Cholesterol cholelithiasis is one of the most prevalent and costly digestive diseases in Western countries. At least 20 million Americans (~12% of adults) have gallstones. The prevalence of gallstones appears to be rising due to the epidemic of obesity, associated with insulin resistance and the metabolic syndrome. Each year, roughly 1 million new cases are discovered. Although many gallstones are “silent,” about one third eventually cause symptoms and complications. An estimated 700,000 cholecystectomies are performed for gallstone disease, and medical expenses for the treatment of gallstones exceeds $6 billion annually. In addition, unavoidable complications of gallstones result in 3000 deaths (0.12% of all deaths) per year. In the United States, persons with gallstone disease have increased overall, cardiovascular disease, and cancer mortality.

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TYPES OF GALLSTONES

Based on chemical composition and macroscopic appearance, gallstones are divided into 3 types: cholesterol, pigment, and rare stones. The majority (~75%) of gallstones in the United States and Europe are cholesterol stones, which consist mainly of cholesterol monohydrate crystals and precipitates of amorphous calcium bilirubinate, often with calcium carbonate or phosphate in one of the crystalline polymorphs. These stones are usually subclassified as either pure cholesterol or mixed stones that contain at least 50% cholesterol by weight. The remaining gallstones are pigment stones that contain mostly calcium bilirubinate and are subclassified into two groups: black pigment stones (~20%) and brown pigment stones (~4.5%). Rare gallstones (~0.5%) include calcium carbonate stones and fatty acid–calcium stones. Gallstones also are classified by their location as intrahepatic, gallbladder, and bile duct (choledocholithiasis) stones. Intrahepatic stones are predominantly brown pigment stones. Gallbladder gallstones are mainly cholesterol stones, with a small group of black pigment stones. Bile duct stones are composed mostly of mixed cholesterol stones.
EPIDEMIOLOGY

Investigations of gallstone prevalence are more common than those of gallstone incidence because of the nature of the statistical analyses. Prevalence is often defined as the number of cases of gallstones at any one point or period of time divided by the population at risk for gallstones. Incidence is usually defined as the number of new cases of gallstones occurring in a time period divided by the population at risk for forming stones. Therefore, the determination of incidence requires that investigation for gallstones be performed at a minimum of two different times—that is, at the beginning and at the end of an interval of time. By contrast, prevalence can be determined by sampling at only one point in time—for example, at US screening or autopsy.

Although determining the true incidence of gallstones in a given population is not easy, a large study of the incidence of gallstones in the Danish population has been performed. The 5-year incidence of gallstones was 0.3%, 2.9%, 2.5%, and 3.3% for Danish men, and 1.4%, 3.6%, 3.1%, and 3.7% for Danish women ages 30, 40, 50, and 60, respectively. Women have a higher incidence than men at ages 30 and 40, but the difference declines with increasing age. These incidence rates may reflect an interaction between genetic and environmental factors on gallstone formation in the specific populations studied because they are in accordance with estimated prevalence rates reported for Denmark and other populations. In a major Italian study, the incidence of gallstones was obtained at 10 years’ follow-up in an originally gallstone-free cohort in the town of Sirmione. This study revealed that new cases of gallstones developed at a rate of 0.5% per year. Although age, female gender, parity, obesity, and hypertriglyceridemia were associated with gallstones in the cross-sectional prevalence study of Sirmione, multivariate analysis of risk factors for the formation of gallstones in the longitudinal study identified only age and obesity as risk factors.

Differences in the incidence of gallstone formation among different populations are striking, suggesting that genetic factors play a crucial role in the pathogenesis of cholesterol gallstones. Pathogenic factors are likely to be multifactorial and to vary among populations. Most relevant studies have found that the prevalence of gallstones in women ranges from 5% to 20% between the ages of 20 and 55 and from 25% to 30% after the age of 50. The prevalence in men is approximately half that of women of the same age.

US screening or autopsy data are often used to estimate the prevalence of gallstone disease in different populations, as illustrated in Figure 65-1. Although US screening cannot be used to distinguish cholesterol from pigment stones, 70% to 80% of detected gallbladder gallstones are assumed to be cholesterol stones.

The prevalence of gallstones in American Pima Indians was investigated by oral cholecystography. The well-studied Pima Indians in southern Arizona exhibit a high prevalence of gallstones, which occur in 70% of the women after the age of 25 years. Subsequently, real-time US was used for screening in nationally representative samples of civilian Mexicans, Hispanic white Americans, non-Hispanic white Americans, and non-Hispanic black Americans of both genders ages 20 to 74. The cross-sectional prevalence rates of gallstones were found to be highest in certain tribes of Native Americans (e.g., Pima Indians), higher in Hispanic Americans than in whites, and lowest in black Americans.

Figure 65-2 shows the world distribution of cholesterol gallstones. American Pima Indians are an extremely high-risk population. Other high-risk populations include Native American groups in North and South America and Scandinavians, of whom 50% develop gallstones by age 50. By contrast, African populations show the lowest risk of gallstones. The prevalence of gallstones in Asian populations is intermediate. Within a given population, first-degree relatives of index cases of persons with gallstones are 4.5 times as likely to form gallstones as matched controls, thereby underscoring the importance of genetic predisposition.

Risk Factors

Age and Gender

Epidemiologic and clinical studies have found that cholesterol gallstones occur infrequently in childhood and adolescence,
and the prevalence of cholesterol gallstones increases linearly with age in both genders and approaches 50% at age 70 in women. Furthermore, older adults are at higher risk for complications of gallstones, and mortality from surgery is often unacceptably high in patients older than 65. Cholesterol saturation of bile is significantly higher in older adult Swedes and Chilean women than in younger controls, and age correlates positively with an increased hepatic secretion rate of biliary cholesterol. In animals, aging has been shown to be associated with increased cholesterol gallstone formation as a result of increased biliary secretion and intestinal absorption of cholesterol, decreased hepatic synthesis and secretion of bile salts, and reduced gallbladder contractility.

Epidemiologic investigations have found, and clinical studies have confirmed, that at all ages, women are twice as likely as men to form cholesterol gallstones. The difference between women and men begins during puberty and continues through the childbearing years because of the effects of female sex hormones and differences between the sexes in metabolism of cholesterol by the liver in response to estrogen. Human and animal studies have shown that estrogen increases the risk of cholesterol gallstones by augmenting hepatic secretion of biliary cholesterol, thereby leading to an increase in cholesterol saturation of bile.

**Pregnancy and Parity**

Pregnancy is a risk factor for the development of biliary sludge and gallstones. During pregnancy, bile becomes more lithogenic because of a significant increase in estrogen levels, which result in increased hepatic cholesterol secretion and supersaturated bile. In addition, gallbladder motility is impaired, with a resulting increase in gallbladder volume and bile stasis. These alterations promote the formation of sludge and stones in the gallbladder. Increased progestogen concentrations also reduce gallbladder motility. Because plasma concentrations of sex hormones, especially estrogen, increase linearly with duration of gestation, the risk of gallstone formation is high in the third trimester of pregnancy. Increasing parity is probably a risk factor for gallstones, especially in younger women.

**Rapid Weight Loss**

Rapid weight loss is a well-known risk factor for the formation of cholesterol gallstones. As many as 50% of obese patients who undergo gastric bypass surgery form biliary sludge and eventually gallstones within 6 months after surgery. Gallstones also develop in 25% of patients who undergo strict dietary restriction. Furthermore, about 40% of these patients display symptoms related to gallstones within the same 6-month period. The mechanisms by which rapid weight loss causes gallstone formation include enhanced hepatic secretion of biliary cholesterol during caloric restriction, increased production of mucin by the gallbladder, and impaired gallbladder motility. Gallstones may be prevented in this high-risk population by prophylactic administration of ursodeoxycholic acid (UDCA), which, in a dose of 600 mg/day, has been reported to reduce the prevalence of gallstones from 28% to 3% in obese patients on a very-low-calorie diet.

**Total Parenteral Nutrition**

TPN is associated with development of cholelithiasis and acalculous cholecystitis. As early as 3 weeks after initiation of TPN, biliary sludge often forms in the gallbladder because of...
prolonged fasting. In addition, the sphincter of Oddi may fail to relax, leading to preferential flow of bile into the gallbladder. Approximately 45% of adults and 43% of children form gallstones after 3 to 4 months of TPN.38,39 Because patients who receive TPN often have serious medical problems and are not good candidates for abdominal surgery, prophylactic treatment to prevent gallstones should be prescribed if no contra-indication exists. CCK octapeptide administered twice daily via an IV line to patients on long-term TPN has proved to be safe and cost effective30 and should be used routinely in TPN-treated patients.

**Biliary Sludge**

Biliary sludge is a crucial intermediate stage in the pathogenesis of both cholesterol and pigment gallstones because it facilitates crystallization and agglomeration of solid plate-like cholesterol monohydrate crystals, as well as precipitation of calcium bilirubinate, and ultimately develops into macroscopic stones.31,32 In addition, biliary sludge can induce acute cholecystitis, cholangitis, and acute pancreatitis. Furthermore, biliary sludge is associated with many conditions that predispose to gallstone formation, including pregnancy, rapid weight loss, spinal cord injury, long-term TPN, and thermore, biliary sludge is associated with many conditions that predispose to gallstone formation, including pregnancy, thermore, biliary sludge is associated with many conditions that predispose to gallstone formation, including pregnancy, rapid weight loss, spinal cord injury, long-term TPN, and treatment with octreotide. Although biliary sludge is reversible in most cases, it persists or disappears and reappears in 12% to 20% of affected persons and eventually leads to gallstones.33 UDCA treatment of patients with persistent biliary sludge decreases the frequency of clinical complications of biliary sludge.

**Drugs**

**Estrogens**

Most but not all relevant clinical studies have shown that use of oral contraceptive steroids and conjugated estrogens in premenopausal women doubles the prevalence of cholesterol gallstones.34,35 Moreover, in a large French study of 45,984 postmenopausal women, use of hormone replacement therapy was associated with an increased risk of cholecystectomy hazard ratio (HR), 1.10); the increase in risk was limited to women receiving unopposed estrogen (HR, 1.38).36 Administration of estrogen to postmenopausal women and estrogen therapy to men with prostatic carcinoma have similar lithogenic effects.37,38 Therefore, estrogen has been proposed to be an important risk factor for the formation of cholesterol gallstones. In mice, the hepatic estrogen receptor α, but not β, plays a crucial role in cholesterol gallstone formation in response to estrogen.39 The hepatic estrogen receptor α, which is activated by estrogen, interferes with the negative feedback regulation of cholesterol biosynthesis by stimulating the sterol-regulatory element binding protein-2 (SREBP-2) pathway, with the resulting activation of the SREBP-2-responsive genes in the cholesterol biosynthetic pathway.40 These alterations lead to increased hepatic secretion of newly synthesized cholesterol and supersaturation of bile, thereby predisposing to precipitation of solid cholesterol monohydrate crystals and formation of gallstones. In addition, estrogen induces a decrease in plasma low-density lipoprotein (LDL) cholesterol levels and an increase in plasma high-density lipoprotein (HDL) cholesterol concentrations. The decrease in plasma LDL levels is a result of increased expression of the hepatic LDL receptor, which increases the clearance of plasma LDL. The increased uptake of LDL by the liver may also result in increased secretion of cholesterol into bile. High levels of estrogen may induce gallbladder hypomotility and consequently bile stasis.

**Lipid-Lowering Drugs**

Lipid-lowering drugs may influence the formation of gallstones because they regulate key pathways in cholesterol and bile salt metabolism. Clofibrate is a lipid-lowering drug associated with gallstone formation. Clofibrate induces cholesterol supersaturation in bile and diminishes bile salt concentrations by reducing the activity of cholesterol 7α-hydroxylase (the rate-limiting enzyme in bile salt synthesis of classical pathway) (see Chapter 64).41 The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) reduce the biliary cholesterol saturation index, but their role in the prevention or therapy of gallstone disease requires further investigation in humans.42 The potent cholesterol absorption inhibitor ezetimibe prevents formation of cholesterol gallstones and facilitates dissolution of gallstones in gallstone-susceptible C57L mice. Ezetimibe also may act as a potent biliary cholesterol-desaturating agent in patients with gallstones.43,44 Cholestyramine and nicotinic acid have no association with gallstone formation.

**Octreotide**

The somatostatin analog octreotide increases the prevalence of gallstones when administered to patients as treatment for acromegaly, with some 28% of treated acromegalic patients forming gallstones. Acromegalic patients who are treated with octreotide display dysfunctional gallbladder motility, sluggish intestinal transit, and increased colonic deoxycholic acid formation and absorption,45 all of which facilitate formation of cholesterol gallstones.

**Ceftriaxone**

The third-generation cephalosporin ceftriaxone has a long duration of action, with much of the drug excreted in the urine. Approximately 40% of the drug, however, is secreted in an unmetabolized form into bile, where its concentration reaches 100 to 200 times that of the concentration in plasma and exceeds its saturation level in bile. Once the saturation level of ceftriaxone is exceeded, it complexes with calcium to form insoluble salts, thereby resulting in formation of biliary sludge. Up to 43% of children who receive high doses of ceftriaxone (60 to 100 mg/kg/d) have been reported to form biliary sludge, and about 19% of these patients experience biliary symptoms.46 The sludge usually disappears after ceftriaxone is discontinued.

**Lipid Abnormalities**

Epidemiologic investigations have shown that plasma HDL cholesterol levels are inversely correlated with the prevalence of cholesterol gallstones.47 By contrast, hypertriglyceridemia is positively associated with an increased prevalence of gallstones.48 These seemingly independent variables are actually interrelated because high plasma triglyceride levels tend to increase with increasing body mass and are inversely correlated with plasma HDL levels. Interestingly, high plasma total and LDL cholesterol levels are not likely to be risk factors for the formation of gallstones.

**Systemic Diseases**

**Obesity and Insulin Resistance**

 Obesity is a well-known risk factor for cholelithiasis and gallstone prevalence and is rising in frequency with the worldwide obesity epidemic and the increasing incidence of insulin
resistance. A large prospective study of obese women demonstrated a strong linear association between BMI and the prevalence of cholelithiasis. In this study, the risk of gallstones was 7-fold higher in women with the highest BMI (>45 kg/m²) than in nonobese control women. Obesity is associated with increased hepatic secretion of cholesterol into bile, possibly because of higher enzymatic activity of HMG-CoA reductase and increased cholesterol synthesis in the liver. As a result, gallbladder bile is more lithogenic in obese than in nonobese persons, and a higher ratio of cholesterol to solubilizing lipids (bile acids and phospholipids) is observed in the former group. These alterations predispose to cholesterol crystallization and gallstone formation. Gallbladder motility is often impaired in obese persons, thereby promoting mucin secretion and accumulation, as well as cholesterol crystallization. The effect of pronucleating and antinucleating factors on cholesterol crystallization and gallstone formation warrants further investigation in gallbladder bile of obese and nonobese subjects.

Diabetes Mellitus

Patients with diabetes mellitus have long been considered to be at increased risk of developing gallstones because hypertriglyceridemia and obesity are associated with diabetes mellitus and because gallbladder motility is often impaired in patients with diabetes mellitus. Proving diabetes mellitus is an independent risk factor for gallstones has been difficult, however. Mice with hepatic insulin resistance induced by liver-specific disruption of the insulin receptor are markedly predisposed to formation of cholesterol gallstones. Hepatic insulin resistance promotes hepatic secretion of biliary cholesterol by increasing expression of the hepatic cholesterol transporters Abcg5 and Abcg8 through the forkhead transcription factor FoxO1 pathway. It also reduces expression of the bile salt synthetic enzymes, particularly oxysterol 7α-hydroxylase, thereby resulting in a lithogenic bile salt profile.

Diseases of the ileum

Disease or resection of the terminal ileum has been found to be a risk factor for gallstone formation. For example, intestinal bile salt absorption is often impaired in patients with Crohn’s disease, who are at increased risk of gallstones. The loss of specific bile salt transporters (e.g., ileal apical sodium-dependent bile acid transporter) in the terminal ileum may result in excessive bile salt excretion in feces and a diminished bile salt pool size, presumably with a consequent increase in the risk of cholesterol gallstones. These changes may also lead to formation of pigment gallstones because increased bile salt delivery to the colon enhances solubilization of unconjugated bilirubin, thereby increasing bilirubin concentrations in bile.

Spinal Cord Injuries

Spinal cord injuries are associated with a high prevalence of gallstones, which have been reported in some 31% of such patients, who have an annual rate of biliary complications of 2.2%. Although the complication rate associated with gallstones in patients with spinal cord injuries is at least 2-fold higher than the rate of gallstones in the general population, the relative risk is still low enough that prophylactic cholecystectomy is probably not justified. The mechanisms responsible for the association between spinal cord injuries and gallstone formation remain unclear. Gallbladder relaxation is impaired in these patients, but gallbladder contraction in response to a meal is normal. Therefore, the increased risk of gallstones is unlikely to be due to biliary stasis alone.

Protective Factors

Statins

Use of statins has been associated with a decreased risk of gallstone disease in 2 large case-control studies. The first study compared 27,035 patients with gallstone disease requiring cholecystectomy with 106,531 matched controls and showed a benefit to long-term statin use (>20 prescriptions filled and use of statins for >1.5 years); statin use was associated with a decreased risk of gallstone disease requiring cholecystectomy (adjusted odds ratio [OR], 0.64). Similar results were observed in a population study from Denmark of 32,494 patients with gallstone disease matched with 324,929 controls. The odds ratio of having gallstone disease in current and prior users of statins (>20 prescriptions filled) was 0.76 and 0.79, respectively, compared with controls.

Ascorbic Acid

The observation that deficiency of ascorbic acid (vitamin C) is associated with development of gallstones in guinea pigs prompted investigation of the relationship between ascorbic acid levels and gallstones in humans. Serum ascorbic acid levels have been correlated with clinical or asymptomatic gallstones in 7042 women and 6088 men who were enrolled in the Third National Health and Nutrition Examination Survey. Among women, but not men, each standard deviation increase in serum ascorbic acid levels was associated with a 13% lower prevalence of clinical gallbladder disease.

Coffee

In a 10-year follow up of 46,000 male health professionals, subjects who consistently drank 2 to 3 cups of regular coffee per day were approximately 40% less likely to develop symptomatic gallstones. Drinking 4 or more cups per day was even more beneficial (relative risk 0.55), but there was no benefit to drinking decaffeinated coffee. A similar benefit to regular coffee was noted in a cohort study involving 81,000 women.

