a variety of organic solutes, including

bilirubin, cholesterol, fatty acids, and phospholipid (see Table 45-3). The predominant cation in bile is Na^+, and the major inorganic anions are Cl^- and HCO_3^-. Solutes whose presence in bile is functionally important include micelle-forming bile acids, phospholipids, and immunoglobulin A.

Figure 45-9 Synthesis of bile acids. The liver converts cholesterol to the "primary bile acids"-cholic acid and chenodeoxycholic acid-in a series of 14 reactions occurring in four different cellular organelles. The first reaction is the 7α hydroxylation of cholesterol. In addition, the action of bacteria in the terminal ileum and colon may *de*hydroxylate bile acids, yielding the "secondary bile acids" deoxycholic acid and lithocholic acid. The hepatocytes conjugate most of the primary bile acids to small molecules such as glycine and taurine (not shown) before secreting them into the bile. In addition, those secondary bile acids that return to the liver via the enterohepatic circulation may also be conjugated to glycine or taurine, as shown in the figure. The liver may also conjugate some primary and secondary bile acids to sulfate or glucuronate (not shown).

Bile acids promote dietary lipid absorption through their micelle-forming properties (p. 965). As shown in Figure 45-9, hepatocytes synthesize the so-called **primary bile acids-**cholic acid and chenodeoxycholic acid-from cholesterol. Indeed, biliary excretion of cholesterol and conversion of **cholesterol** to bile acids are the principal routes of cholesterol excretion and catabolism, thus making bile formation pivotal for total-body cholesterol balance. The first step in this conversion is catalyzed by cholesterol 7α-hydroxylase, a specific cytochrome P-450 enzyme that is located in the SER. As we shall see later, **secondary bile acids** are the products of bacterial dehydroxylation in the terminal ileum and colon. After being absorbed and returning to the liver ("enterohepatic circulation," discussed later), these secondary bile acids may also undergo conjugation. Figure 45-9 shows typical examples of conjugation reactions. ***Cholesterol 7α-Hydroxylase***

Phospholipids in bile help to solubilize cholesterol as well as diminish the cytotoxic effects of other bile acids on hepatocytes and bile duct cells. **Immunoglobulin A** inhibits bacterial growth in bile.

Excretory or **waste products** found in bile include cholesterol, bile pigments, trace minerals, plant sterols, lipophilic drugs and metabolites, antigen-antibody complexes, and oxidized glutathione.

Bile is also the excretory route for compounds that do not readily enter the renal glomerular filtrate, either because they are associated with proteins such as albumin or because they are associated with formed elements in blood. Although these compounds are generally lipophilic, they also include the heavy metals. Some bile acids (e.g., the trihydroxy bile acid cholic acid) are only partly bound to serum albumin and may therefore enter the glomerular filtrate. However, they are actively reabsorbed by the renal tubule. In health, bile acids are virtually absent from the urine.

Canalicular Bile Flow Has a Constant Component Driven by the Secretion of Small Organic Molecules and a Component Driven by the Secretion of Bile Acids

Total bile flow is the sum of the bile flow from hepatocytes into the canaliculi ("canalicular flow") and the additional flow from cholangiocytes into the bile ducts ("ductular flow"). In most species, the rate of **canalicular bile secretion** (i.e., milliliters per minute) increases more or less linearly with the rate of bile acid secretion (i.e., moles per minute). Canalicular bile flow is the sum of two components (Fig. 45-10): (1) a "constant" component that is independent of bile acid secretion (bile acid-*in*dependent flow) and (2) a rising component that increases linearly with bile acid secretion (bile acid-*dependent* flow). In humans, most of the canalicular bile flow is bile acid dependent. If we now add the **ductular secretion**, which is also "constant," we have the **total bile flow** in Figure 45-10. We will discuss the canalicular secretion in the remainder of this section, and ductular secretion in the following section.

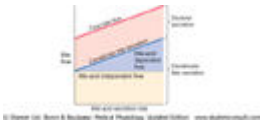

Figure 45-10 Components of bile flow.

BILE ACID-INDEPENDENT FLOW IN THE CANALICULI.