COMPOSITION AND ABNORMALITIES OF BILE

Physical Chemistry of Bile

Chemical Composition of Bile

Cholesterol, phospholipids, and bile salts are the 3 major lipid species in bile, and bile pigments are minor solutes. Cholesterol accounts for up to 95% of the sterols in bile and gallstones; the remaining 5% of the sterols are cholesterol precursors and dietary sterols from plant and shellfish sources. Concentrations of cholestereryl esters are negligible in bile and account for less than 0.02% of total sterols in gallstones. The major phospholipids are lecithins (phosphatidylcholines), which account for more than 95% of total phospholipids; the remainder consists of cephalins (phosphatidylethanolamines) and a trace amount of sphingomyelin. Phospholipids constitute 15% to 25% of total lipids in bile. Lecithins are insoluble amphiphilic molecules with a hydrophilic zwitterionic phosphocholine head group and hydrophobic tails that include 2 long fatty acyl chains. Biliary lecithins possess a saturated C-16 acyl chain in the sn-1 position and an unsaturated C-18 or C-20 acyl chain in the sn-2 position. The major molecular...
species of lecithins (with corresponding frequencies) in bile are 16:0 to 18:2 (40% to 60%), 16:0 to 18:1 (5% to 25%), 18:0 to 18:2 (1% to 16%), and 16:0 to 20:4 (1% to 10%). Lecithins are synthesized principally in the endoplasmic reticulum of the hepatocyte from diacylglycerols through the cytidine diphosphate-choline pathway. The common bile salts typically contain a steroid nucleus of 4 fused hydrocarbon rings with polar hydroxyl functions and an aliphatic side chain conjugated in amide linkage with glycine or taurine. In bile, more than 95% of bile salts are 5β,7,12-24-hydroxylated acidic steroids that are amide-linked to glycine or taurine in an approximate ratio of 3:1. Bile salts constitute approximately two thirds of the solute mass of normal human bile by weight. The hydrophilic (polar) areas of bile salts are the hydroxyl groups and hydrophobic (nonpolar) area is the ringed steroid nucleus. Because they possess both hydrophilic and hydrophobic surfaces, bile salts are highly soluble, detergent-like, amphiphilic molecules. Their high aqueous solubility is due to their capacity to self-assemble into micelles when a critical micellar concentration is exceeded.

The primary bile salts are hepatic catalytic products of cholesterol and are composed of cholate (a trihydroxy bile salt) and chenodeoxycholate (a dihydroxy bile salt) (see Chapter 64). The secondary bile salts are derived from the primary bile salt species by the action of intestinal bacteria in the ileum and colon and include deoxycholate, Ursodeoxycholate, and lithocholate. The most important of the conversion reactions is 7α-dehydroxylation of primary bile salts to produce deoxycholate from cholate and lithocholate from chenodeoxycholate. Another important conversion reaction is the 7α-dehydrogenation of chenodeoxycholate to form 7α-oxo-lithocholate. This bile salt does not accumulate in bile but is metabolized by hepatic or bacterial reduction to form the tertiary bile salt chenodeoxycholate (mainly in the liver) or its 7β-epimer Ursodeoxycholate (primarily by bacteria in the colon).

Bile pigments are minor solutes and formed as a metabolic product of certain porphyrins. They account for roughly 0.5% of total lipids in bile by weight. They are mainly bilirubin conjugates with traces of porphyrins and unconjugated bilirubin. Bilirubin can be conjugated with a molecule of glucuronic acid, which makes it soluble in water. In human bile, bilirubin monoglucuronides and diglucuronides are the major bile pigments. Other bile pigments are monoconjugates and diconjugates of xyllose, glucose, and glucuronic acid and various homoclonjugates and heteroconjugates of them.

Proteins and elements are also found in bile. Albumin appears to be the most abundant protein in bile, followed by immunoglobulins G and M, apolipoproteins AI, AII, B, C1, and CII, transferrin, and α1-macroglobulin. Other proteins that have been identified but not quantitated in bile include EGF, insulin, haptoglobin, CCK, lysosomal hydrolyase, and amylose. Elements detected in bile include sodium, phosphorus, potassium, calcium, copper, zinc, iron, manganese, molybdenum, magnesium, and strontium.

**Physical States of Biliary Lipids**

Cholesterol is nearly insoluble in water, and the mechanism by which cholesterol is solubilized in bile is complex because bile is an aqueous solution. The 2 main types of macromolecular aggregates in bile are micelles and vesicles, which greatly enhance the solubilization of cholesterol in bile.

Bile salts are soluble in an aqueous solution because they are amphiphilic, in that they have both hydrophilic and hydrophobic areas. This unique property of bile salts is dependent on the number and characteristics of the hydroxyl groups and side chains, as well as the composition of the particular aqueous solution. When bile salt concentrations exceed the critical micellar concentration, their monomers can spontaneously aggregate to form simple micelles. The simple micelles (~3 nm in diameter) are small, disk-like, and thermodynamically stable aggregates that can solubilize cholesterol. They can also solubilize and incorporate phospholipids to form mixed micelles that are capable of solubilizing at least triple the amount of cholesterol compared with that solubilized by simple micelles. Mixed micelles (4 to 8 nm in diameter) are large, thermodynamically stable aggregates composed of bile salts, phospholipids, and cholesterol. Their size depends on the relative proportion of bile salts and phospholipids. The mixed micelle is a lipid bilayer with the hydrophobic groups of the bile salts and phospholipids aligned on the “outside” of the bilayer, interfacing with the aqueous bile, and the hydrophilic groups on the “inside.” Therefore, cholesterol molecules can be solubilized on the inside of the bilayer away from the aqueous areas on the outside. The amount of cholesterol that can be solubilized is dependent on the relative proportions of bile salts, and the maximal solubility of cholesterol occurs when the molar ratio of phospholipids to bile salts is between 0.2 and 0.3. Furthermore, the solubility of cholesterol in mixed micelles is enhanced when the concentration of total lipids in bile is increased.

When model and native bile are examined by quasi-elastic light-scattering spectroscopy and electron microscopy, it is found that, besides micelles, vesicles solubilize cholesterol in bile. Biliary vesicles are unilamellar spherical structures that contain phospholipids, cholesterol, and little if any bile salts. Vesicles are substantially larger than either simple or mixed micelles (40 to 100 nm in diameter) but much smaller than liquid crystals (~500 nm in diameter) that are composed of multimembrane spherical structures. Because vesicles are present in large quantities in hepatic bile, they could be secreted by hepatocytes. Unilamellar vesicles are often detected in freshly collected samples of unsaturated bile and are physically indistinguishable from those identified in supersaturated bile. Dilute hepatic bile, in which solid cholesterol crystals and gallstones never form, is always supersaturated with cholesterol because vesicles solubilize biliary cholesterol in excess of what could be solubilized in mixed micelles. Cholesterol-rich vesicles are remarkably stable in dilute bile. In vivo, the critical micellar concentration of cholesterol decreases, and the absence of cholesterol crystallization in hepatic bile. The unilamellar vesicles can fuse and form large multimembrane vesicles (also known as liposomes or liquid crystals). Solid cholesterol monohydrate crystals may nucleate from multimembrane vesicles in concentrated gallbladder bile.

Vesicles are relatively static structures that are affected by several factors, including biliary lipid concentrations and the relative ratios of cholesterol, phospholipids, and bile salts. The relative concentrations of these 3 important lipids in bile are influenced by their hepatic secretion rates, which vary with fasting and feeding. For example, during the fasting period, hepatic output of biliary bile salts is relatively low. As a result, the ratio of cholesterol to bile salts is increased, and more cholesterol is carried in vesicles than in micelles. By contrast, with feeding, hepatic output of biliary bile salts is increased and more cholesterol is solubilized in micelles than in vesicles. In addition, when the concentration of bile salts is relatively low, especially in dilute hepatic bile, vesicles are relatively stable, and only some vesicles are converted to micelles. By contrast, with increasing bile salt concentrations in concentrated gallbladder bile, vesicles may be converted completely into mixed micelles. Because relatively more phospholipids than cholesterol can be transferred from vesicles to mixed micelles, the residual vesicles are remodeled and may be enriched in cholesterol relative to phospholipids. If the
remaining vesicles have a relatively low ratio (<1) of cholesterol to phospholipids, they are relatively stable, but if the ratio of cholesterol to phospholipids in vesicles is greater than 1, vesicles become increasingly unstable. These cholesterol-rich vesicles may transfer some cholesterol to less cholesterol-rich vesicles or to micelles or may fuse or aggregate to form larger (~500 nm in diameter) multimembranellar vesicles (i.e., liposomes or liquid crystals). Liquid crystals are often visible by polarizing light microscopy as lipid circular droplets with characteristic birefringence in the shape of a Maltese cross. Liquid crystals are inherently unstable and may form solid plate-like cholesterol monohydrate crystals, a process termed cholesterol nucleation. Therefore, nucleation of cholesterol monohydrate crystal results in a decrease in the amount of cholesterol contained in vesicles but not in micelles, and vesicles may serve as the primary source of cholesterol for nucleation.

Under normal physiologic conditions, bile is concentrated gradually within the biliary tree so that the bile salt concentration approaches its critical micellar concentration. When this occurs, bile salts begin to modify the structure of phospholipid-rich vesicles that are secreted into bile by hepatocytes. These interactions signify the start of a complex series of molecular rearrangements that ultimately lead to formation of simple and mixed micelles. In supersaturated bile, 2 pathways result in formation of cholesterol-rich vesicles from phospholipid-rich vesicles at the canalicular membrane of hepatocyte. Because bile salts solubilize phospholipids more efficiently than cholesterol, cholesterol-rich vesicles may form when bile salts preferentially extract phospholipid molecules directly from phospholipid-rich vesicles. The alternative pathway is the rapid dissolution of phospholipid-rich vesicles by bile salts with the production of unstable mixed micelles that contain excess cholesterol. Obviously, structural rearrangements of these unstable micellar particles result in the formation of cholesterol-rich vesicles.

**Phase Diagrams and Cholesterol Solubility in Bile**

In the 1960s, Small and colleagues defined the maximal solubility (saturation) limits for cholesterol in model quaternary bile systems that consisted of varying proportions of cholesterol, phospholipids, bile salts, and water. The relative proportions (as molar percentages) of the 3 lipids in bile play a critical role in determining the maximal solubility of cholesterol. When the relative proportions of the 3 lipids at a fixed total lipid concentration are plotted in a triangular coordinate, the solubility of cholesterol for any given solute concentration can be determined. The triangular coordinate diagram also illustrates the physical phases of cholesterol in bile. For example, the phase diagram shown in Figure 65-3 is specific for a total lipid concentration of 7.5 g/dL, which is typical of human gallbladder bile. For hepatic bile, with a typical total lipid concentration of 3 g/dL, the phase boundaries would be different, with a smaller micellar zone, all phase boundaries shifted to the left, and an expanded 2-phase zone on the right (i.e., region E in Fig. 65-3). The effect of total lipid concentration on cholesterol solubilization in the micellar zone explains why hepatic bile tends to be more saturated with cholesterol than is gallbladder bile in the same subject. Because hepatic bile contains a large number of cholesterol-phospholipid vesicles that are relatively stable, solid plate-like cholesterol monohydrate crystals never occur in hepatic bile.

**FIGURE 65-3.** Equilibrium phase diagram of a cholesterol-phospholipid (lecithin)-mixed bile salt system (37°C, 0.15 M NaCl, pH 7.0, total lipid concentration 7.5 g/dL) showing positions and configuration of crystallization regions. Components are expressed in moles percent. The 1-phase micellar zone at the bottom is enclosed by a solid angulated line, and above it, 2 solid lines divide the two-phase zones from a central 3-phase zone. Based on the solid and liquid crystallization sequences present in the bile, the left two-phase and central three-phase regions are divided by dashed lines into regions A to D. The number of phases given represents the equilibrium state. The phases are cholesterol monohydrate crystals and saturated micelles for crystallization regions A and B; cholesterol monohydrate crystals, saturated micelles, and liquid crystals for regions C and D; and liquid crystals of variable composition and saturated micelles for region E. Of note is that decreases in temperature (37°C → 4°C), total lipid concentration (7.5 g/dL → 2.5 g/dL), and bile salt hydrophobicity (3a, 12α→3α, 7α→3α, 7α, 12α→3α, 7β-hydroxylated taurine conjugates) progressively shift all crystallization pathways to lower phospholipid contents, retard crystallization, and reduce micellar cholesterol solubilities. These changes generate a series of new condensed-phase diagrams with an enlarged region E. (Reproduced with permission from Wang DQ, Carey MC. Complete mapping of crystallization pathways during cholesterol precipitation from model bile: Influence of physical-chemical variables of pathophysiologic relevance and identification of a stable liquid crystalline state in cold, dilute and hydrophilic bile salt-containing systems. J Lipid Res 1996; 37:606-30.)

occurs only in gallbladder bile. For example, in unsaturated bile, all cholesterol can be solubilized in both simple and mixed micelles, and relative biliary lipid compositions are located in the micellar zone of the phase diagram. By contrast, in supersaturated bile, cholesterol cannot be completely solubilized by simple and mixed micelles, and relative biliary lipid compositions are located outside the micellar zone of the phase diagram. Under these circumstances, high vesicular cholesterol concentrations and high total lipid concentrations in bile can work together to produce the solid crystalline phase. Therefore, with typical physiologic lipid ratios, at equilibrium, cholesterol monohydrate crystals are present with saturated simple and mixed micelles or with saturated micelles plus vesicles that have become multimellar liquid crystals. The final physical state of bile is also influenced by the ratio of the concentration of bile salts to that of phospholipids and...
that has a CSI of 1 is saturated; bile with a saturation index
actual amount of cholesterol present in a bile sample to the
for a sample of bile can be estimated directly from the diagram
of saturation of bile with cholesterol can be quantitated. A CSI
ation index (CSI) (or lithogenic index).
cholesterol apex is often calculated as the cholesterol satura-
outside the micellar zone directed along an axis joined to the
precipitate in bile. Furthermore, the proportional distance
pholipids, solid plate-like cholesterol monohydrate crystals
which can be solubilized by the available bile salts and phos-
visually cloudy. Obviously, relatively stable unilamellar
porter ABCA1 may translocate, either directly or indirectly, cholesterol and phospholipids to the cell surface, where they appear to
form lipid domains that interact with amphipathic α-helices in apolipoproteins. This interaction solubilizes these lipids and generates
nascent HDL particles that dissociate from the cell. A proportion of cholesterol is used for synthesis of bile salts (BS) via the classical and alternative pathways, as regulated by 2 rate-limiting enzymes, cholesterol 7α-hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27A1), respectively. Hepatic secretion of biliary cholesterol, bile salts, and phospholipids (PL) across the canalicular membrane is determined by 3 lipid transporters, ABCG5/G8, ABCB11, and ABCB4, respectively. The Niemann-Pick C1-like 1 (NPC1L1) protein may have a weak role in taking cholesterol back from hepatic bile to the hepatocyte. A vesicle is shown in the canalculus.

FIGURE 65-4. Uptake, biosynthesis, catabolism, and biliary secretion of cholesterol at the hepatocyte level. Hepatic uptake of cholesterol is mediated by the low-density lipoprotein (LDL) receptor (LDLR) for LDL, by scavenger receptor class B type I (SR-BI) for high-density lipoprotein (HD), and by the chylomicron remnant receptor (CMRR) for chylomicron remnants (CMR). Biosynthesis of hepatic choles-
terol (CH) from acetate is regulated by the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). Part of the cholesterol is esterified by acyl-coenzyme A:cholesterol acyltransferase (ACAT) for storage in the liver. Some of the cholesterol is used for the formation of very-low-density lipoprotein (VLDL), which is secreted into the blood. The ATP-binding cassette (ABC) transport-
er ABCA1 may translocate, either directly or indirectly, cholesterol and phospholipids to the cell surface, where they appear to
form lipid domains that interact with amphipathic α-helices in apolipoproteins. This interaction solubilizes these lipids and generates
nascent HDL particles that dissociate from the cell. A proportion of cholesterol is used for synthesis of bile salts (BS) via the classical and alternative pathways, as regulated by 2 rate-limiting enzymes, cholesterol 7α-hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27A1), respectively. Hepatic secretion of biliary cholesterol, bile salts, and phospholipids (PL) across the canalicular membrane is determined by 3 lipid transporters, ABCG5/G8, ABCB11, and ABCB4, respectively. The Niemann-Pick C1-like 1 (NPC1L1) protein may have a weak role in taking cholesterol back from hepatic bile to the hepatocyte. A vesicle is shown in the canalculus.

the overall hydrophilic-hydrophobic balance of both bile salt and phospholipid species.
Within the micellar zone (see Fig. 65-3), bile is a visually clear, stable solution that is considered unsaturated because all cholesterol can be solubilized in thermodynamically stable simple and mixed micelles. At the boundary line of the micel-
lar zone, bile is saturated because all the solubilizing capacity for cholesterol is utilized and no further cholesterol can be

carried in micelles. Outside the micellar zone, bile is supersatu-
rated because excess cholesterol cannot be solubilized by micelles and exists in more than one phase (micelles, liquid crystals, and solid monohydrate crystals); the solution is visually cloudy. Obviously, relatively stable unilamellar cholesterol-phospholipid vesicles solubilize a significant pro-
portion of cholesterol outside the micellar zone. The term metastable zone refers to the area in the phase diagram (above
but near the micellar zone) in which bile is supersaturated with cholesterol but may not form solid cholesterol monohy-
drate crystals even after many days. The diagram also sug-

gests that when the quantity of cholesterol in bile exceeds that
which can be solubilized by the available bile salts and phos-
pholipids, solid plate-like cholesterol monohydrate crystals
precipitate in bile. Furthermore, the proportional distance
outside the micellar zone directed along an axis joined to the
cholesterol apex is often calculated as the cholesterol satu-
ration index (CSI) (or lithogenic index). Therefore, the degree
of saturation of bile with cholesterol can be quantitated. A CSI
for a sample of bile can be estimated directly from the diagram
or calculated by using a formula. The CSI is the ratio of the actual amount of cholesterol present in a bile sample to the
maximal amount of cholesterol that can be dissolved in it. Bile

that has a CSI of 1 is saturated; bile with a saturation index
less than 1 is unsaturated; and bile with a saturation index
greater than 1 is supersaturated. The degree of saturation can
also be expressed as percent saturation by multiplying the
saturation index by 100. For example, at the boundary of the
micellar zone, bile is saturated, and the CSI is 100%. Super-
saturated bile has a CSI above 100%, and unsaturated bile has
a CSI below 100%. The CSI values are also useful for predict-
ing the proportion of lipid particles and the metastable and
equilibrium physical states in bile.

Hepatic Secretion of Biliary Lipids
Source of Lipids Secreted in Bile
The supply of hepatic cholesterol molecules that can be
recruited for biliary secretion depends on the balance of input
d and output of cholesterol and its metabolism in the liver (Fig.
65-4) (also see Chapter 72). Input is related to the amount of
cholesterol (both unesterified and esterified) taken up by the
liver from plasma lipoproteins (LDL > HDL > chylomicron
remnants) plus de novo hepatic cholesterol synthesis. Output
is related to the amount of cholesterol disposed of within the
liver by conversion to cholesteryl ester (to form new very-low-
density lipoprotein [VLDL] and for storage) minus the amount
of cholesterol converted to primary bile salts. An appreciable
fraction of cholesterol in bile may also be derived from the diet
via apolipoprotein E–dependent delivery of chylomicron rem-
nants to the liver. Under low or no dietary cholesterol condi-
tions, bile contains newly synthesized cholesterol from the
liver and preformed cholesterol that reaches the liver in several
different ways. Approximately 20% of the cholesterol in bile
comes from de novo hepatic biosynthesis, and 80% is from
pools of preformed cholesterol within the liver. De novo cholesterol synthesis in the liver uses acetate as a substrate and is mainly regulated by the rate-limited enzyme HMG-CoA reductase. This enzyme can be up- or down-regulated depending on the overall cholesterol balance in the liver. An increase in the activity of this rate-limiting enzyme leads to excessive cholesterol secretion in bile. The major sources of preformed cholesterol are hepatic uptake of plasma lipoproteins (mainly HDL and LDL) through their receptors on the basolateral membranes of hepatocytes. Consistent with their central role in reverse cholesterol transport, HDL particles are the main lipoprotein source of cholesterol that is targeted for biliary secretion. Under conditions of a high cholesterol diet, dietary cholesterol reaches the liver through the intestinal lymphatic pathway as chylomicrons and then chylomicron remnants, after chylomicrons are hydrolyzed by plasma lipoprotein lipase and hepatic lipase. The synthesis of new cholesterol in the liver is reduced and comprises only about 5% of biliary cholesterol. Overall, the liver can systematically regulate the total amount of cholesterol within it, and any excess cholesterol is handled efficiently.

Although biliary phospholipid is derived from the cell membranes of hepatocytes, the composition of biliary phospholipid differs markedly from that of hepatocyte membranes. The membranes of hepatocytes contain phosphatidylcholines (lecithins), phosphatidylethanolamines, phosphatidylinositols, phosphatidylserines, and sphingomyelins. The major source of phosphatidylcholine molecules destined for secretion into bile is hepatic synthesis. A fraction of biliary phosphatidylcholines may also originate in the phospholipid coat of HDL particles. From 10 to 15 g of phospholipids are secreted into bile each day in humans.

More than 95% of bile salt molecules, after secretion into bile, return to the liver through the enterohepatic circulation by absorption mostly from the distal ileum via an active transport system such as apical sodium-dependent bile acid transporter and organic solute transporters α and β (see Chapter 64). Consequently, newly synthesized bile salts in the liver contribute only a small fraction (<5%) to biliary secretion and compensate for bile salts that escape intestinal absorption and are lost in feces. Fecal excretion of bile salts is increased when the enterohepatic circulation of bile salts is partially or completely interrupted by surgery, disease states, or drugs (e.g., bile salt-binding resins such as cholestyramine). Complete interruption of the enterohepatic circulation results in up-regulation of bile salt synthesis in the liver, which restores bile salt secretion rates to approximately 25% of their usual values. Cholesterol from 2 sources serves as substrate for bile salt synthesis: cholesterol that is newly synthesized in the smooth endoplasmic reticulum and cholesterol that is preformed outside the smooth endoplasmic reticulum. The first step in this process is catalyzed by cholesterol 7α-hydroxylase. In the basal state, bile salt synthesis uses principally newly synthesized cholesterol as substrate. When de novo cholesterol biosynthesis is suppressed by long-term therapy with an HMG-CoA reductase inhibitor like a statin, preformed cholesterol originating from plasma lipoprotein substitutes for newly synthesized cholesterol.

**Biliary Lipid Secretion**

Bile salts have been shown to stimulate hepatic secretion of vesicles, which are always detected in freshly collected hepatic bile. When cultured under specified conditions, rat hepatocytes form couplets with isolated “bile canaliculi” at the interface between adjoining cells. With the use of laser light-scattering techniques, vesicle formation can be observed within these bile canaliculi after exposure to bile salts. In addition, rapid fixation techniques and electronic microscopy have provided direct morphologic evidence of vesicle formation and secretion at the outer surface of the canicular membrane of hepatocytes. Most if not all bile salts are thought to enter canicular spaces as monomers, whereas biliary phospholipids and cholesterol enter as unilamellar vesicles (see Fig. 65-4). A study on the molecular genetics of sitosterolemia (see Chapter 64) has shown that efflux of biliary cholesterol from the canicular membrane of the hepatocyte is a protein-mediated process. Two plasma membrane proteins—ATP-binding cassette (ABC) sterol transporters ABCG5 and ABCG8—promote cellular efflux of cholesterol. The significance of this process for bile formation has been examined in genetically modified mice in which overexpression of the human ABCG5 and ABCG8 genes in the liver was shown to increase the cholesterol content of gallbladder bile. Despite a reduced prevalence of gallstones, formation of gallstones is still observed in Abcg5/g8 double-knockout mice, as well as in Abcg5 or Abcg8 single-knockout mice fed a lithogenic diet. These findings strongly support the existence of an ABCG5/G8-independent pathway for hepatic secretion of biliary cholesterol and its role in formation of cholesterol gallstones.

The Niemann-Pick C1-like 1 (NPC1L1) protein is expressed in the canicular membrane of hepatocytes as well as the apical membrane of enterocytes; however, its expression levels are significantly lower in the liver than in the small intestine in humans. These observations suggest that hepatic NPC1L1 may have a weak role in the regulation of biliary cholesterol secretion. In addition, scavenger receptor class B type 1 (SR-BI) is localized in sinusoidal and possibly canalicular membranes of hepatocytes, and in transgenic and knockout mice fed a chow diet, biliary secretion of cholesterol varies in proportion to hepatic expression of SR-BI and to the contribution of SR-BI to sinusoidal uptake of HDL cholesterol destined for secretion into bile. Attenuation of the SR-BI, however, does not influence gallstone formation in mice. These results suggest that although HDL cholesterol is a principal source of biliary cholesterol in the basal state, uptake of cholesterol from chylomicron remnants appears to be the major contributor to biliary cholesterol hypersecretion during diet-induced cholesterol lithogenesis in the mouse.

Deletion of the Abcb4 gene completely inhibits hepatic secretion of bile salts by surgery, disease states, or drugs (e.g., bile salt-binding resins such as cholestyramine). Complete interruption of the enterohepatic circulation results in up-regulation of bile salt synthesis in the liver, which restores bile salt secretion rates to approximately 25% of their usual values. Cholesterol from 2 sources serves as substrate for bile salt synthesis: cholesterol that is newly synthesized in the smooth endoplasmic reticulum and cholesterol that is preformed outside the smooth endoplasmic reticulum. The first step in this process is catalyzed by cholesterol 7α-hydroxylase. In the basal state, bile salt synthesis uses principally newly synthesized cholesterol as substrate. When de novo cholesterol biosynthesis is suppressed by long-term therapy with an HMG-CoA reductase inhibitor like a statin, preformed cholesterol originating from plasma lipoprotein substitutes for newly synthesized cholesterol.

**Deletion of the Abcb4 gene completely inhibits hepatic secretion of bile salts by surgery, disease states, or drugs (e.g., bile salt-binding resins such as cholestyramine). Complete interruption of the enterohepatic circulation results in up-regulation of bile salt synthesis in the liver, which restores bile salt secretion rates to approximately 25% of their usual values. Cholesterol from 2 sources serves as substrate for bile salt synthesis: cholesterol that is newly synthesized in the smooth endoplasmic reticulum and cholesterol that is preformed outside the smooth endoplasmic reticulum. The first step in this process is catalyzed by cholesterol 7α-hydroxylase. In the basal state, bile salt synthesis uses principally newly synthesized cholesterol as substrate. When de novo cholesterol biosynthesis is suppressed by long-term therapy with an HMG-CoA reductase inhibitor like a statin, preformed cholesterol originating from plasma lipoprotein substitutes for newly synthesized cholesterol.**
secretion is curvilinear: At low bile salt secretion rates (usually <10 μmol/hr/kg), more cholesterol is secreted per molecule of bile salt than at higher rates. Although bile salt secretion rates are not low in normal subjects, they may diminish during prolonged fasting, during the overnight period, and with substantial bile salt losses, as occur with a biliary fistula or ileal resection when the liver cannot compensate sufficiently by increasing bile salt synthesis. At high bile salt secretion rates, for example, during and after eating, biliary cholesterol saturation is less than that during interprandial periods. In laboratory animals, biliary secretion of organic anions does not influence bile salt secretion but does inhibit hepatic secretion of phospholipids and cholesterol into bile because organic anions bind bile salts within bile canaliculi and prevent interactions with the canalicular membrane of hepatocytes.

**PATHOPHYSIOLOGY**

Figure 65-5 shows interactions of 5 primary defects that lead to formation of cholesterol gallstones: (1) certain genetic factors, including LITH genes, (2) hepatic hypersecretion of biliary cholesterol, (3) gallbladder hypomotility, (4) rapid phase transitions of cholesterol, and (5) certain intestinal factors. These defects act together to facilitate cholesterol nucleation and crystallization, and ultimately promote formation of cholesterol gallstones.

**Hepatic Hypersecretion of Biliary Cholesterol**

Hepatic hypersecretion of biliary cholesterol plays a primary role in the pathogenesis of cholesterol gallstone formation. By definition, supersaturated bile contains cholesterol that cannot be solubilized at equilibrium by bile salts and phospholipids. Cholesterol supersaturation could result from (1) excessive hepatic secretion of biliary cholesterol, (2) decreased hepatic secretion of biliary bile salts or phospholipids with relatively normal cholesterol secretion, or (3) a combination of hypersecretion of cholesterol and hyposecretion of the solubilizing lipids. With the passage of time and in the presence of heterogeneous pronucleating agents (usually mucin gel), cholesterol supersaturation leads to precipitation of solid plate-like cholesterol monohydrate crystals in bile, followed by agglomeration and growth of the crystals into mature and macroscopic stones.

**Rapid Cholesterol Nucleation and Crystallization**

Cholesterol nucleation and crystallization is a process by which solid plate-like cholesterol monohydrate crystals precipitate from supersaturated bile. The crystals can be detected by polarizing light microscopy in a sample of bile previously rendered crystal-free (“isotropic”). Bile from patients with cholesterol gallstones and from certain normal controls is supersaturated with cholesterol, and the degree of cholesterol supersaturation is not a reliable predictor of gallstones. On the other hand, rapid in vitro cholesterol nucleation and crystallization from the isotropic phase of gallbladder bile distinguishes the lithogenic bile of patients with cholesterol gallstones from cholesterol-supersaturated bile of non-gallstone control subjects. The phase diagram of cholesterol, phospholipids, and bile salts discussed earlier (see Fig. 65-3) is often used to study the phase transitions where metastable intermediates form. Five crystallization pathways can be identified on the basis of the phospholipid-to-bile salt ratio, total lipid concentration, bile salt species (hydrophilic and hydrophobic properties), temperature, and CSL. Furthermore, these crystallization pathways have been confirmed in fresh human and mouse gallbladder biles. In Figure 65-3, which shows the cholesterol-phospholipid–mixed bile salt model bile system, the 5 distinct crystallization pathways are designated A to E, with each representing a different sequence of phase transitions, including an anhydrous cholesterol pathway and a liquid crystalline pathway that leads to formation of solid plate-like cholesterol monohydrate crystals. Transient arc-like crystals appear in some of the pathways and are consistent with crystalline anhydrous cholesterol. Why anhydrous cholesterol crystals should precipitate in an aqueous environment is unknown, but they are characteristic of the pathways that seem to originate from unilamellar, as opposed to multilamellar, vesicles. These pathways, the critical nucleus may be a unilamellar vesicle that could contain
liquid anhydrous cholesterol molecules in its core, possibly reflecting internal nucleation. In essence, these early vesicular “nuclei” may already have initiated the nucleation cascade by the time bile enters the gallbladder. The current paradigm for cholesterol nucleation and crystallization, based principally on observations from video-enhanced polarized light microscopy, suggests that biliary vesicles must fuse or at least aggregate to form crystalline cholesterol monohydrate. Because cholesterol nucleation and crystallization are apparently initiated in vesicles, the stability of the vesicle determines the stability of bile. Unstable vesicles can fuse, aggregate, and grow into multilamellar liquid crystalline structures (liposomes) in which cholesterol crystallizes out of solution. Furthermore, evidence from quasi-elastic light-scattering spectroscopy shows that nucleation of solid cholesterol crystals may occur directly from supersaturated micelles in conjugated deoxycholate-rich bile in vitro without an intervening vesicle or liquid crystalline phase.

In bile with the lowest phospholipid content (region A in Fig. 65-3), arc-like crystals with a density \( (d = 1.030 \, \text{g/mL}) \) consistent with anhydrous cholesterol appear first and evolve via helical and tubular crystals to form plate-like cholesterol monohydrate crystals \( (d = 1.045 \, \text{g/mL}) \). With higher phospholipid contents (region B), cholesterol monohydrate crystals appear earlier than arc-like crystals and other transitional crystals. With typical physiologic phospholipid contents (region C), early liquid crystals \( (d = 1.020 \, \text{g/mL}) \) are followed by cholesterol monohydrate crystals; subsequently, arc-like and other intermediate crystals appear. With still higher phospholipid contents (region D), liquid crystals are followed by cholesterol monohydrate crystals only. At the highest phospholipid mole fractions (region E), liquid crystals are quite stable and no solid crystals form. Decreases in temperature (37°C \( \rightarrow \) 4°C), total lipid concentration (7.5 g/dL \( \rightarrow \) 2.5 g/dL), and bile salt hydrophobicity (3α,12α \( \rightarrow \) 3α,7α,12α,1α \( \rightarrow \) 3α,7β-hydroxylated taurine conjugates) progressively shift all crystallization pathways to lower phospholipid contents, reduce micellar cholesterol solubilization, and retard crystallization.

Cholesterol crystallization pathways and sequences in human gallbladder bile are identical to those of model bile samples matched for appropriate physical-chemical conditions and the physiologic state, 3 of the 5 sequences observed in model bile samples are found in human and mouse gallbladder bile. Notably, the kinetics of all these phase transitions are faster in lithogenic human bile than in identically patterned model bile samples, most likely a result in part of the combined influences of increased levels of cholesterol, secondary bile salts, and mucin glycoproteins. In addition, biliary lipid, electrolyte, and protein factors may be important in stabilizing supersaturated bile. Nonprotein factors that retard cholesterol nucleation and crystallization include (1) a total lipid concentration less than 3 g/dL, (2) reduced hydrophobicity of the bile salt pool, (3) low bile salt-to-phospholipid ratios, (4) low cholesterol-to-phospholipid ratios in vesicles, and (5) low total calcium ion concentrations. The states opposite to these conditions accelerate cholesterol nucleation and crystallization.

**Imbalance of Pronucleating and Antinucleating Factors**

Cholesterol crystallization is significantly more rapid in the gallbladder bile of patients with gallstones than in that of control subjects even though CSI values are similar. These findings imply that lithogenic bile may contain pronucleating agents that accelerate crystallization or that normal bile may contain antinucleating agents that inhibit crystallization. Furthermore, bile may contain both accelerators and inhibitors of crystallization, and imbalances between them can induce rapid cholesterol crystallization in gallbladder bile in patients with cholesterol gallstones.

Mucin was the first biliary protein shown to promote cholesterol crystallization. The epithelial cells of the gallbladder secrete mucin that serves as a protective layer over the mucosa in the normal physiologic state. Mucin or mucin glycoproteins are large molecules that consist of a protein core and many carbohydrate side chains. An important property of mucin is its ability to form a gel phase in higher concentrations, and the gel has greatly increased viscosity compared with the sol (soluble) phase.

Gallbladder mucins, a heterogeneous family of O-linked glycoproteins, are divided into 2 classes: epithelial and gel-forming mucins. The epithelial mucins, which are produced by mucin gene 1 (MUC1), MUC3, and MUC4, are not able to form aggregates and are integral membrane glycoproteins located on the apical surface of epithelial cells. The gel-forming mucins MUC2, MUC5AC, and MUC5B, which are secreted by specialized gallbladder mucin-producing cells, provide a protective coating on the underlying mucosa. They form disulfide-stabilized oligomers or polymers, a phenomenon that favors their viscoelastic properties. Mucins from different organs vary in carbohydrate side chain, protein composition, and charge but generally have similar properties. Mucins have hydrophilic domains to which many water molecules bind. They have an overall charge and are capable of binding other charged species like calcium. Hydrophobic domains in the mucin molecule (on the nonglycosylated regions of the polypeptide core) allow binding of lipids such as cholesterol, phospholipids, and bilirubin.

Evidence shows that gallbladder mucins play an important role in the early stages of gallstone formation and are a potent pronucleating agent for accelerating cholesterol crystallization in native and model biles. Indeed, hypersecretion of gallbladder mucins is a prerequisite for gallstone formation, and increased amounts of gallbladder mucins are consistently observed in gallbladder bile of several animal models of gallstones. Mucins are also found within gallstones, where they act as a matrix for stone growth. The mucins in gallstones have been found to extend from the amorphous center to the periphery in either a radial or laminated fashion. Mucins are also a major component of gallbladder sludge. Gallbladder sludge has been shown to be a precursor of gallstones. Therefore, 2 roles in the formation of gallstones have been proposed for mucins: (1) a pronucleating agent for accelerating the nucleation and crystallization of cholesterol from saturated bile and (2) a scaffolding for the deposition of solid cholesterol monohydrate crystals during the growth of stones.

The synthesis of mucin glycoproteins that are secreted by the epithelial cells of the gallbladder and bile ducts may be regulated by mucosal prostaglandins derived from arachidonic acid–containing biliary phospholipids. During gallstone formation, the gallbladder hypersecretes mucins, mostly as a result of stimulation by some components of saturated bile. Then, the carbohydrate polymers of the mucins avidly bind water to form gels. The hydrophobic polypeptides in the core of mucin glycoproteins also can bind bilirubin and calcium in bile. The resulting water-insoluble complex of mucin glycoproteins and calcium bilirubinate provides a surface for nucleation of cholesterol monohydrate crystals and a matrix for the growth of stones.

Mucin secretion and accumulation in the gallbladder is determined by multiple mucin genes. Targeted disruption of the Muc1 gene reduces MUC1 mucin in the gallbladder of mice, thereby leading to a decrease in susceptibility to cholesterol gallstone formation. Also, expression levels of the gallbladder Muc5ac gel-forming mucin gene are significantly reduced in Muc1-knockout mice in response to a lithogenic
diet. As a result, cholesterol crystallization and the development of gallstone formation are significantly retarded. These findings suggest that gene–gene interactions between the Muc1 and Muc5ac genes might affect mucin secretion and accumulation in the gallbladder. Furthermore, increased gallbladder epithelial MUC1 mucin enhances cholelithogenesis, mostly by promoting gallbladder cholesterol absorption and impairing gallbladder motility in mice that are transgenic for the human MUC1 gene; this lithogenic mechanism is completely different from that associated with the gel-forming mucins. Collectively, these findings support the concept that inhibition of the secretion and accumulation of not only the gel-forming mucins but also the epithelial mucins in the gallbladder may completely prevent formation of cholesterol gallstones.

Many glycoproteins that bind reversibly to concanavalin A–sepharose also speed up cholesterol crystallization. These glycoproteins include aminopentadecapeptide N, immunoglobulins, α1-acid glycoprotein, phospholipase C, fibronectin, and haptoglobin. Other pronucleating agents are the amphipathic anionic polypeptide fraction/calcium-binding protein, albumin-lipid complexes, and group II phospholipase A2. Nonprotein components of bile also expedite cholesterol crystallization by promoting fusion of cholesterol-rich vesicles. Precipitation of calcium salts in bile that is supersaturated with calcium salts and cholesterol may lead to rapid cholesterol crystallization, an effect enhanced by the presence of mucins. The rapidity of cholesterol crystal formation also varies in proportion to the deoxycholate content of bile and is related to the effect of deoxycholate on the equilibria of rapidly growing microdomains on a crystal face and interfering with further solute attachment. It may inhibit growth of solid cholesterol crystals by attaching to the most rapidly growing microdomains on a crystal face and interfering with further solute attachment. It is still uncertain whether only one or several antinucleating factors exist and how they may inhibit the initiation of cholesterol crystal formation, but unilamellar vesicles have been proposed to be the key sites of action.