The secretion of *organic* compounds probably provides the major driving force for bile acid-independent flow. For example, glutathione, present in bile in high concentrations, may generate a potent osmotic driving force for canalicular bile formation. **Contribution of Inorganic Solutes to Bile-Acid-Independent Flow**

BILE ACID-DEPENDENT FLOW IN THE CANALICULI.

The negatively charged bile salts in bile are in a micellar form and are-in a sense-large polyanions. Thus, they are effectively out of solution and have a low **osmotic activity coefficient**. However, the positively charged counterions accompanying these micellar bile acids are still in aqueous solution and may thus represent the predominant osmotic driving force for water movement in bile acid-dependent flow. If one infuses an animal with a nonphysiological bile acid that does not form micelles or one that forms micelles only at a rather high concentration, the osmotic activity will be higher and thus the exogenous bile acid will be more effective in producing bile acid-dependent flow. In other words, the slope of the *blue* "bile acid-dependent" line in Figure 45-10 would be steeper than for physiological bile acids.

Bile flow does not always correlate with the osmotic activity of the bile acid. In some cases, bile acids increase electrolyte and water flux by other mechanisms, such as by stimulating Na^+-coupled cotransport mechanisms or by modulating the activity of other solute transporters. For example, the bile acid ursodeoxycholic acid produces a substantial increase in bile flow by markedly stimulating biliary HCO_3^- excretion.

Bile acids in the lumen may also stimulate the secretion of other solutes by trapping them in the lumen. These solutes include bilirubin and other organic anions, as well as lipids such as cholesterol and phospholipids. The mixed micelles formed by the bile acids apparently sequester these other solutes, thus lowering their effective luminal concentration and favoring their entry. Thus, excretion of cholesterol and phospholipid is negligible when bile acid output is low, but it increases and approaches maximum values as bile acid output increases.

Secretin Stimulates the Cholangiocytes of Ductules and Ducts to Secrete a Watery, HCO_3^- Rich Fluid

As we saw in the previous section, biliary epithelial cells, or **cholangiocytes**, are the second major source of the fluid in hepatic bile. Experimentally, one can isolate cholangiocytes from normal liver or from the liver of experimental animals in which ductular hyperplasia has been induced by ligating the bile duct. These cholangiocytes have a number of transporters (Fig. 45-11), including an apical Cl-HCO_3 exchanger, and several apical Cl^- channels, including CFTR (p. 916). In a mechanism that may be similar to that in pancreatic duct cells (p. 914), the Cl-HCO_3 exchanger, in parallel with the Cl^- channels for Cl^- recycling, can secrete an HCO_3^--rich fluid. **Bicarbonate Secretion by Cholangiocytes**

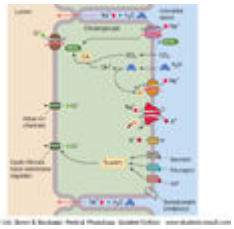

Figure 45-11 Secretion of an HCO_3^--rich fluid by cholangiocytes. The apical step of HCO_3^- secretion by the duct cell is mediated by a Cl-HCO_3 exchanger. The Cl^- recycles back to the lumen via Cl^- channels, such as cystic fibrosis trans-membrane regulator (CFTR). The basolateral step of HCO_3^- Secretion probably is mediated in part by the uptake of HCO_3^- via an electrogenic Na/HCO_3 cotransporter. The uptake of CO_2, combined with the extrusion of H^+ via an Na-H exchanger and an H^+ pump generates the rest of the HCO_3^- via carbonic anhydrase (CA). Secretin, glucagon, vasoactive intestinal peptide (VIP), and gastrin-releasing peptide (GRP) all are choleretics. Somatostatin either enhances fluid absorption or inhibits secretion.

A complex network of hormones, mainly acting via cAMP, regulate cholangiocyte secretory function.