In summary, although many biliary proteins besides mucin gel have been proposed as either pronucleating or antinucleating factors influencing cholesterol nucleation and crystallization in bile, their in vivo roles (if any) in the pathogenesis of cholesterol gallstone formation remain unclear. Furthermore, proteolysis of soluble biliary glycoproteins does not influence the detection time of cholesterol monohydrate crystals either in normal or abnormal gallbladder and hepatic bile, and soluble biliary proteins may not play an important pathophysiologic role in cholesterol crystallization.

**Gallbladder Dysfunction**

Under normal physiologic conditions, frequent gallbladder contractions occur throughout the day. Between meals, the gallbladder stores hepatic bile (with an average fasting volume of 25 to 30 mL in healthy subjects). Following a meal, depending on the degree of neurohormonal response, the gallbladder discharges a variable amount of bile. Studies using a combination of cholecintigraphy and US have found that after a meal, the gallbladder empties immediately and refills repeatedly. By contrast, an increased fasting gallbladder volume, as well as incomplete emptying and high residual gallbladder volume, is often observed in patients with cholesterol gallstones, regardless of whether they have tiny or large stones or simply lithogenic bile. In patients with cholesterol gallstones and gallbladder motility abnormalities, inflammation in the gallbladder wall is usually mild and cannot account for the impaired dynamics of the gallbladder. Furthermore, the poor interdigestive gallbladder filling is consistent with delivery of a greater percentage of lithogenic bile from the liver directly into the small intestine, leading to augmentation of the enterobiliary effects of increased recycling and bile salt hydrophobicity. These observations show that emptying and filling of the gallbladder are affected in patients with gallbladder hypomotility.

Clinical investigations have confirmed that gallbladder hypomotility is associated principally with the formation of cholesterol gallstones, although a milder degree of gallbladder dysmotility, in the absence of an enlarged gallbladder in the fasting state and any gallbladder inflammation, is also found in patients with pigment gallstones. In patients with cholesterol gallstones, impaired gallbladder motility persists in the stone-free gallbladder following successful extracorporeal shock-wave lithotripsy and oral bile acid dissolution therapy. The degree of impairment of gallbladder emptying has been found to increase in proportion to the cholesterol content of gallbladder bile, even in healthy subjects without gallstones. These findings imply that excess cholesterol molecules in the gallbladder wall may act as myotoxic agents.

In vitro studies have found that compared to that in control subjects, gallbladder function in patients with cholesterol gallstones shows abnormalities in the binding of agonists like CCK to plasma membranes. In the fasting state and any gallbladder inflammation, is also found in patients with pigment gallstones.

UDCA may exert its effect by stabilizing vesicles, perhaps by enhancing the incorporation of apolipoprotein AI into (or onto) the vesicles. In addition, a potential antinucleating factor from normal human gallbladder bile is detected by lectin affinity chromatography and high-performance liquid ion-exchange chromatography and found to be a slightly acidic glycoprotein with an apparent molecular size of 120 kD. The protein may inhibit growth of solid cholesterol crystals by attaching to the most rapidly growing microdomains on a crystal face and interfering with further solute attachment. It is still uncertain whether only one or several antinucleating factors exist and how they may inhibit the initiation of cholesterol crystal formation, but unilamellar vesicles have been proposed to be the key sites of action.

In summary, although many biliary proteins besides mucin gel have been proposed as either pronucleating or antinucleating factors influencing cholesterol nucleation and crystallization in bile, their in vivo roles (if any) in the pathogenesis of cholesterol gallstone formation remain unclear. Furthermore, proteolysis of soluble biliary glycoproteins does not influence the detection time of cholesterol monohydrate crystals either in normal or abnormal gallbladder and hepatic bile, and soluble biliary proteins may not play an important pathophysiologic role in cholesterol crystallization.
viscous mucin gel that forms in the gallbladder lumen may contribute to hypomotility by impairing gallbladder emptying mechanically, possibly at the level of the cystic duct. In particular, sludge contains calcium, pigment, bile salts, and glycoproteins and could serve as a nidus for nucleation and crystallization of cholesterol or precipitation of calcium bilirubinate. The high prevalence of cholelithiasis in patients receiving long-term TPN (see earlier) highlights the importance of gallbladder stasis in the formation of gallstones. For example, 49% of patients with Crohn’s disease who are on TPN have gallstones, whereas only 27% of patients with Crohn’s disease alone have gallstones. During TPN, the gallbladder does not empty completely because the stimulus (ingestion of meals) for CCK release is eliminated. As a result, bile stagnates and sludge develops in the gallbladder, thereby enhancing gallstone formation. Daily IV administration of CCK can completely prevent gallbladder dysmotility and eliminate the inevitable risk of biliary sludge and gallstone formation. In addition, slow emptying and increased volume of the gallbladder, as measured by US, often occur during pregnancy and during administration of oral contraceptives, 2 conditions that predispose to formation of gallstones (see earlier). Acidification of bile by the gallbladder increases cholesterol solubility but also enhances cholesterol nucleation and crystallization in bile and may thereby contribute to gallstone formation. In addition to concentrating bile, the normal gallbladder can acidify bile. Acidification increases the solubility of calcium salts (e.g., bilirubinate and carbonate), which may be promoters of nucleation and crystallization of cholesterol; therefore, defective acidification may promote the formation of gallstones.

Differential absorption rates of cholesterol, phospholipids, and bile salts by the gallbladder epithelial cells may reduce cholesterol saturation of bile in normal subjects; however, defective acidification may promote the formation of gallstones. Daily IV administration of CCK can completely prevent gallbladder dysmotility and eliminate the inevitable risk of biliary sludge and gallstone formation. In addition, slow emptying and increased volume of the gallbladder, as measured by US, often occur during pregnancy and during administration of oral contraceptives, 2 conditions that predispose to formation of gallstones (see earlier). Acidification of bile by the gallbladder increases cholesterol solubility but also enhances cholesterol nucleation and crystallization in bile and may thereby contribute to gallstone formation. In addition to concentrating bile, the normal gallbladder can acidify bile. Acidification increases the solubility of calcium salts (e.g., bilirubinate and carbonate), which may be promoters of nucleation and crystallization of cholesterol; therefore, defective acidification may promote the formation of gallstones.

Intestinal Factors

The high efficiency of intestinal cholesterol absorption correlates significantly with the prevalence of cholesterol gallstones in inbred strains of mice, and gallstone-susceptible C57L mice display significantly higher intestinal cholesterol absorption than do gallstone-resistant AKR mice. These observations show that high dietary cholesterol intake and high efficiency of intestinal cholesterol absorption are 2 independent risk factors for cholesterol gallstone formation. Differences in the metabolism of chylomicron remnant cholesterol between C57L and AKR mice may account for lithogenic bile formation in the former, and the cholesterol absorbed from the small intestine provides an important source for biliary cholesterol hypersecretion in mice fed a lithogenic diet.

Altered intestinal motility also may have a role in gallstone formation. Delayed or impaired small intestinal transit is associated with enhanced intestinal cholesterol absorption, biliary cholesterol secretion, and gallstone formation in CCK-1 receptor-knockout mice. The association of impaired colonic motility with increased biliary deoxycholate levels is found in some patients with cholesterol gallstones. Evidence for a causal relation among impaired intestinal motility, deoxycholate formation, and bile lithogenicity comes from studies in humans and mice. Clinical studies have found that acromegalic patients treated with octreotide (a known risk factor for cholesterol gallstone disease [see earlier]) display a prolonged colonic transit time, high levels of biliary deoxycholate concentration, and rapid precipitation of cholesterol crystals. Furthermore, higher levels of biliary deoxycholate are associated with increased amounts of Gram-positive anaerobic bacteria and increased activity of 7α-dehydroxylation in the cecum of patients with cholesterol gallstones compared with control subjects who have no stones. Biliary deoxycholate and cholesterol concentrations can be lowered by antibiotic treatment that reduces focal 7α-dehydroxylation activity. Compared with resistant AKR mice, gallstone-susceptible C57L mice also have higher biliary levels of deoxycholate, which are associated with cholesterol supersaturation and gallstone formation. Chronic intestinal infection has been proposed to be a potential risk factor in the pathogenesis of cholesterol gallstones. A mouse study has shown that distal intestinal infection with Helicobacter species (but not Hp) is essential for nucleation and crystallization of cholesterol from supersaturated bile. The association of impaired colonic transit and rapid precipitation of cholesterol crystals in CCK-1 receptor-knockout mice may account for lithogenic bile formation in the former, and the cholesterol absorbed from the small intestine provides an important source for biliary cholesterol hypersecretion in mice fed a lithogenic diet.

Growth of Gallstones

Findings in patients who have cholesterol crystals but no gallstones in the gallbladder suggest that the growth of cholesterol crystals into gallstones does not always follow crystallization.
Stone growth may represent a second critical stage in gallstone formation that results from delayed emptying of the gallbladder. When multiple gallstones are found in the gallbladder, they often are equal in size, indicating that cholesterol crystallization for this family of stones occurred simultaneously and the stones grew at the same rate. By contrast, stones of unequal size could represent different generations. The amorphous material in the center of stones contains bilirubin, bile salts, mucins, glycoproteins, calcium carbonate, phosphate, copper, and sulfur, which could have provided a required nidus for cholesterol nucleation and crystallization. Solid plate-like cholesterol monohydrate crystals could assemble about this nidus. Formation of a nidus and subsequent stone growth could be determined by mucins, other biliary proteins, and the cholesterol saturation of bile. The growth of stones is likely a discontinuous process punctuated by deposition of rings of calcium bilirubinate and calcium carbonate. Because cholesterol monohydrate crystals often aggregate randomly in amorphous groupings and layer radially and concentrically, cholesterol stones consist of radially or horizontally oriented cholesterol crystals embedded within an organic matrix. In the outer portion of stones, cholesterol monohydrate crystals are oriented perpendicularly to the surface. Throughout the formation of gallstones, mucins could provide a matrix on which gallstone growth occurs. Furthermore, concentric pigment rings separate layers of cholesterol monohydrate crystals that have different axial orientations. The chemical composition of these rings often resembles the center of gallstones, and the rings may reflect cyclic deposition of calcium bilirubinate, other calcium salts, and mucin glycoproteins.

**GENETICS**

Evidence for a genetic component of cholesterol gallstone disease in humans is mostly indirect and based on geographic and ethnic differences, as well as on family and twin studies. A genetic predisposition is clearly present in the Pima and certain other North and South American Indians, who display the highest prevalence rate (>5%) in the world. By contrast, the overall prevalence of gallstones in white American and European populations is about 20%, and in African populations (<5%) are observed in Asian populations (5% to 20%), as shown in Figures 65-1 and 65-2. Although some independent risk factors (e.g., aging, gender, parity, obesity, insulin resistance, some drugs, rapid weight loss) for gallstone formation have been found, none can explain the striking differences in the prevalence of gallstones among different populations, thereby suggesting a genetic contribution to the etiology of the disease.

Gallstones are more frequent by a ratio of 3:1 in siblings and other family members of affected persons than in spouses or unrelated controls. Using US to detect gallstones in first-degree relatives of index patients, Gilat and colleagues found a 21% prevalence rate in first-degree relatives compared with 9% in matched controls. Sarin and coworkers also observed a prevalence that was 5 times higher in relatives than in controls. Furthermore, cholesterol supersaturation is higher in fasting duodenal bile of older sisters of patients with cholesterol gallstones than in controls. Cholesterol synthesis rates, bile saturation levels, and gallstone prevalence rates are also significantly higher on pair-wise correlations in monozygotic twins than in dizygotic male twins. Despite these observations, a mode of inheritance that fits a Mendelian pattern cannot be shown in most cases.

Study of populations with different incidence rates of gallstones but living in the same environment should provide insights into genetic mechanisms of the disease. Unfortunately, intermarriages between 2 populations result in a rapid loss of the original genetic background within a few generations and make such studies impossible. With use of pedigree data to explore the genetic susceptibility to symptomatic gallbladder disease in a Mexican-American population of 52 families, heritability (i.e., the proportion of the phenotypic variance of the trait that is due to genetic effects) has been estimated to be 44%. A variance component analysis in 1038 persons from 358 families in the United States has determined the heritability of symptomatic gallbladder disease to be 29%. A large study of 43,141 twin pairs in Sweden has provided conclusive evidence for the role of genetic factors in the pathogenesis of cholesterol gallstones. In this study, concordance rates were significantly higher in monozygotic twins than in dizygotic twins, with genetic factors accounting for 25% of the phenotypic variation between twins.

Evidence that human gallstones may be caused by a single gene defect came initially from a study by Lin and colleagues, who reported that among 232 Mexican-Americans, a variant of the cholesterol 7-hydroxylase (CYP7A1) gene was associated with gallstones in Mexican males but not females. CYP7A1 is a candidate gene because it encodes the rate-limiting enzyme in hepatic bile salt synthesis of the classical pathway and because bile salts are essential for forming bile and for keeping cholesterol molecules solubilized in simple and mixed micelles in bile. Pullinger and colleagues found a link between another single gene defect of CYP7A1 and cholesterol gallstones associated with hypercholesterolemia resistant to HMG-CoA reductase inhibitors in 2 male homoyzgotes.

Missense mutations in the ABCB4 gene, which encodes the phosphatidylcholine transporter in the canalicular membrane of hepatocytes, are the basis of a particular type of cholelithiasis. The disorder is characterized by intrahepatic sludge, gallbladder cholesterol gallstones, mild chronic cholestasis, a high cholesterol-to-phospholipid ratio in bile, and recurrent symptoms after cholecystectomy. In patients with hepatothiliasis, a common disease in Asia, low expression levels of ABCB4 and phosphatidylcholine transfer protein occur together, with markedly reduced phospholipid concentrations in bile (see Chapter 68). Additionally, HMG-CoA reductase activity is increased in CYP7A1 activity is reduced in patients with gallstones compared with control subjects. In this disorder, the formation of cholesterol-rich intrahepatic stones could be induced by decreased hepatic secretion of biliary phospholipids in the setting of increased cholesterol synthesis and decreased bile salt synthesis.

Because gallbladder hypermotility favors gallstone formation, the genes for CCK and the CCK-1 receptor (CCK-1R), which regulate gallbladder motility, are attractive candidates. Genetic variation in CCK-1R is associated with gallstone risk, and an aberrant splicing of CCK-1R, which is predicted to result in a nonfunctional receptor, has been found in a few obese patients with gallstones. A search for mutations or polymorphisms in the CCK-1R gene in patients with gallstones has been unsuccessful, however.

Some studies have reported that certain polymorphisms of the apolipoprotein (APOE) and APOB genes and the cholesterol ester transfer protein, all of which are involved in carrying cholesterol in plasma, are associated with gallstone formation. The APOE polymorphisms are the most extensively studied polymorphisms in patients with gallstones, but reports concerning the protective role of the ε2 allele against gallstones have been inconsistent. The ε2 allele appears to protect against gallstones, and the degree of dietary cholesterol absorption in the intestine varies with the APOE isoform (ε2ε3ε3 vs. ε2). Also, the fecal excretion of cholesterol tends to be
higher in persons with the APOE2 phenotype than in those with the APOE3 or APOE4 phenotypes. In a study of polymorphisms at the \( APOB, APOAI, \) and cholesteryl ester transfer protein gene loci in patients with gallbladder disease, a polymorphism of the cholesteryl ester transfer protein gene, in relation to another HDL lowering factor, was found to be associated with cholesterol gallstones. Also, a link was found between the \( X^+ \)-allele of the \( APOB \) gene and an increased risk of cholesterol gallstones. A genomewide association study in a large cohort of patients with gallstones from Germany and a linkage study in affected sibling pairs identified a common variant (D19H) of the sterol transporters ABCG5 and ABCG8 on the canalicular membrane of hepatocytes as a risk factor for gallstones. Subsequently, many studies have shown that ABCG8 variants (T400K, D19H, A632V, M429V, C54Y) and ABCG5 variants (Q646E) may be important risk factors for gallstone formation in European, Asian, and Chilean Hispanic populations.

Table 65-1 summarizes progress in identifying \( LITHT \) genes and the major classes of candidate genes for cholesterol and pigment gallstones in humans. Although some candidate genes have been found in humans, their roles in cholelithogenesis merit further investigation. In general, genes that contribute to cholesterol gallstone formation include those that encode (1) hepatic and intestinal membrane lipid transporters, (2) hepatic and intestinal lipid regulatory enzymes, (3) hepatic and intestinal intracellular lipid transporters, (4) hepatic and intestinal lipid regulatory transcription factors, (5) hepatic lipoprotein receptors and related proteins, (6) hormone receptors in the gallbladder, and (7) biliary mucus.

Changes in the expression and function of one of several ABC transporters in the canalicular membrane may influence gallstone formation by inducing an alteration of biliary lipid secretion and bile composition. In addition, mutations in genes that encode several lipoprotein receptors and related proteins that determine the uptake of HDL and LDL in several intracellular proteins that transport biliary lipids through the cytosol of hepatocytes, as well as transcription factors that regulate hepatic cholesterol and bile salt metabolism and biliary lipid secretion, may cause formation of cholesterol gallstones. Mutations in genes that affect CCK, the CCK receptor, and the secretion and properties of mucin may also play a role in the pathogenesis of pigment stones. A genomewide association study has found that increased hepatic biosynthesis and fecal excretion of cholesterol may precede cholesterol gallstone formation and may be key metabolic features in some ethnic groups at high risk of gallstones. This study strongly suggests that inhibiting both hepatic synthesis and intestinal absorption of cholesterol to reduce biliary output of cholesterol may be a therapeutic strategy for genetically defined subgroups of persons at high risk for gallstones.

The factors that regulate intestinal membrane lipid transporters, lipid regulatory enzymes, intracellular lipid transporters, and lipid regulatory transcription factors may influence the amount of cholesterol of intestinal origin contributing to biliary secretion by the liver. Direct evidence for the role of intestinal factors in mouse gallstones comes from a study of ACAT2-knockout mice. Because of the deletion of the \( Acat2 \) gene, the lack of cholesteryl ester synthesis in the small intestine significantly reduces intestinal cholesterol absorption and leads to complete resistance to diet-induced cholesterol gallstones. Furthermore, the potent cholesterol absorption inhibitor ezetimibe prevents gallstones by effectively reducing intestinal absorption and biliary secretion of cholesterol and protects gallbladder motility by desaturating bile in mice. Moreover, ezetimibe significantly reduces biliary cholesterol saturation and retards cholesterol crystallization in bile of patients with gallstones. Therefore, reduced intestinal absorption of cholesterol or hepatic uptake of chylomicron remnants may induce a decrease in biliary cholesterol secretion and saturation. In addition, reduced expression levels of the genes encoding the ileal apical sodium-dependent bile acid transporter (ASBT), the cytosolic ileal lipid binding protein (ILBP), and organic solute transporters \( \alpha \) and \( \beta \) (OS16 and \( \beta \)) may contribute to gallstone formation by decreased ileal bile acid reabsorption and an altered bile acid pool and composition in female and nonobese patients with gallstones compared with control subjects. The single nucleotide polymorphism rs9514089 in the apical sodium-dependent bile acid transporter gene (gene symbol SLC10A2) has been identified as a susceptibility variant for cholelithiasis in humans, although the effect of rs9514089 genotype on gallstone risk was not replicated in Sorbs. Further analyses in larger cohorts are required to evaluate the role of genetic variants of SLC10A2 as a risk factor for gallstone formation.

**PIGMENT STONES**

Although the pathogenesis of black and brown pigment gallstones is not as well understood as that of cholesterol gallstones, and each type of stone probably has a distinctive pathogenesis, both types of pigment stones result from abnormalities in the metabolism of bilirubin and are pigmented as a result of bilirubin precipitation. In general, the bile of patients with both types of pigment stones contains an excess of unconjugated bilirubin, analogous to the saturation of bile with cholesterol in patients with cholesterol stones. Also, both types of pigment stones are composed primarily of bile pigment and contain a matrix of mucin glycoproteins. In black stones, however, the pigment is predominantly an insoluble highly cross-linked polymer of calcium bilirubinate, whereas in brown stones, the main pigment is monomeric calcium bilirubinate. The 2 types of pigment stones also differ in radioactivity, density, location within the biliary system, and geographic distribution.

Results of studies of susceptibility genes for pigment stones are summarized in Table 65-1. Several candidate genes involved in the pathogenesis of pigment stones by increasing enterohepatic cycling of bilirubin. Persons with Gilbert’s syndrome have mild, chronic, unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis because of reduced expression of bilirubin uroporphyrinogen I diehydrase. The \( UGT1A1 \) gene is due to an abnormality in the promoter region of the gene for this enzyme (see Chapter 21). A genomewide association study has identified a variant of the \( UGT1A1 \) gene as a major risk factor for gallstone disease in humans. The \( UGT1A1 \) promoter variant increases the susceptibility to pigment stone formation in patients with sickle cell disease or \( CF \). A genome-wide association study has shown that serum bilirubin levels and the prevalence of gallstones are strongly associated with the number of \( UGT1A1 \) promoter \( [TA] \) repeats in patients with sickle cell disease, with each additional repeat correlating with an increase in serum bilirubin levels of 21% and in cholesterol gallstone risk of 87%. Moreover, \( UGT1A1 \) gene variants in linkage disequilibrium with the variant are associated with the risk of developing cholesterol gallstones. These findings imply that the supersaturation of bile with bilirubin may be a risk factor for the formation of both pigment and cholesterol gallstone. As discussed earlier, increased biliary bilirubin levels and enhanced precipitation of calcium bilirubinate in bile provide a critical nidus for cholesterol nucleation and crystallization.
<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCG5/G8</td>
<td>ATP-binding cassette transporters G5/G8</td>
<td>2p21</td>
<td>ABCG8 p.D19H (rs1188753)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>ABCB4</td>
<td>ATP-binding cassette transporter B4</td>
<td>7q21.1</td>
<td>Multiple</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>ABCB11</td>
<td>ATP-binding cassette transporter B11</td>
<td>2q24</td>
<td>Multiple</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>SLC10A2 (IBAT)</td>
<td>Solute carrier family 10, member 2 ( ileal sodium-dependent bile salt transporter)</td>
<td>13q33</td>
<td>c.378-105A&gt;G (#s9540689)</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>SLC01B1 (OATP1B1)</td>
<td>Solute carrier organic anion transporter family, member 1B1</td>
<td>12p12</td>
<td>p.155Thr (rs11045819)</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

**Lipid Regulatory Enzymes**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP7A1</td>
<td>Cholesterol 7α-hydroxylase ( Cytochrome P450 7A1)</td>
<td>8q11-q12</td>
<td>Promoter SNP-204A&gt;C</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>Bilirubin UDP glucuronosyltransferase</td>
<td>2q37</td>
<td>Promoter A(TA)7TAA</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

**Intracellular Lipid Regulatory Transporters**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CETP</td>
<td>Cholesteryl ester transfer protein</td>
<td>16q12-q21</td>
<td>RFLP</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

**Lipid Regulatory Transcription Factors**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR1H4 (FXR)</td>
<td>Nuclear receptor 1H4 (Farnesoid X receptor)</td>
<td>12q23.1</td>
<td>Promoter SNPs −1G&gt;T and −20647T&gt;G, IVS7−31 A&gt;T</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ Biliary cholesterol secretion</td>
</tr>
</tbody>
</table>

**Lipoprotein Receptors and Related Genes**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOA1</td>
<td>Apolipoprotein A1</td>
<td>11q23-q24</td>
<td>−75G&gt;A, RFLP</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>APOB</td>
<td>Apolipoprotein B</td>
<td>2p24-p23</td>
<td>c.2486C&gt;T, c.4154G&gt;A</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

Continued
### TABLE 65-1 Human Gallstone (LITH) Genes and Gene Products That Have Been Identified as of 2014—cont’d

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
</table>
| **APOC1**  | Apolipoprotein C1 | 19q13.2 | RFLP | − − + ↑ | ↑ APOC1 remnant-like particle cholesterol 
↓ Hepatic cholesterol uptake from chylomicron remnants via LRP |
| **LRPAP1** | Low-density lipoprotein receptor-related protein-associated protein 1 | 4p16.3 | Intron 5 insertion/deletion (rs11267919) | − − − + | |

**Hormone Receptors**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
</table>
| **CCK1R** (CCKAR) | Cholecystokinin 1 receptor (Cholecystokinin A receptor) | 4p15.1-p15.2 | RFLP | + − − + | ↓ Gastric and small intestinal motility 
↑ Hepatic cholesterol biosynthesis 
↓ Gastric bilirubin motility |
| **ESR2 (ERβ)** | Estrogen receptor 2 | 14q23.2 | c.1092+3607(CA), c.172(CAG), p.R64W (rs4944) | − − − + | |
| **ARβ** | Androgen receptor | 8p12 | Multiple | − + − + | |

**Black Pigment Stones**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
</table>
| **ANK1** | Ankyrin 1 | 8p11.1 | Multiple ΔE508 | − − + − | Spherocytosis → hemolysis 
↑ Enterohpatic bilirubin circulation 
↓ Bile pH 
↑ Fecal bile salt excretion 
↑ Hemolysis |
| **G6PD** | Glucose-6-phosphate dehydrogenase | 7q31.2 | Multiple | + − − + | |
| **GPI** | Glucose-6-phosphate isomerase | 19q13.1 | p.Leu339Pro | − − − + | TBD |
| **PKLR** | Pyruvate kinase | 1q21 | p.R510Q | − − + + | TBD |
| **HBA1/2** | Hemoglobin alpha chain complex | 16p13.3 | HbH | − − + + | TBD 
α-Thalassemia/β-thalassemia intermediate/minor/sickle cell disease → hemolysis |
| **HBB** | Hemoglobin beta chain complex | 11p15.5 | p.E26K (HbE) p.E6V (HbS) | − − + + | TBD 
Hemolysis |
| **UGT1A1** | Bilirubin UDP glucuronosyltransferase | 2q37 | Promotor A(TA)7TAA | − − − + | ↑ Hepatic bilirubin conjugation |

**Biliary Tract Stones**

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Chromosome Location</th>
<th>Gene Variants</th>
<th>Inheritance Pattern</th>
<th>Potential Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMT</strong></td>
<td>Catechol-O-methyltransferase</td>
<td>22q11.21</td>
<td>Exons4–76C&gt;G (rs4818) c.811C&gt;T (rs2230054) c.123ST&gt;C (rs1126579)</td>
<td>− − − +</td>
<td>↑ Estrogen levels</td>
</tr>
<tr>
<td><strong>CXXCR2</strong></td>
<td>Chemokine (C-X-C motif) receptor 2</td>
<td>2q35</td>
<td>c.123ST&gt;C (rs1126579)</td>
<td>− − + +</td>
<td>TBD</td>
</tr>
<tr>
<td><strong>IL8</strong></td>
<td>Interleukin-8</td>
<td>4q13-2q1</td>
<td>Exons16+14C&gt;T (rs297518)</td>
<td>− − − +</td>
<td>↑ IL8 expression → inflammation</td>
</tr>
<tr>
<td><strong>NOS2</strong></td>
<td>Nitric oxide synthase 2</td>
<td>17q11.2-q12</td>
<td>Exons1-9A-G (rs486907)</td>
<td>− − + +</td>
<td>TBD</td>
</tr>
<tr>
<td><strong>RNASEL</strong></td>
<td>Ribonuclease L</td>
<td>1q25</td>
<td></td>
<td>− − − +</td>
<td>TBD</td>
</tr>
</tbody>
</table>

HDL, high-density lipoprotein; LRP, low-density lipoprotein receptor-related protein; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; TBD, to be determined; UDP, uridine diphosphate; VLDL, very-low-density lipoprotein.

The frequency of gallstones in patients with CF is 10% to 30% compared with less than 5% in age-matched control subjects, but biliary cholesterol saturation does not differ between patients with and without gallstones. In fact, gallstones in patients with CF are generally black pigment stones (i.e., composed of calcium bilirubinate with an appreciable cholesterol admixture) but rarely cause symptoms. In a mouse (ΔF508 mutant) model of CF, increased fecal bile salt loss induces more hydrophobic bile salts in hepatic bile and augments enterohepatic cycling of bilirubin.256 These alterations lead to hyperbilirubinemia and significantly higher levels of all bilirubin conjugates and unconjugated bilirubin, followed by hydrolysis and precipitation of divergent metal salts of unconjugated bilirubin in bile. In addition, lower gallbladder bile pH values and elevated levels of calcium bilirubinate ion products in bile increase the likelihood of supersaturating bile with bilirubin and forming black pigment gallstones. The pancreatic duodenal homeobox gene-1 (Pdx1) is required for proper development of the major duodenal papilla, peribiliary glands, and mucin-producing cells in the bile duct and for maintenance of the periamplulatory duodenal epithelial cells during the perinatal period. Loss of the major duodenal papilla allows duodenobiliary reflux and bile infection, resulting in formation of brown pigment stones in Pdx1-knockout mice, and treatment with antibiotics significantly reduces the frequency of brown pigment stones.210

### Black Stones

Black pigment stones are formed in unaffected gallbladders, particularly in patients with chronic hemolytic anemia (e.g., β-thalassemia, hereditary spherocytosis, sickle cell disease), ineffective erythropoiesis (e.g., pernicious anemia), ileal diseases (e.g., Crohn’s disease) with spillage of excess bile salts into the large intestine, extended ileal resections, and liver cirrhosis. These alterations promote formation of black pigment stones because higher colonic bile salt concentrations enhance the solubilization of unconjugated bilirubin, thereby increasing bilirubin concentrations in bile.204 The resulting unconjugated bilirubin is precipitated as calcium bilirubinate to form stones.255 This type of stone is composed of either pure calcium bilirubinate or polymer-like complexes consisting of unconjugated bilirubin, calcium bilirubinate, and copper. Mucin glycoproteins account for as much as 20% of the weight of black pigment stones. A regular crystalline structure is not present in this type of stone.

For hepatic secretion, bilirubin is first mono- or diglucuronidated by UGT1A1 and subsequently secreted by ABC transporter C2 (ABCC2), also called multidrug resistance associated protein 2 (MRP2) (see Chapters 64 and 77). Under normal physiologic conditions, unconjugated bilirubin is not secreted into bile. Although bilirubin glucuronidates are hydrolyzed by endogenous β-glucuronidase, unconjugated bilirubin constitutes less than 1% of total bile pigment, primarily because the activity of the enzyme is inhibited by β-glucaro-1,4-lactone in the biliary system.201 The unifying predisposing factor in black pigment stone formation is hepatic hypersecretion of bilirubin conjugates (especially monoglucuronidates) into bile. In the presence of hemolysis, hepatic secretion of these bilirubin conjugates increases 10-fold. Unconjugated monohydroxylated bilirubin is formed by the action of endogenous β-glucuronidase, which coprecipitates with calcium as a result of supersaturation. A 1% hydrolysis rate may give rise to high concentrations of unconjugated bilirubin that often greatly exceed the solubility of bilirubin in bile. A defect in acidification of bile may also be induced by gallbladder inflammation or the reduced buffering capacity of sialic acid and sulfate moieties in the mucin gel. The reduction in buffering capacity facilitates supersaturation of calcium carbonate and calcium phosphate that would not occur at a more acidic pH. Gallbladder motility defects are not observed in patients with black pigment stones.

### Brown Stones

Brown pigment stones are composed mainly of calcium salts of unconjugated bilirubin, with varying amounts of cholesterol, fatty acids, pigment fraction, and mucin glycoproteins, as well as small amounts of bile salts, phospholipids, and bacterial residues. Brown pigment stones may be easily distinguished grossly from black pigment stones by their reddish brown to dark brown color and lack of brightness. Their shape is irregular or molded and occasionally spherical. Most of the stones are muddy in consistency, and some show facet formation. Brown pigment stones are either smooth or rough without any surface luster and are soft, fragile, and light in comparison with other gallstones. The cut surface is generally a stratified structure (lamellation) or is amorphous without the radiating crystalline structure seen in cholesterol stones. Almost invariably, brown pigment stones have a lamellated cross-sectional surface with calcium bilirubinate-rich layers alternating with calcium palmitate-rich layers.

Brown pigment stones are formed not only in the gallbladder but also commonly in other portions of the biliary tract, especially in intrahepatic bile ducts. Formation of brown pigment stones requires the presence of structural or functional stasis of bile associated with biliary infection, especially with Escherichia coli.208 These stones are quite prevalent in Asia, where Clonorchis sinensis and roundworm infestations are common, and parasitic elements have been considered to be kernels of brown pigment stone formation (see Chapter 84).209 Bile stasis predisposes to bacterial infection as well as accumulation of mucins and bacterial cytoskeletons in the bile ducts. Bile stasis may be induced by bile duct stenosis and parasitic elements have been considered to be kernels of brown pigment stone formation.211,212 The frequency of gallstones in patients with CF is 10% to 24% since the 1960s, and similar changes have been reported from other Asian populations prone to development of brown pigment stones, the ratio of cholesterol stones to pigment stones has also changed in these populations. The percentage of brown pigment stones in Japan has fallen from 60% to 24% since the 1960s, and similar changes have been reported from other Asian countries.211-213

Enteric bacteria produce β-glucuronidase, phospholipase A₁, and conjugated bile acid hydrolase. Activity of β-glucuronidase results in production of unconjugated bilirubin from bilirubin glucuronide; phospholipase A₁ liberates palmitic and stearic acids from phospholipids; and bile acid hydrolases produce unconjugated bile salts from glycine or taurine-conjugated bile salts. Partially ionized saturated fatty acids, unconjugated bilirubin, and unconjugated bile salts may precipitate as calcium bilirubinate. Mucin gel can trap these complex precipitates and facilitate their growth into macroscopic brown pigment stones. Figure 65-6 shows the postulated mechanisms underlying the formation of brown pigment stones. Under normal physiologic conditions, bilirubin in bile exists mainly as bilirubin glucuronide, which is soluble in aqueous media. Bile also contains β-glucuronidase of tissue origin, the activity of which is inhibited by β-glucaro-1,4-lactone, which is also formed in the liver. If infection with E. coli occurs, the concentration of bacterial β-glucuronidase increases significantly and exceeds the inhibitory power of β-glucaro-1,4-lactone. As a result, bilirubin glucuronide is hydrolyzed to produce unconjugated bilirubin and glucuronic acid; the former is water-insoluble and combines with calcium to form calcium bilirubin at its carboxyl radical, thereby leading to the formation of brown pigment gallstones.
NATURAL HISTORY

The natural history of gallstones is typically described in 2 separate groups of patients: those who have symptoms and those who are asymptomatic. Autopsy studies clearly show that the vast majority of patients with gallstones are asymptomatic and remain so. Ascertaining the true frequency of complications in persons with asymptomatic stones (as well as those with symptomatic stones) is critical to providing rational, cost-effective recommendations regarding therapy (see later). Unfortunately, the information available on the natural history of gallstones has been sparse and somewhat varied.214-216

Asymptomatic Stones

The study that changed our understanding of the course and appropriate therapy of gallstone disease was performed by Gracie and Ransohoff.214 They monitored 123 University of Michigan faculty members for 15 years after they had been found to have gallstones on routine screening US. At 5, 10, and 15 years of follow-up, 10%, 15%, and 18% of the patients, respectively, had become symptomatic, and none had experienced serious complications. The investigators suggested that the rate at which biliary pain develops in persons with asymptomatic gallstones is about 2% per year for 5 years and then decreases over time. Biliary complications developed in only 3 patients in this study, and all complications were preceded by episodes of biliary pain. Biliary pain, not a biliary complication, is the initial manifesting symptom in 90% of people with previously asymptomatic gallstones.214 Therefore, in patients with asymptomatic stones, the frequency of complications is low, and prophylactic cholecystectomy is not necessary.

Subsequent studies have reported slightly higher rates of biliary pain and complications in patients with initially asymptomatic gallstones,215 but only 1 was a long-term and prospective study:216 The Group for Epidemiology and Prevention of Cholelithiasis (GREPCO) in Rome reported the courses of 151 subjects with gallstones, 118 of whom were asymptomatic on entering the study. In those who were initially asymptomatic, the frequency of biliary pain was 12% at 2 years, 17% at 4 years, and 26% at 10 years, and the cumulative rate of biliary complications was 3% at 10 years.216

In a 1987 study, incidental gallstones were discovered in 285 (21%) of 1371 patients from Norway who had not had a cholecystectomy.217 Twenty-four years later, a follow-up study included 134 of the patients who had gallstones.218 Gallstones were present on US in 25 of 89 patients (28% overall, 31% of women and 25% of men), and there was no correlation between initial size and number of gallstones and persistence of stones on follow up. Nine of 134 patients (7%) had undergone cholecystectomy, as had 5 of 91 patients who had died prior to follow-up (6%). During follow-up, abdominal pain developed in 44%, and 29% had what were deemed to be functional abdominal complaints. This study illustrates again both the frequent resolution and relatively benign nature of asymptomatic gallstone disease.
Stones in Patients with Diabetes Mellitus

Diabetic patients have been considered at increased risk of gallstone complications; however, the natural history of gallstones in diabetic patients follows the same pattern observed in nondiabetic persons. A prospective study of patients with insulin-resistant diabetes mellitus showed that after 9 years of follow up, symptoms had developed in 15% of the asymptomatic patients. Moreover, the complication and mortality rates were comparable to those in studies of nondiabetic patients with gallstones. Therefore, prophylactic cholecystectomy is not recommended in patients with insulin-resistant diabetes mellitus and asymptomatic gallstones.

Symptomatic Stones

The cardinal symptom of gallstones is biliary pain (“colic”), which is described as pain in the right upper quadrant (RUQ) often radiating to the back, with or without nausea and vomiting. The pain is usually not true colic (see Chapter 11) and is almost never associated with fever. The natural history of symptomatic gallstones has a more aggressive course than that of asymptomatic stones. The U.S. National Cooperative Gallstone Study showed that in persons who had an episode of uncomplicated biliary pain in the year before entering the study, the rate of recurrent biliary pain was 38% per year. Other investigators have reported a rate of recurrent biliary pain as high as 50% per year in persons with symptomatic gallstones. As noted earlier, biliary complications are also more likely to develop in persons with symptomatic gallstones. The risk of biliary complications is estimated to be 1% to 2% per year and is believed to remain relatively constant over time. Therefore, cholecystectomy should be offered to patients after biliary symptoms develop. In patients with high operative risk, an alternative approach is close observation, because 30% will have no further episodes of biliary pain.