Secretin receptors (p. 902) are present on the basolateral membranes of cholangiocytes, which explains why **secretin** produces a water-rich choleresis-that is, a bile rich in HCO_3^- (i.e., alkaline) but diluted in bile acids. Similarly, the hormones **glucagon** (p. 1081) and **VIP** (vasoactive intestinal peptide; p. 898) also produce an HCO_3^--rich choleresis at the level of the ducts. These hormones raise $[cAMP]_i$ and thus stimulate apical Cl^- channels and the Cl-HCO_3 exchanger. A Ca^{2+}-activated Cl^- channel is also present in the apical membrane. **Regulation of Cholangiocyte Secretion**

Cholangiocytes are also capable of reabsorbing fluid and electrolytes, as suggested by the adaptation that occurs after removal of the gallbladder (i.e., cholecystectomy). Bile found within the common bile duct of cholecystectomized, fasting animals is similar in composition to the concentrated bile typically found in the gallbladder. Thus, the ducts have partially taken over the function of the gallbladder (see later).

The hormone **somatostatin** inhibits bile flow by lowering $[cAMP]_i$, an effect opposite that of secretin. This inhibition may be caused by enhancing fluid reabsorption by bile ducts or by inhibiting ductular secretion of the HCO_3^--rich fluid discussed earlier.

Solutes reabsorbed from bile by cholangiocytes can be returned to the hepatocyte for resecretion. As shown earlier in Figure 45-2, the intralobular bile ducts are endowed with a rich peribiliary vascular plexus that is supplied by the hepatic artery. The blood draining this plexus finds its way into the hepatic sinusoids. This plexus is analogous to the capillaries of the gut, which via the portal vein, also find their way into the hepatic sinusoids. Thus, some solutes, such as the hydrophilic bile acid ursodeoxycholic acid, may be absorbed by the cholangiocytes from bile and returned to the hepatocytes for resecretion, thus inducing significant choleresis.

The Gallbladder Stores and Concentrates Bile and Delivers It to the Duodenum During a Meal

The gallbladder is not an essential structure of bile secretion, but it does serve to concentrate bile acids up to 10-or even 20-fold during interdigestive periods. Tonic contraction of the sphincter of Oddi facilitates gallbladder filling by maintaining a positive pressure within the common bile duct. As we noted earlier, up to 50% of hepatic bile-or approximately 450 ml/day-is diverted to the gallbladder during fasting. The remaining approximately 450 ml/day passes directly into the duodenum. Periods of gallbladder filling between meals are interrupted by brief periods of partial emptying of concentrated bile and probably aspiration of dilute hepatic bile in a process analogous to the function of a bellows.

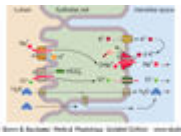

Figure 45-12 Isotonic fluid reabsorption by the gallbladder epithelium. The gallbladder epithelium performs the isotonic absorption of NaCl. The apical step is parallel Na-H exchange and Cl-HCO_3 exchange. Because Na-H exchange is somewhat faster, there is a net secretion of acid into the lumen. The basolateral step of NaCl absorption is mediated by the Na-K pump and Cl^- channels. K^+ channels provide a route for basolateral K^+ recycling. Water follows passively through the tight junctions and through the basolateral membrane.

Bile salts and certain other components of bile are concentrated up to 20-fold within the gallbladder lumen because they are left behind during the isotonic reabsorption of NaCl and $NaHCO_3$ by the leaky gallbladder epithelium (Figure 45-12). The apical step of NaCl uptake and transport is electroneutral and mediated by parallel Na-H and Cl-HCO_3 exchangers. At the basolateral membrane, Na^+ exits via the Na-K pump, whereas Cl^- most likely exits by Cl^- channels. Both water and HCO_3^- move passively from lumen to blood via the tight junctions, which are rather leaky. Water can also move through the cell. The net transport is isotonic, which leaves behind gallbladder bile that is also isotonic but has a higher concentration of bile salts, K^+, and Ca^{2+}. Net fluid and electrolyte transport across the gallbladder epithelium is under hormonal regulation. Both VIP (released from neurons innervating the gallbladder) and serotonin inhibit net fluid and electrolyte absorption. Conversely, α-adrenergic blockade of neuronal VIP release increases fluid absorption.