Special Patient Populations

The clinical manifestations of gallstones are shown schematically in Figure 65-7 and summarized in more detail in Table 65-2. Biliary pancreatitis is discussed in Chapter 58. Although the standard approach to asymptomatic gallstones is observation, some patients with asymptomatic gallstones may be at increased risk of complications and may require consideration of prophylactic cholecystectomy.

An increased risk of cholangiocarcinoma and gallbladder carcinoma has been associated with certain disorders of the biliary tract and in some ethnic groups (e.g., Native Americans) (see Chapter 69). Risk factors include choledochal cysts, Caroli’s disease, anomalous pancreatic ductal drainage (in which the pancreatic duct drains into the bile duct), large gallbladder adenomas, and porcelain gallbladder (see Chapters 62 and 67). Patients at increased risk of biliary cancer may benefit from prophylactic cholecystectomy. If abdominal surgery is planned for another indication, an incidental cholecystectomy should be performed.

Pigment gallstones are common and often asymptomatic in patients with sickle cell disease. Prophylactic cholecystectomy is not recommended, but an incidental cholecystectomy...
### TABLE 65-2 Pathophysiology, Clinical Manifestations, Diagnosis, and Treatment of Gallstone Disease

<table>
<thead>
<tr>
<th>Pathophysiology</th>
<th>Biliary Pain</th>
<th>Acute Cholecystitis</th>
<th>Choledocholithiasis</th>
<th>Cholangitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent obstruction of the cystic duct</td>
<td>Severe, poorly localized, epigastric or RUQ visceral pain growing in intensity over 15 minutes and remaining constant for 1-6 hours, often with nausea</td>
<td>75% of cases are preceded by attacks of biliary pain</td>
<td>Fever, but usually to &lt;102°F unless complicated by gangrene or perforation</td>
<td>Fever in 95%</td>
</tr>
<tr>
<td>No acute inflammation of the gallbladder</td>
<td>Frequency of attacks varies from days to months</td>
<td>Visceral epigastric pain gives way to moderately severe localized pain in the RUQ, back, right shoulder, or, rarely, chest</td>
<td>Right subcostal tenderness with inspiratory arrest (Murphy’s sign)</td>
<td>RUQ tenderness in 90%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nausea with some vomiting is frequent</td>
<td>Palpable gallbladder in 33% of patients, especially those having their first attack</td>
<td>Jaundice in 80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain lasting &gt;6 hours favors cholecystitis over biliary pain alone</td>
<td>Mild jaundice in 20%; higher frequency in older adults</td>
<td>Peritoneal signs in 15%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often asymptomatic</td>
<td>Often findings are completely normal if the obstruction is intermittent</td>
<td>Fever in 15% of patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Symptoms (when present) are indistinguishable from biliary pain</td>
<td>Jaundice with pain suggests stones; painless jaundice and a palpable gallbladder favor malignancy</td>
<td>Mental confusion, lethargy, and delirium suggest sepsis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Predisposes to cholangitis and acute pancreatitis</td>
<td>Fever in 80%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Charcot’s triad (pain, jaundice, and fever) is present in 70% of patients</td>
<td>Pancreatitis (forming Reynolds’ pentad in combination with Charcot’s triad) coexist in 15% and suggest Gram-negative sepsis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain may be mild and transient and is often accompanied by chills</td>
<td>Leukocytosis in 80%, but the remainder may have a normal white blood cell count with or without band forms</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Often findings are completely normal if the obstruction is intermittent</td>
<td>Serum bilirubin level is &gt;2 mg/dL in 80%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jaundice with pain suggests stones; painless jaundice and a palpable gallbladder favor malignancy</td>
<td>Elevated serum bilirubin and alkaline phosphatase levels are seen with BD obstruction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fever in 80%</td>
<td></td>
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</tr>
</tbody>
</table>

### Symptoms
- Fever, but usually to <102°F unless complicated by gangrene or perforation
- Right subcostal tenderness with inspiratory arrest (Murphy’s sign)
- Palpable gallbladder in 33% of patients, especially those having their first attack
- Mild jaundice in 20%; higher frequency in older adults
- Often findings are completely normal if the obstruction is intermittent
- Jaundice with pain suggests stones; painless jaundice and a palpable gallbladder favor malignancy

### Physical findings
- Fever in 95%
- RUQ tenderness in 90%
- Jaundice in 80%
- Peritoneal signs in 15%
- Hypotension and mental confusion (forming Reynolds’ pentad in combination with Charcot’s triad) coexist in 15% and suggest Gram-negative sepsis

### Laboratory findings
- Leukocytosis in 80%, but the remainder may have a normal white blood cell count with or without band forms
- Serum bilirubin level is >2 mg/dL in 80%
- Elevated serum bilirubin and alkaline phosphatase levels are seen with BD obstruction
- A transient “spike” in serum amylase or lipase levels suggests the passage of a stone

### Diagnostic studies
- US
- Oral cholecystography
- Meltzer-Lyon test (see Chapter 67)
- US
- Hepatobiliary scintigraphy
- Abdominal CT
- ERCP
- EUS
- MRC
- Percutaneous THC
- ERCP
- Percutaneous THC
should be considered if abdominal surgery is performed for other reasons. Some authorities recommend combined prophylactic splenectomy and cholecystectomy in young asymptomatic patients with hereditary spherocytosis if gallstones are present.

Morbidly obese persons who undergo bariatric surgery are at high risk of complications of gallstones (see Chapters 7 and 8). These patients have a frequency of gallstones of greater than 30%. An incidental cholecystectomy is recommended at the time of surgery.

Some investigators have proposed that patients with incidental cholelithiasis who are awaiting heart transplantation undergo a prophylactic cholecystectomy irrespective of the presence or absence of biliary tract symptoms because they are at increased risk of post-transplant gallstone complications. A retrospective study that addressed this issue in renal transplant recipients, however, concluded that complications of gallstones could be managed safely after symptoms emerged.

**DIAGNOSIS**

Imaging studies play a central role in the diagnosis of gallstones and associated conditions. Table 65-3 shows the wide array of imaging techniques available to evaluate the biliary tract. Each modality has its strengths and limitations, and the methods vary widely in relative cost and risk to the patient. With the possible exception of US, none of the modalities should be ordered routinely in the evaluation of a patient with suspected gallstone disease; rather, the diagnostic evaluation should proceed in a rational stepwise fashion based on the individual patient’s symptoms, signs, and results of laboratory studies (see later).

Notably absent from the list of imaging studies of the biliary tract is the plain abdominal film. Although useful on occasion for evaluating patients with abdominal pain, plain abdominal films are limited by a lack of sensitivity and specificity. Only 50% of pigment stones and 20% of cholesterol stones contain enough calcium to be visible on a plain abdominal film. Because 80% of gallstones in the Western world are of the cholesterol type, only 25% of stones can be detected by simple radiographs. Plain abdominal films have their greatest usefulness in evaluating patients with some of the unusual complications of gallstones (e.g., emphysematous cholecystitis, cholecystenteric fistula, gallstone ileus) or in detecting a porcelain gallbladder (see later).

**US**

Since its introduction in the 1970s, US examination of the biliary tract has become the principal imaging modality for the diagnosis of cholelithiasis. US requires only an overnight or 8-hour fast, involves no ionizing radiation, is simple to perform, and provides accurate anatomic information. It has the additional advantage of being portable and thus available at the bedside of a critically ill patient.

The diagnosis of gallstones relies on detection of echogenic objects within the lumen of the gallbladder that produce an acoustic shadow (Fig. 65-8A). The stones are mobile and generally congregate in the dependent portion of the gallbladder. Modern US is able to detect stones as small as 2 mm in diameter routinely. Smaller stones may be missed or may be confused with biliary sludge (layering echogenic material that does not cast acoustic shadows).

The sensitivity of US for detection of gallstones in the gallbladder is better than 95% for stones larger than 2 mm. The specificity is greater than 95% when stones produce acoustic shadows. Rarely, advanced scarring and contraction of the gallbladder around gallstones make locating the gallbladder or the stones impossible, raising the possibility of gallbladder cancer. The contracted gallbladder filled with stones may give a “double-arc shadow” or “wall-echo shadow”
### TABLE 65-3 Imaging Studies of the Biliary Tract

<table>
<thead>
<tr>
<th>Technique</th>
<th>Condition Tested For</th>
<th>Findings/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US</strong></td>
<td>Cholelithiasis</td>
<td>Stones manifest as mobile, dependent echogenic foci within the gallbladder lumen with acoustic shadowing. Sludge appears as layering echogenic material without shadows. Sensitivity &gt;95% for stones &gt;2 mm. Specificity &gt;95% for stones with acoustic shadows. Rarely, a stone-filled gallbladder may be contracted and difficult to see, with a “wall-echo-shadow” sign. <strong>Best single test for stones in the gallbladder</strong>. Stones are seen in the BD in only ~50% of cases but can be inferred from the finding of a dilated BD (&gt;6 mm diameter), with or without gallstones, in another ~25% of cases. Can confirm, but not exclude, BD stones. Pericholecystic fluid (in the absence of ascites) and gallbladder wall thickening to &gt;4 mm (in the absence of hypoalbuminemia) are nonspecific findings but are suggestive of acute cholecystitis.</td>
</tr>
<tr>
<td></td>
<td>Choledocholithiasis</td>
<td>Highly accurate for excluding or confirming stones in the BD. Concordance of EUS with the ERCP diagnosis ~95%; many studies suggest slightly higher sensitivity rates for EUS than for ERCP. Specificity ~97%. Positive predictive value ~99%, negative predictive value ~98%, accuracy ~97%. With experienced operators, EUS can be used in lieu of ERCP to exclude BD stones, particularly when the clinical suspicion is low or intermediate. <strong>Considered for patients with low to moderate clinical probability of choledocholithiasis</strong>.</td>
</tr>
<tr>
<td>EUS</td>
<td>Choledocholithiasis</td>
<td>Stones manifest as mobile filling defects in an opacified gallbladder. Sensitivity and specificity exceed 90% when the gallbladder is opacified, but nonvisualization occurs in 25% of studies and can result from multiple causes other than stones. Opacification of the gallbladder indicates cystic duct patency. May be useful in the evaluation of acalculous gallbladder diseases such as cholesterolosis and adenomyomatosis (see Chapter 67).</td>
</tr>
<tr>
<td>Oral cholecystography’</td>
<td>Cholelithiasis</td>
<td>Stones manifest as mobile filling defects in an opacified gallbladder. Sensitivity and specificity exceed 90% when the gallbladder is opacified, but nonvisualization occurs in 25% of studies and can result from multiple causes other than stones. Opacification of the gallbladder indicates cystic duct patency. May be useful in the evaluation of acalculous gallbladder diseases such as cholesterolosis and adenomyomatosis (see Chapter 67).</td>
</tr>
<tr>
<td>Cholescintigraphy (hepatobiliary scintigraphy; hydroxyiminodiacetic acid or diisopropyl iminodiacetic acid scan)</td>
<td>Acute cholecystitis</td>
<td>Assesses patency of the cystic duct. A normal scan shows radioactivity in the gallbladder, BD, and small bowel within 30-60 minutes. A positive result is defined as nonvisualization of the gallbladder, with preserved hepatic excretion of radionuclide into the BD or small bowel. Sensitivity is ~95% and specificity is ~90%, with false-positive results seen in fasted critically ill patients. With cholecystokinin stimulation, the gallbladder “ejection fraction” can be determined and may help evaluate patients with acalculous biliary pain (see Chapter 67). A normal scan result virtually excludes acute cholecystitis.</td>
</tr>
<tr>
<td>ERCP</td>
<td>Choledocholithiasis</td>
<td>ERCP is the standard diagnostic test for stones in the BD, with sensitivity and specificity of ~95%. Use of ERCP to extract stones (or at least drain infected bile) is life-saving in severe cholangitis and reduces the need for BD exploration at the time of cholecystectomy. Recommended for patients with a high clinical probability of choledocholithiasis. When contrast agent flows retrograde into the gallbladder, stones appear as filling defects and can be detected with a sensitivity rate of ~80%, but US remains the mainstay for confirming cholelithiasis.</td>
</tr>
<tr>
<td></td>
<td>Cholelithiasis</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 65-3 Imaging Studies of the Biliary Tract—cont’d

<table>
<thead>
<tr>
<th>Technique</th>
<th>Condition Tested For</th>
<th>Findings/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRCP</td>
<td>Chohledocholitiasis</td>
<td>A rapid, noninvasive modality that provides detailed bile duct and pancreatic duct images equal to those of ERCP. Sensitivity =93% and specificity =94%, comparable with those for ERCP. Useful for examining nondilated ducts, particularly at the distal portion, which often is not well visualized by US. Adjacent structures such as the liver and pancreas can be examined at the same time. Recommended for patients with low to moderate clinical probability of chohledocholitiasis.</td>
</tr>
<tr>
<td>CT</td>
<td>Complications of gallstones</td>
<td>Not well suited for detecting uncomplicated stones but excellent for detecting complications such as abscess, perforation of gallbladder or BD, and pancreatitis. Spiral CT may prove useful as a noninvasive means of excluding BD stones; some studies suggest improved diagnostic accuracy when CT is combined with an oral cholecystographic contrast agent.</td>
</tr>
</tbody>
</table>

*Performed infrequently. BD, bile duct.

Intraluminal imaging provides several advantages over transabdominal US, including closer proximity to the bile duct, higher resolution, and lack of interference by bowel gas or abdominal wall layers (Fig. 65-9). In several studies, EUS had a positive predictive value of 99%, negative predictive value of 98%, and accuracy rate of 97% for the diagnosis of bile duct stones compared with ERCP. If bile duct stones are found on EUS, endoscopic removal of the stones is necessary, and it can be argued that ERCP should be the initial study if choledocholithiasis is strongly suspected. Nevertheless, several studies that compared EUS with ERCP have found both techniques to be accurate for confirming or excluding choledocholithiasis, with EUS having advantages in both safety and cost.

EUS has also been found to be superior to MRCP (or simply magnetic resonance cholangiography [MRC]) in detecting the presence or absence of bile duct stones (see later). The major benefit of EUS in patients with a clinical suspicion of choledocholithiasis is the ability to avoid unnecessary ERCP and sphincterotomy, which is not without risk. Use of EUS to determine if ERCP is indicated may avoid a significant number of ERCPs and result in fewer complications. A systematic review of randomized controlled trials compared EUS-guided ERCP with ERCP alone for detection of bile duct stones. Patients randomized to EUS were able to avoid ERCP in 67% of cases and had lower rates of complications and pancreatitis compared with those randomized to ERCP alone (OR, 0.35 and 0.21, respectively). EUS failed to detect common bile duct stones in only 2 of 213 patients (0.9%). Therefore, EUS is currently considered an appropriate modality for excluding bile duct stones, especially if the pretest probability of finding stones is low to intermediate.

**Oral Cholecystography**

Once the mainstay of imaging studies of the gallbladder, oral cholecystography (OCG) now has limited application as a secondary approach to identifying stones in the gallbladder. The only useful clinical indications for OCG are the evaluation of patients in whom medical dissolution of stones or lithotripsy is being considered (see Chapter 66) and the evaluation of patients for unsuspected gallbladder disease, such as sign, with the gallbladder wall, echogenic stones, and acoustic shadowing seen in immediate proximity. If the gallbladder cannot be identified ultrasonographically, then a complementary imaging modality such as oral cholecystography or abdominal CT is warranted.

US is the standard for the diagnosis of stones in the gallbladder but is distinctly less sensitive for the detection of stones in the bile duct (common bile duct). Because of the proximity of the distal bile duct to the duodenum, luminal bowel gas often interferes with the US image, and the entire length of the bile duct cannot be examined. As a result, only about 50% of bile duct stones are actually seen on US. The presence of an obstructing bile duct stone, however, can be inferred when a dilated duct is found in the absence of cholecystectomy. Now that ERCP has uncovered a rising frequency of falsely negative US, the upper limit of normal of the diameter of the bile duct has declined from 10 mm to 6 mm. Even so, inferring choledocholithiasis from a dilated bile duct on US has a sensitivity of only 75%.

US is quite useful for diagnosing acute cholecystitis. Pericholecystic fluid (in the absence of ascites) and gallbladder wall thickening to more than 4 mm (in the absence of hypoalbuminemia) are suggestive of acute cholecystitis (see Fig. 65-8D). Unfortunately, in the critical care setting, these nonspecific findings are seen frequently in patients with no other evidence of gallbladder disease. A more specific finding is the so-called sonographic Murphy’s sign, in which the ultrasonographer elicits focal gallbladder tenderness under the ultrasound transducer. Eliciting a sonographic Murphy’s sign is somewhat operator dependent and requires an alert patient. Presence of the sign has a positive predictive value of greater than 90% for detecting acute cholecystitis if gallstones are present. US may help localize other abdominal diseases, such as abscesses or pseudocysts, that may be in the differential diagnosis.

**EUS**

EUS is highly accurate for detecting choledocholithiasis. More invasive and more expensive than standard US, EUS has the advantage of being able to visualize the bile duct from within the GI lumen and is comparable to ERCP in this respect.
may allow imaging of the biliary tree in a patient with a serum bilirubin value as high as 20 mg/dL.

An abnormal or “positive” scan result is defined as nonvisualization of the gallbladder, with preserved excretion into the bile duct or small intestine. The accuracy of the test for detecting acute cholecystitis is 92%, superior to that for US.

**Cholescintigraphy**

Cholescintigraphy (hepatobiliary scintigraphy) is a radionuclide imaging test of the gallbladder and biliary tract that is most useful for evaluating patients with suspected acute cholecystitis. By demonstrating patency of the cystic duct, cholescintigraphy can exclude acute cholecystitis rapidly (within 90 minutes) in a patient who presents with abdominal pain. The procedure can be performed on an emergency basis in a nonfasting patient after IV administration of gamma-emitting \(^{99m}\)Tc-labeled hydroxyl iminodiacetic acid (HIDA) or diisopropyl iminodiacetic acid (DISIDA), which is taken up rapidly by the liver and secreted into bile. As shown in Figure 65-10, serial scans after injection normally should show radioactivity in the gallbladder, bile duct, and small intestine within 30 to 60 minutes. In the past, imaging of jaundiced patients with this technique was limited, but use of DISIDA may allow imaging of the biliary tree in a patient with a serum bilirubin value as high as 20 mg/dL.
administration of CCK may be useful in identifying patients with chronic acalculous biliary pain who are likely to benefit from empirical cholecystectomy (see Chapter 67). An additional important role for cholescintigraphy is the noninvasive detection of bile leakage from the cystic duct as a complication of cholecystectomy (see Chapter 66).

ERCP

ERCP is one of the most effective modalities for detecting choledocholithiasis. The technique is discussed in more detail in Chapter 70. Stones within the bile duct appear as filling defects and can be detected with a sensitivity of around 95% (Fig. 65-11). Care should be taken to avoid inadvertent injection of air into the biliary tract, because bubbles may mimic gallstones. The specificity of ERCP for the detection of bile duct stones is approximately 95%.

CT and Magnetic Resonance Cholangiography

In patients with cholelithiasis or choledocholithiasis, CT has been used principally for detecting complications like pericholecystic fluid in acute cholecystitis, gas in the gallbladder wall (suggesting emphysematous cholecystitis), gallbladder perforation, and abscesses (Fig. 65-12). Spiral CT cholangiography (CTC) with use of an oral cholecystographic contrast agent has been studied for the detection of choledocholithiasis alone, EUS and MRC are equal in accuracy to ERCP.

False-positive results occur primarily in fasting or critically ill patients, in whom gallbladder motility is decreased. The reduction in gallbladder motility leads to greater water resorption, which results in a gelatinous bile. In critically ill patients, cholestasis and hepatocyte dysfunction result in reduced clearance of radionuclide imaging agents. Although nonvisualization of the gallbladder because of cystic duct obstruction is the hallmark of acute cholecystitis, pericholecystic hepatic uptake of radionuclide is a useful secondary sign.

In some patients (e.g., those with chronic cholecystitis, liver disease, or choledocholithiasis), imaging of the gallbladder by radionuclide scanning is delayed for several hours, and scanning must be repeated in 4 or more hours to confirm absence of acute cholecystitis. This delay in visualization of the gallbladder is problematic in the acutely ill patient but has largely been overcome with the administration of IV morphine sulfate to patients in whom the gallbladder fails to be visualized within 60 minutes. Morphine raises the pressure within the sphincter of Oddi, thereby leading to the preferential flow of bile into the gallbladder if the cystic duct is not obstructed. Another scan is obtained 30 minutes after injection of morphine, and if the gallbladder is visualized, cystic duct obstruction, and hence acute cholecystitis, is excluded. The gallbladder may not be visualized in approximately half of critically ill patients even after injection of morphine, thereby leading to false-positive cholescintigraphy results.

Although primarily a tool for evaluating acutely ill patients with suspected acute cholecystitis, cholescintigraphy after
Biliary Pain and Chronic Cholecystitis

Biliary pain is the most common presenting symptom of cholecystitis, and about 75% of patients with symptomatic gallstone disease seek medical attention for episodic abdominal pain. In patients who present with a complication of gallstones, such as acute cholecystitis, a history of recurrent episodes of abdominal pain in the months preceding the complication is often elicited.

Pathogenesis

Biliary pain (conventionally referred to as biliary “colic,” a misnomer) is caused by intermittent obstruction of the cystic duct by 1 or more gallstones. Biliary pain does not require that inflammation of the gallbladder accompany the obstruction. The term “chronic cholecystitis” to describe biliary pain should be avoided because it implies the presence of a chronic inflammatory infiltrate that may or may not be present in a given patient. Indeed, the severity and frequency of biliary pain and the pathologic changes in the gallbladder do not correlate.

The most common histologic changes observed in patients with biliary pain are mild fibrosis of the gallbladder wall with a chronic inflammatory cell infiltrate and intact mucosa. Recurrent episodes of biliary pain can also be associated with a scarred, shrunken gallbladder and Rokitansky-Aschoff sinuses (intramural diverticula). Bacteria can be cultured from gallbladder bile or gallstones themselves in about 10% of patients with biliary pain, but bacterial infection is not believed to contribute to the symptoms (see Chapter 67).

Clinical Features

Biliary pain is visceral in nature and thus poorly localized. In a typical case, the patient experiences episodes of upper abdominal pain, usually in the epigastrium or RUQ, but sometimes in other abdominal locations. Ingestion of a meal often precipitates pain, but more commonly no inciting event is apparent. The onset of biliary pain is more likely to occur during periods of weight reduction and marked physical inactivity such as prolonged bed rest than at other times.

The term “biliary colic,” used in the past, is a misnomer because the pain is steady rather than intermittent, as would be suggested by the word colic. The pain increases gradually over a period of 15 minutes to an hour and then remains at a plateau for an hour or more before slowly resolving. In one third of patients, the onset of pain may be more sudden, and on rare occasions, the pain may cease abruptly. Pain lasting more than 6 hours suggests acute cholecystitis rather than simple biliary pain.

In order of decreasing frequency, biliary pain is felt maximally in the epigastrium, RUQ, left upper quadrant, and various parts of the precordium or lower abdomen. Therefore, the notion that pain not located in the RUQ is atypical of gallstone disease is incorrect. Radiation of the pain to the scapula, right shoulder, or lower abdomen occurs in half of patients. Diaphoresis and nausea with some vomiting are common, although vomiting is not as protracted as in intestinal obstruction or acute pancreatitis. Like patients with other kinds of visceral pain, the patient with biliary pain is usually restless and active during an episode.

Complaints of gas, bloating, flatulence, and dyspepsia, which are common in patients with gallstones, are probably not related to the stones themselves. These nonspecific
symptoms are found with similar frequencies in persons without gallstones. Accordingly, patients with gallstones whose only symptoms are dyspepsia and other nonspecific upper GI tract complaints are not candidates for cholecystectomy. Physical findings are usually normal, with only mild to moderate gallbladder tenderness during an attack and perhaps mild residual tenderness lasting several days after an attack.

Diagnosis

In a patient with uncomplicated biliary pain, laboratory parameters are usually normal. Elevations of serum bilirubin, alkaline phosphatase, or amylase levels suggest coexisting cholelithiasis.

In general, the first, and often the only, imaging study recommended in patients with biliary pain is US of the RUQ. Despite the impressive diagnostic accuracy of US, a clinically important stone is occasionally missed and the correct diagnosis delayed because of the large number of patients who undergo US for any reason. Given the relatively benign natural history of biliary pain, patients with suspected gallstones but a negative US result can safely be observed, with further diagnostic testing reserved for those in whom symptoms recur.

Differential Diagnosis

The differential diagnosis of recurrent episodic upper abdominal symptoms includes reflux esophagitis, peptic ulcer, pancreatitis, renal colic, diverticulitis, carcinoma of the colon, IBS, radiculopathy, and angina pectoris (see Chapter 11). Usually a carefully taken history assists in narrowing the differential diagnosis. In a study of 1008 patients who underwent cholecystectomy for gallstones, clinical features associated with biliary pain (“episodic gallbladder pain”) were episodic pain (usually once a month or less), pain lasting 30 minutes to 2 hours, pain during the evening or at night, and onset of symptoms one year or less before presentation.

Treatment

Patients with recurrent uncomplicated biliary pain and documented gallstones are generally treated with elective laparoscopic cholecystectomy (see Chapter 66). Acute biliary pain improves with administration of meperidine, with or without ketorolac or diclofenac. Aspirin taken prophylactically has been reported to prevent gallstone formation as well as acute attacks of biliary pain in patients with gallstones, but long-term use of other NSAIDs does not prevent gallstone formation.

Acute Cholecystitis

Acute cholecystitis is the most common complication of gallstone disease. Inflammation of the gallbladder wall associated with abdominal pain, RUQ tenderness, fever, and leukocytosis is the hallmark of acute cholecystitis. In some 90% of cases, the underlying cause is obstruction of the outlet of the gallbladder by a gallstone in the cystic duct, gallbladder neck, or Hartman’s pouch. In the remaining 10% of cases, cholecystitis occurs in the absence of gallstones (acalculous cholecystitis [see Chapter 67]). Acute cholecystitis caused by gallstones is a disease of young, otherwise healthy women and generally has a favorable prognosis, whereas acute acalculous cholecystitis occurs more commonly in critically ill patients and is associated with high morbidity and mortality rates.

Pathogenesis

Acute cholecystitis generally occurs when a stone becomes embedded in the cystic duct and causes chronic obstruction, rather than transient obstruction as in biliary pain. Stasis of bile within the gallbladder lumen results in damage of the gallbladder mucosa, with consequent release of intracellular enzymes and activation of a cascade of inflammatory mediators.

In animal studies, if the cystic duct is ligated, the usual result is gradual absorption of the gallbladder contents without the development of inflammation; the additional instillation of a luminal irritant (e.g., concentrated bile or lysosome) or trauma from an indwelling catheter is required to cause acute cholecystitis in an obstructed gallbladder. Phospholipase A is believed to be released by gallstone-induced mucosal trauma and converts lecithin to lyssolecithin. Although normally absent from gallbladder bile, lyssolecithin is present in the gallbladder contents of patients with acute cholecystitis. In animal models, installation of lyssolecithin into the gallbladder produces acute cholecystitis associated with increased protein secretion, decreased water absorption, and evidence of WBC invasion associated with elevated production of prostaglandins E and F. Administration of indomethacin, a COX inhibitor, has been shown to block this inflammatory response. Studies of human tissue obtained at cholecystectomy have demonstrated enhanced prostaglandin production in the inflamed gallbladder. Additionally, administration of IV indomethacin and oral ibuprofen to patients with acute cholecystitis has been shown to diminish both luminal pressure in the gallbladder and pain.

Supporting evidence for the role of prostaglandins in the development of acute cholecystitis comes from a prospective study in which patients who presented with biliary pain were randomized to receive diclofenac, a prostaglandin synthetase inhibitor, or placebo. Ultimately, acute cholecystitis developed in 9 of 40 patients who received placebo, whereas episodes of biliary pain resolved in all 20 patients who received diclofenac. These data suggest a chain of events in which obstruction of the cystic duct in association with one or more intraluminal factors damages the gallbladder mucosa and stimulates prostaglandin synthetase. The resulting fluid secretion and inflammatory changes promote a cycle of further mucosal damage and inflammation.

Enteric bacteria can be cultured from gallbladder bile in roughly half of patients with acute cholecystitis. Bacteria are not believed to trigger the actual onset of acute cholecystitis, however.

Pathology

If examined in the first few days of an attack of acute cholecystitis, the gallbladder is usually distended and contains a stone embedded in the cystic duct. After the gallbladder is opened, inflammatory exudate and, rarely, pus are present. Later in the attack, the bile pigments that are normally present are absorbed and replaced by thin mucoid fluid, pus, or blood. If the attack of acute cholecystitis is left untreated for a long period but the cystic duct remains obstructed, the lumen of the gallbladder may become distended with clear mucoid fluid, a condition known as hydrops of the gallbladder.

Histologic changes range from mild acute inflammation with edema to necrosis and perforation of the gallbladder wall. Surprisingly, the severity of histologic changes correlates little with the patient’s symptoms. If the gallbladder is resected for acute cholecystitis and no stones are found, the specimen should be carefully examined histologically for evidence of vasculitis or cholesterol emboli, because these
systemic disorders may manifest as acalculous cholecystitis (see Chapter 36).

**Clinical Features**

Approximately 75% of patients with acute cholecystitis report prior attacks of biliary pain (see Table 65-2). Often, such a patient is alerted to the possibility that more than simple biliary pain is occurring by the prolonged duration of the pain. If biliary pain has been constant for more than 6 hours, acute cholecystitis should be suspected.

In contrast to uncomplicated biliary pain, the physical findings can, in many cases, suggest the diagnosis of acute cholecystitis. Fever is common, but body temperature is usually less than 102°F unless the gallbladder has become gangrenous or has perforated (Fig. 65-14). Mild jaundice is present in 20% of patients with acute cholecystitis and 40% of older adult patients. Serum bilirubin levels usually are less than 4 mg/dL. Bilirubin levels above this value suggest the possibility of bile duct stones, which may be found in 50% of jaundiced patients with acute cholecystitis. Another cause of pronounced jaundice in patients with acute cholecystitis is Mirizzi’s syndrome, which is associated with inflammatory obstruction of the common hepatic duct (see later).

The abdominal examination often demonstrates right subcostal tenderness with a palpable gallbladder in a third of patients; a palpable gallbladder is more common in patients having a first attack of acute cholecystitis. Repeated attacks usually result in a scarred, fibrotic gallbladder that is unable to distend. For unclear reasons, the gallbladder is usually palpable lateral to its normal anatomic location.

A relatively specific finding of acute cholecystitis is Murphy’s sign. During palpation in the right subcostal region, pain and inspiratory arrest may occur when the patient takes a deep breath that brings the inflamed gallbladder into contact with the examiner’s hand. The presence of Murphy’s sign in the appropriate clinical setting is a reliable predictor of acute cholecystitis, although gallstones should still be confirmed by US.

**Natural History**

The pain of untreated acute cholecystitis generally resolves in 7 to 10 days. Not uncommonly, symptoms remit within 48 hours of hospitalization. One study has shown that acute cholecystitis resolves without complications in about 83% of patients but results in gangrenous cholecystitis in 7%, gallbladder empyema in 6%, perforation in 3%, and emphysematous cholecystitis in fewer than 1%.277

**Diagnosis**

Perhaps because it is so common, acute cholecystitis is often at the top of the differential diagnosis of abdominal symptoms and is actually overdiagnosed when clinical criteria alone are considered. In a prospective series of 100 patients with RUQ pain and tenderness and suspected acute cholecystitis, this diagnosis was correct in only two thirds of cases. The clinician must therefore use laboratory and imaging studies to confirm the presence of acute cholecystitis, exclude complications such as gangrene and perforation, and look for alternative causes of the clinical findings.

Table 65-3 shows the most common laboratory findings in acute cholecystitis. Leukocytosis with a shift to immature neutrophils is common. Because a diagnosis of bile duct stones with cholangitis usually is in the differential diagnosis, attention should be directed to results of liver biochemical tests. Even without detectable bile duct obstruction, acute cholecystitis often causes mild elevations in serum aminotransferase and alkaline phosphatase levels. As noted earlier, the serum bilirubin level may also be mildly elevated (2 to 4 mg/dL), and even serum amylase and lipase values may be elevated nonspecifically. A serum bilirubin value above 4 mg/dL or amylase value above 1000 U/L usually indicates coexisting bile duct obstruction or acute pancreatitis, respectively, and warrants further evaluation.

When the level of leukocytosis exceeds 15,000/mm³, particularly in the setting of worsening pain, high fever (temperature > 102°F), and chills, suppurative cholecystitis (empyema) or perforation should be suspected, and urgent surgical intervention may be required. Such advanced gallbladder disease may be present even if local and systemic manifestations are unimpressive.

US is the single most useful imaging study in acutely ill patients with RUQ pain and tenderness. It accurately establishes the presence or absence of gallstones and serves as an extension of the physical examination. Presence of sonographic Murphy’s sign, defined as focal gallbladder tenderness under the transducer, has a positive predictive value better than 90% for detecting acute cholecystitis if gallstones are also present, the operator is skillful, and the patient is alert. Additionally, US can detect nonspecific findings suggestive of acute cholecystitis, such as pericholecystic fluid and gallbladder wall thickening greater than 4 mm. Both findings lose specificity for acute cholecystitis if the patient has ascites or hypoalbuminemia.

Because the prevalence of gallstones is high in the population, many patients with nonbiliary tract diseases that manifest as acute abdominal pain (e.g., acute pancreatitis and complications of peptic ulcer) may have incidental and clinically irrelevant gallstones. The greatest usefulness of cholescintigraphy in these patients is its ability to exclude acute cholecystitis and allow the clinician to focus on nonbiliary causes of the patient’s acute abdominal pain. A normal cholescintigraphy result shows radioactivity in the gallbladder, bile duct, and small intestine within 30 to 60 minutes of injection of the isotope. With rare exceptions, a normal result excludes acute cholecystitis due to gallstones. Several studies have suggested that the sensitivity and specificity of scintigraphy in the setting of acute cholecystitis are approximately 94% each. However, sensitivity and specificity are reduced considerably in patients who have liver disease, are receiving

![FIGURE 65-14. US demonstrating a complex fluid collection adjacent to the gallbladder (GB), consistent with gallbladder perforation. (Courtesy Julie Champine, MD, Dallas, Tex.)](image)
parenteral nutrition, or are fasting. These conditions can lead to a false-positive result, defined as the absence of isotope in the gallbladder in a patient who does not have acute cholecystitis. If a positive result is defined as the absence of isotope in the gallbladder, then a false-negative result is defined as filling of the gallbladder with isotope in the setting of acute cholecystitis, a situation that virtually never occurs. Therefore, scintigraphy should not be used as the initial imaging study in a patient with suspected cholecystitis but rather should be used as a secondary imaging study in patients who already are known to have gallstones and in whom a nonbiliary cause of acute abdominal pain is possible.232

The greatest usefulness of abdominal CT in patients with acute cholecystitis is to detect complications such as emphysematous cholecystitis and perforation of the gallbladder. At the same time, CT can exclude other intra-abdominal processes that may engender a similar clinical picture. For example, abdominal CT is highly sensitive for detecting pneumoperitoneum, acute pancreatitis, pancreatic pseudocysts, hepatic or intra-abdominal abscesses, appendicitis, and obstruction or perforation of a hollow viscus. Abdominal CT usually is not warranted in patients with obvious acute cholecystitis, but if the diagnosis is uncertain or the optimal timing of surgery is in doubt, CT may be invaluable.

Differential Diagnosis

The principal conditions to consider in the differential diagnosis of acute cholecystitis are appendicitis, acute pancreatitis, pyelonephritis or renal calculi, peptic ulcer, acute hepatitis, pneumonia, hepatic abscess or tumor, and gonococcal or chlamydial pericholangitis. These possibilities should be considered before a cholecystectomy is recommended.

Treatment

The patient in whom acute cholecystitis is suspected should be hospitalized. The patient is often hypovolemic from vomiting and poor oral intake, and fluid and electrolytes should be administered IV. Oral feeding should be withheld and an NG tube inserted if the patient has a distended abdomen or persistent vomiting.

In uncomplicated cases of acute cholecystitis, antibiotics need not be given. Antibiotics are warranted if the patient appears toxic or is suspected of having a complication such as perforation of the gallbladder or emphysematous cholecystitis. Broad-spectrum antibiotic coverage is usually indicated to cover Gram-negative organisms and anaerobes, with multiple possible regimens. The most commonly used regimens include piperacillin-tazobactam, ceftriaxone plus metronidazole, or levofloxacin plus metronidazole.

Definitive therapy of acute cholecystitis consists of cholecystectomy. The safety and effectiveness of a laparoscopic approach in the setting of acute cholecystitis have been demonstrated (see Chapter 66).241

Choledocholithiasis

Choledocholithiasis is defined as the occurrence of stones in the bile ducts. Like stones in the gallbladder, stones in the bile ducts may remain asymptomatic for years, and stones from the bile duct are known to pass silently into the duodenum, perhaps frequently. Unlike stones in the gallbladder, which usually become clinically evident as relatively benign episodes of recurrent biliary pain, stones in the bile duct, when they do cause symptoms, tend to manifest as life-threatening complications such as cholangitis and acute pancreatitis (see Chapter 58). Therefore, discovery of choledocholithiasis generally should be followed by an intervention to remove the stones (see Chapter 70).