Although the gallbladder reabsorbs NaCl by parallel Na-H and Cl-HCO_3 exchange at the apical membrane, Na-H exchange outstrips Cl-HCO_3 exchange, with the end result being net secretion of **H^+ ions**. This action

neutralizes the
HCO_3^- and acidifies the bile. The H^+ secreted by the gallbladder protonates the intraluminal contents. This action greatly increases the solubility of calcium salts in bile and reduces the likelihood of calcium salt precipitation and **gallstone formation**. Common "pigment gallstones" contain one or more of several calcium salts, including carbonate, bilirubinate, phosphate, and free fatty acids. The solubility of each of these compounds is significantly increased by the acidification of bile.

Integration link: Gallstones - two principal types

Taken from Clinical Medicine 5E

Mucus secretion by gallbladder epithelial cells results in the formation of a polymeric gel that protects the apical surface of the gallbladder epithelium from the potentially toxic effects of bile salts. However, excessive mucin synthesis can be deleterious. For example, in animal models of cholesterol cholelithiasis (i.e., formation of gallstones made of cholesterol), a marked increase in mucin release precedes crystal and stone formation.

page 992
page 993

The Relative Tone of the Gallbladder and Sphincter of Oddi Determines Whether Bile Secreted by the Liver Flows from the Common Hepatic Duct into the Gallbladder or into the Duodenum

Bile exiting the liver and flowing down the common hepatic duct reaches a bifurcation that permits flow either into the cystic duct and then into the gallbladder or into the common bile duct, through the sphincter of Oddi, and into the duodenum (see Fig. 45-4). The extent to which bile takes either path depends on the relative resistance of the two pathways.

The sphincter of Oddi-which also controls the flow of pancreatic secretions into the duodenum-corresponds functionally to a short (4 to 6 mm) zone within the wall of the duodenum. The basal pressure within the lumen of the duct at the level of the sphincter is 5 to 10 mm Hg. The pressure in the lumen of the resting common bile duct is also 5 to 10 mm Hg, compared with a pressure of approximately 0 mm Hg inside the duodenum.

CHOLESTASIS

Ther term *cholestasis* refers to the suppression of bile secretion. Biliary constituents may therefore be retained within the hepatocyte and regurgitated into the systemic circulation. Cholestasis causes three major groups of negative effects: first, regurgitation of bile components (bile acids, bilirubin) into the systemic circulation gives rise to the symptoms of jaundice and pruritus (itching). Second, cholestasis damages hepatocytes, as evidenced by the release of "liver enzymes" (e.g., alkaline phosphatase) into the plasma. Third, because the bile acids do not arrive in the duodenum, lipid digestion and absorption may be impaired.

Many acute and chronic liver diseases produce cholestasis by mechanically obstructing the **extrahepatic** bile ducts or impairing bile flow at the level of the hepatocytes or **intrahepatic** bile ducts. The mechanisms underlying the obstructive and functional forms of cholestasis are complex and have not been completely defined. Experimental models of cholestasis have produced multiple abnormalities: (1) altered plasma membrane composition and fluidity; (2) inhibition of membrane proteins, including the Na-K pump; (3) reduced expression of genes encoding transporters for bile acids and other organic anions; (4) increased permeability of the paracellular pathway, with backdiffusion of biliary solutes into the plasma; (5) altered function of microfilaments, with decreased contractions of bile canaliculi; and (6) loss of the polarized distribution of some plasma membrane proteins.

The basal contraction of the sphincter prevents reflux of the duodenal contents into the common bile duct. In its basal state, the sphincter exhibits high-pressure, phasic contractions several times per minute. These contractions are primarily peristaltic and antegrade to provide a motive force toward the duodenum. Thus, the sphincter of Oddi acts principally as an adjustable occluding mechanism and regulator of bile flow.

Both hormonal and cholinergic mechanisms appear to be involved in gallbladder emptying. Dietary lipid

stimulates the release of cholecystokinin (CCK) from duodenal I cells (p. 920). This CCK not only stimulates pancreatic secretion but also causes smooth-muscle contraction and evacuation of the gallbladder. The coordinated response to CCK also includes relaxation of the sphincter of Oddi, thus enhancing bile flow into the duodenum.