Etiology

Gallstones may pass from the gallbladder into the bile duct or form de novo in the duct. Generally, all gallstones from one patient, whether from the gallbladder or bile duct, are of one type, either cholesterol or pigment. Cholesterol stones form only in the gallbladder, and any cholesterol stones found in the bile duct must have migrated there from the gallbladder. Black pigment stones, which are associated with old age, hemolysis, alcoholism, and cirrhosis, also form in the gallbladder but only rarely migrate into the bile duct. The majority of pigment stones in the bile duct are the softer brown pigment stones. These stones form de novo in the bile duct as a result of bacterial action on phospholipid and bilirubin in bile (see earlier).232 They are often proximal to a biliary stricture and are frequently associated with cholangitis. Brown pigment stones are found in patients with hepatolithiasis and recurrent pyogenic cholangitis (see Chapter 68).282

Even a percent of patients with gallbladder stones also have bile duct stones. Conversely, of patients with ductal stones, 95% also have gallbladder stones.244 In patients who present with choledocholithiasis months or years after a cholecystectomy, determining whether the stones were overlooked at the earlier operation or have subsequently formed may be impossible. In fact, formation of pigment stones in the bile duct is also a late complication of endoscopic sphincterotomy.263 In a study of the long-term consequences of endoscopic sphincterotomy in more than 400 patients, the cumulative frequency of recurrent bile duct stones was 12%; all the recurrent stones were of the brown pigment type, irrespective of the chemical composition of the original gallstones. This observation suggests that sphincterotomy permits chronic bacterial colonization of the bile duct that results in deconjugation of bilirubin and precipitation of pigment stones.

Stones in the bile duct usually come to rest at the lower end of the ampulla of Vater. Obstruction of the bile duct raises bile pressure proximally and causes the duct to dilate. Pressure in the bile duct is normally 10 to 15 cm H2O and rises to 25 to 40 cm H2O with complete obstruction. When pressure exceeds 15 cm H2O, bile flow decreases, and at 30 cm H2O, bile flow stops.

The bile duct dilates to the point that dilatation can be detected on either US or abdominal CT in about 75% of cases. In patients who have had recurrent bouts of cholangitis, the bile duct may become fibrotic and unable to dilate. Moreover, dilatation of the duct is sometimes absent in patients with choledocholithiasis because the obstruction is low-grade and intermittent.

Clinical Features

The morbidity of choledocholithiasis stems principally from biliary obstruction, which raises biliary pressure and diminishes bile flow. The rate of onset of obstruction, its extent, and the amount of bacterial contamination of the bile are the major factors that determine resulting symptoms. Acute obstruction usually causes biliary pain and jaundice, whereas obstruction that develops gradually over several months may manifest initially as pruritus or jaundice alone.263 If bacteria proliferate, life-threatening cholangitis may result (see later).

Physical findings are usually normal if obstruction of the bile duct is intermittent. Mild to moderate jaundice may be noted when obstruction has been present for several days to a few weeks. Deep jaundice without pain, particularly with a
palpable gallbladder (Courvoisier’s sign), suggests neoplastic obstruction of the bile duct, even when the patient has stones in the gallbladder. With longstanding obstruction, secondary biliary cirrhosis may result, leading to physical findings of chronic liver disease.

As shown in Table 65-2, the results of laboratory studies may be the only clue to the presence of choledocholithiasis. With bile duct obstruction, serum bilirubin and alkaline phosphatase levels both increase. Bilirubin accumulates in serum because of blocked excretion, whereas alkaline phosphatase levels rise because of increased synthesis of the enzyme by the canalicular epithelium. The rise in the alkaline phosphatase level is more rapid than and precedes the rise in bilirubin level. The absolute height of the serum bilirubin level is proportional to the extent of obstruction, but the height of the alkaline phosphatase level bears no relation to either the extent of obstruction or its cause. In cases of choledocholithiasis, the serum bilirubin level is typically in the range of 2 to 5 mg/dL and rarely exceeds 12 mg/dL. Transient “spikes” in serum aminotransferase or amylase levels suggest passage of a bile duct stone into the duodenum. The overall sensitivity of liver biochemical testing for detecting choledocholithiasis is reported to be 94%; serum levels of GGTP are elevated most commonly but may not be assessed in clinical practice.

Natural History
Little information is available on the natural history of asymptomatic bile duct stones. In many patients, such stones remain asymptomatic for months or years, but available evidence suggests the natural history of asymptomatic bile duct stones is less benign than that of asymptomatic gallstones.

Diagnosis
US actually visualizes bile duct stones in only about 50% of cases; whereas dilatation of the bile duct to a diameter greater than 6 mm is seen in about 75% of cases. US can confirm, or at least suggest, the presence of bile duct stones but cannot exclude choledocholithiasis definitively. EUS, although clearly more invasive than standard US, has the advantage of visualizing the bile duct more accurately. In preliminary studies, EUS has excluded or confirmed choledocholithiasis with sensitivity and specificity rates of approximately 98% as compared with ERCP.

ERCP is the standard method for diagnosis and therapy of bile duct stones, with sensitivity and specificity rates of about 95%. When the clinical probability of choledocholithiasis is low, however, less invasive studies like EUS and MRCP should be performed first.

Percutaneous transhepatic cholangiography (percutaneous THC) is also an accurate test for confirming the presence of choledocholithiasis. The procedure is most readily accomplished when the intrahepatic bile ducts are dilated and is performed primarily when ERCP is unavailable or has been technically unsuccessful.

Laparoscopic US may be used in the surgical suite immediately before mobilization of the gallbladder during cholecystectomy. Laparoscopic US may be as accurate as surgical cholangiography in detecting bile duct stones and may thereby obviate the need for the latter.

Differential Diagnosis
Symptoms caused by obstruction of the bile duct cannot be distinguished from those caused by obstruction of the cystic duct. Therefore, biliary pain is always in the differential diagnosis in patients with an intact gallbladder. The presence of jaundice or abnormal liver biochemical test results strongly points to the bile duct rather than the gallbladder as the source of the pain.

In patients who present with jaundice, malignant obstruction of the bile duct or obstruction from a choledochal cyst may be indistinguishable clinically from choledocholithiasis (see Chapters 62 and 69). AIDS-associated cholangiopathy and papillary stenosis should be considered in HIV-positive patients with RUQ pain and abnormal liver biochemical test results (see Chapter 34).

Treatment
Because of its propensity to result in serious complications such as cholangitis and acute pancreatitis, choledocholithiasis warrants treatment in nearly all cases. The optimal therapy for a given patient depends on the severity of symptoms, presence of coexisting medical problems, availability of local expertise, and presence or absence of the gallbladder.

Bile duct stones discovered at the time of a laparoscopic cholecystectomy present a dilemma to the surgeon. Some surgeons may attempt laparoscopic exploration of the bile duct. In other cases, the operation can be converted to an open cholecystectomy with bile duct exploration, but this approach results in greater morbidity and a more prolonged hospital stay. Alternatively, the laparoscopic cholecystectomy can be carried out as planned, and the patient can return for ERCP with removal of the bile duct stones. Such an approach, if successful, cures the disease but runs the risk of necessitating a third procedure, namely a bile duct exploration, if the stones cannot be removed at ERCP. In general, the greater the expertise of the therapeutic endoscopist, the more inclined the surgeon should be to complete the laparoscopic cholecystectomy and have the bile duct stones removed endoscopically.

In especially high-risk patients, endoscopic removal of bile duct stones may be performed without cholecystectomy. This approach is particularly appropriate for older adult patients with other severe concurrent illnesses. Cholecystectomy is required subsequently for recurrent symptoms in only 10% of patients. Surgical management and endoscopic treatment of gallstones are discussed in detail in Chapters 66 and 70, respectively.

Cholangitis
Of all the common complications of gallstones, the most serious and lethal is acute bacterial cholangitis. Pus under pressure in the bile ducts leads to rapid spread of bacteria via the liver into the blood, with resulting septicemia. Moreover, the diagnosis of cholangitis is often problematic (especially in the critical early phase of the disease) because clinical features that point to the biliary tract as the source of sepsis are often absent. Table 65-2 delineates the symptoms, signs, and laboratory findings that can aid in an early diagnosis of cholangitis.

Etiology and Pathophysiology
In approximately 85% of cases, cholangitis is caused by a stone embedded in the bile duct, with resulting bile stasis. Other causes of bile duct obstruction that may result in cholangitis are neoplasms (see Chapters 60 and 69), biliary strictures (see Chapters 68 and 70), parasitic infections (see Chapters 68 and 84), and congenital abnormalities of the bile ducts (see Chapter 62). This discussion deals specifically with cholangitis caused by gallstones in the bile duct.
Bile duct obstruction is necessary but not sufficient to cause cholangitis. Cholangitis is relatively common in patients with choledocholithiasis and nearly universal in patients with a post-traumatic bile duct stricture, but is seen in only 15% of patients with neoplastic obstruction of the bile duct. It is most likely to result when a bile duct that already contains bacteria becomes obstructed, as in most patients with choledocholithiasis and stricture but in few patients with neoplastic obstruction. Malignant obstruction is more often complete than obstruction by a stricture or a bile duct stone and less commonly permits reflux of bacteria from duodenal contents into the bile ducts.296

The bacterial species most commonly cultured from the bile are *E. coli*, *Klebsiella*, *Pseudomonas*, *Proteus*, and enterococci. Anaerobic species such as *Bacteroides fragilis* and *Clostridium perfringens* are found in about 15% of appropriately cultured bile specimens. Anaerobes usually accompany aerobes, especially *E. coli*. The shaking chills and fever of cholangitis are due to bacteremia from bile duct organisms. The degree of regurgitation of bacteria from bile into hepatic venous blood is directly proportional to the biliary pressure and, hence, the degree of obstruction.296 For this reason, decompression alone often effectively treats the illness.

**Clinical Features**

The hallmark of cholangitis is Charcot’s triad, consisting of RUQ pain, jaundice, and fever (see Table 65-2). The full triad is present in only 70% of patients. The pain of cholangitis may be surprisingly mild and transient but is often accompanied by chills and rigors. Older adult patients in particular may present solely with mental confusion, lethargy, and delirium. Altered mental status and hypotension in combination with Charcot’s triad, known commonly as Reynolds’ pentad, occur in severe suppurative cholangitis.

On physical examination, fever is almost universal, occurring in 95% of patients, and usually greater than 102°F. RUQ tenderness is elicited in about 90% of patients, but jaundice is clinically detectable in only 80%. Notably, peritoneal signs are found in only 15% of patients. The combination of hypotension and mental confusion indicates Gram-negative sepsisemia. In overlooked cases of severe cholangitis, intrahepatic abscesses may manifest as a late complication (see Chapter 84).

Laboratory study results are often helpful in pointing to the biliary tract as the source of sepsis. In particular, the serum bilirubin level exceeds 2 mg/dL in 80% of patients. When the bilirubin level is normal initially, the diagnosis of cholangitis may not be suspected.296 The WBC count is elevated in 80% of patients. In many patients who have a normal WBC count, examination of the peripheral blood smear reveals a dramatic shift to immature neutrophil forms. The serum alkaline phosphatase level is usually elevated, and the serum amylase level may also be elevated if pancreatitis is also present.

In the majority of cases, blood culture results are positive for enteric organisms, especially if culture specimens are obtained during chills and fever spikes. The organism found in the blood is invariably the same as that found in the bile.

**Diagnosis**

The principles of radiologic diagnosis of cholangitis are the same as those for choledocholithiasis. Stones in the bile duct are seen ultrasonographically in only about 50% of cases113 but can be inferred by detection of a dilated bile duct in about 75% of cases (see Table 65-3). Normal US findings do not exclude the possibility of choledocholithiasis in a patient in whom the clinical presentation suggests cholangitis.296

Abdominal CT is an excellent test for excluding complications of gallstones such as acute pancreatitis and abscess, but standard abdominal CT is not capable of excluding bile duct stones. EUS and MRC, as noted earlier, have a much higher accuracy rate than CT for detecting and excluding stones in the bile duct.

ERCP is the definitive test for the diagnosis of bile duct stones and cholangitis. Moreover, the ability of ERCP to establish drainage of infected bile under pressure can be life-saving. If ERCP is unsuccessful, percutaneous THC can be performed (see Chapter 70).

**Treatment**

In cases of suspected bacterial cholangitis, blood culture specimens should be obtained immediately and therapy started with antibiotics effective against the likely causative organisms.297 In mild cases, initial therapy with a single drug (e.g., cefoxitin 2.0 g IV every 6 to 8 hours) is usually sufficient. In severe cases, more intensive therapy (e.g., gentamicin, ampicillin, and metronidazole or a broad-spectrum agent such as piperacillin-tazobactam 3.575 g IV every 6 hours or, if resistant organisms are suspected, meropenem 1 g IV every 8 hours) is indicated.

The patient’s condition should improve within 6 to 12 hours, and in most cases, the infection comes under control within 2 to 3 days, with defervescence, relief of discomfort, and a decline in WBC count. In these cases, definitive therapy can be planned on an elective basis. If, however, after 6 to 12 hours of careful observation, the patient’s clinical status declines, with worsening fever, pain, mental confusion, or hypotension, the bile duct must be decompressed immediately.297 If available, ERCP with stone extraction, or at least decompression of the bile duct with an intrabiliary stent, is the treatment of choice. Controlled studies in which ERCP and decompression of the bile duct were compared with emergency surgery and bile duct exploration have shown dramatically lower morbidity and mortality rates in patients treated endoscopically.298 The surgical treatment and endoscopic management of cholangitis are discussed in detail in Chapters 66 and 70, respectively.

**UNCOMMON COMPLICATIONS**

Table 65-4 describes the clinical manifestations, diagnosis, and treatment of several uncommon complications of gallstone disease.

**Emphysematous Cholecystitis**

Patients who have emphysematous cholecystitis present with the same clinical manifestations as patients with uncomplicated acute cholecystitis, but in the former, gas-forming organisms have secondarily infected the gallbladder wall. Pockets of gas are evident in the area of the gallbladder fossa on plain abdominal films, US, and abdominal CT (see Fig. 65-13).298 Emphysematous cholecystitis often occurs in diabetic persons or older men who do not have gallstones, in whom atherosclerosis of the cystic artery with resulting ischemia may be the initiating event (see Chapter 67). Emergency antibiotic therapy with anaerobic coverage and early cholecystectomy are warranted because the risk of gallbladder perforation is high.

**Cholecystoenteric Fistula**

A cholecystoenteric fistula occurs when a stone erodes through the gallbladder wall (usually the neck) and into a hollow
viscera. The most common entry point into the bowel is the duodenum, followed in frequency by the hepatic flexure of the colon, the stomach, and the jejunum. Symptoms are initially similar to those of acute cholecystitis, although at times the stone may pass into the bowel and may be excreted without causing any symptoms. Because the biliary tract is decompressed, cholangitis is not common, despite gross seeding of the gallbladder wall with gas-forming organisms (Escherichia coli, Clostridium welchii, and anaerobic streptococci). More common in older adult diabetic men; can occur without stones (see Chapter 67).

If the gallstone exceeds 25 mm in diameter, it may manifest similar to those of acute cholecystitis, although at times the stone may pass into the bowel and may be excreted without causing any symptoms. Because the biliary tract is decompressed, cholangitis is not common, despite gross seeding of the gallbladder wall with gas-forming organisms (Escherichia coli, Clostridium welchii, and anaerobic streptococci). More common in older adult diabetic men; can occur without stones (see Chapter 67).

<table>
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<th>Complication</th>
<th>Pathogenesis</th>
<th>Clinical Features</th>
<th>Diagnosis/Treatment</th>
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<tr>
<td>Emphysematous cholecystitis</td>
<td>Secondary infection of the gallbladder wall with gas-forming organisms (Clostridium welchii, Escherichia coli, and anaerobic streptococci)</td>
<td>Symptoms and signs similar to those of severe acute cholecystitis</td>
<td>Plain abdominal films may show gas in the gallbladder fossa. US and CT are sensitive for confirming gas. Treatment is with IV antibiotics, including anaerobic coverage, and early cholecystectomy. High morbidity and mortality rates.</td>
</tr>
<tr>
<td>Cholecystoenteric fistula</td>
<td>Erosion of a (usually large) stone through the gallbladder wall into the adjacent bowel, most often the duodenum, followed in frequency by the hepatic flexure, stomach, and jejunum</td>
<td>Symptoms and signs similar to those of acute cholecystitis, although sometimes a fistula may be clinically silent. Stones &gt; 25 mm, especially in older adult women, may produce a bowel obstruction, or “gallstone ileus”; the terminal ileum is the most common site of obstruction. Gastric outlet obstruction (Bouveret’s syndrome) may occur rarely.</td>
<td>Plain abdominal films may show gas in the biliary tree and/or a small bowel obstruction in gallstone ileus, as well as a stone in the RLQ if the stone is calcified. Contrast upper GI series may demonstrate the fistula. A fistula from a solitary stone that passes may close spontaneously. Cholecystectomy and bowel closure are curative. Gallstone ileus requires emergency laparotomy; the diagnosis is often delayed, with a resulting mortality rate of ~20%.</td>
</tr>
<tr>
<td>Mirizzi’s syndrome</td>
<td>An impacted stone in the gallbladder neck or cystic duct, with extrinsic compression of the common hepatic duct from accompanying inflammation or fistula</td>
<td>Jaundice and RUQ pain</td>
<td>ERCP demonstrates dilated intrahepatic ducts and extrinsic compression of the common hepatic duct and possible fistula. Preoperative diagnosis is important to guide surgery and minimize the risk of BD injury.</td>
</tr>
<tr>
<td>Porcelain gallbladder</td>
<td>Intramural calcification of the gallbladder wall, usually in association with stones</td>
<td>No symptoms attributable to the calcified wall per se, but carcinoma of the gallbladder is a late complication in ~20% (see Chapter 69)</td>
<td>Plain abdominal films or CT show intramural calcification of the gallbladder wall. Prophylactic cholecystectomy is indicated to prevent carcinoma.</td>
</tr>
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BD, bile duct; RLQ, right lower quadrant; RUQ, right upper quadrant.

**TABLE 65-4 Uncommon Complications of Gallstone Disease**

**Mirizzi’s Syndrome**

Mirizzi’s syndrome is a rare complication in which a stone embedded in the neck of the gallbladder or cystic duct extrinsically compresses the common hepatic duct, with resulting jaundice, bile duct obstruction, and in some cases a fistula. Typically the gallbladder is contracted and contains stones. ERCP usually demonstrates the characteristic extrinsic compression of the common hepatic duct. Treatment is traditionally by an open cholecystectomy, although endoscopic stenting and laparoscopic cholecystectomy have been performed successfully. Preoperative diagnosis of Mirizzi’s syndrome is important so that bile duct injury can be avoided (see Chapter 66).
Porcelain Gallbladder

Strictly speaking, *porcelain gallbladder*, defined as intramural calcification of the gallbladder wall, is not a complication of gallstones but is mentioned here because of the remarkable tendency of carcinoma to develop as a late complication of gallbladder calcification (specifically, a gallbladder with focal rather than diffuse wall calcification). Diagnosis of a porcelain gallbladder can be made with a plain abdominal film or abdominal CT, which shows intramural calcification of the gallbladder wall. In occasional persons, hypersecretion of calcium into bile results in a “milk of calcium” or “limy” bile that can mimic the radiologic features of porcelain gallbladder. Prophylactic cholecystectomy, preferably through a laparoscopic approach, is indicated to prevent subsequent development of carcinoma, which may otherwise occur in up to 20% of cases (see Chapter 69).²⁰⁶

KEY REFERENCES

Full references for this chapter can be found on <www.expertconsult.com>.

REFERENCES