Section 15

LECTURE

Biliary Secretion, Cholestasis, and Gallstone Formation

BILIARY SECRETION, CHOLESTASIS AND GALLSTONE FORMATION

A. Composition of Bile

Bile is the "exocrine" secretion of the liver. Its solute composition (3 g/dL for hepatic bile, 10 g/dL for gallbladder (GB) bile) consists principally of four lipid species in an aqueous electrolyte solution (Figure 1). They are:

a. Bile Salts (BS) - a "quintet" of closely related molecules - 2/3 of solute mass
b. Phospholipids (PL) - mostly (>95%) phosphatidylcholine (lecithin, PC)
c. Cholesterol (CH) - all unesterified (free sterol)
d. Bilirubin - 90% mono- and di-glucuronic acid conjugates (bile pigments); 10% glucose/xylose conjugates; <1% unconjugated

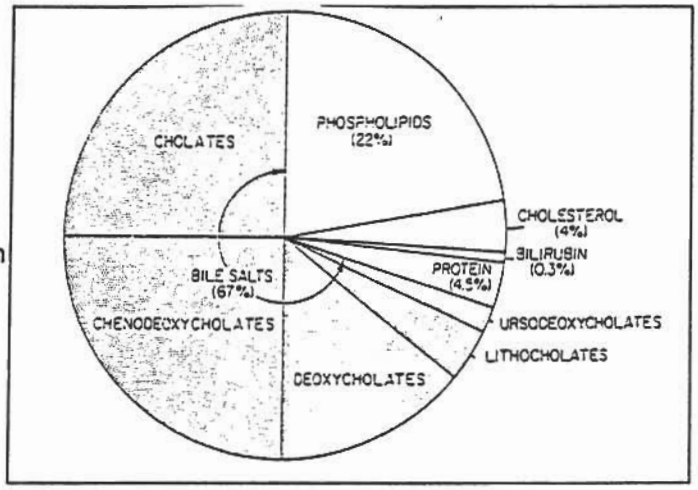

FIGURE 1: Typical solute composition of human gallbladder bile, by weight percentage

B. Principal Functions of Bile

a. To aid the excretion of endogenous lipids (through the biliary tree)
b. To aid the absorption of dietary lipids (from upper small intestine)

C. Bile Water Production

1. Production of bile is ~500-1200 mL per day.

2. Water transport is entirely passive (as in the intestine) and occurs at hepatocellular (canalicular), ductular and gallbladder epithelial sites in response to active secretion/absorption of a number of solutes. These can be compartmentalized, as shown in Figure 2.

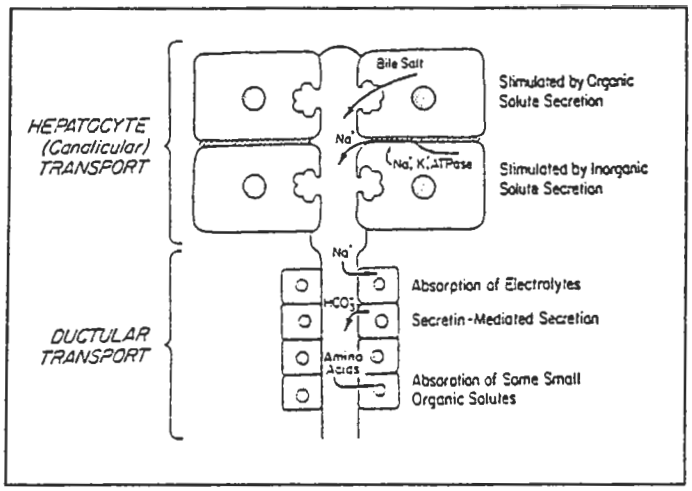

FIGURE 2: Bile water production at different levels of the biliary system. Direction of water movement and principal stimuli for water transport shown by labeled arrows.

3. Canalicular H_2O transport:

   Mediated by active BS secretion (BS-dependent H_2O transport) and the active transport of glutathione (a glutamyl-cysteinyl-glycine tripeptide, or GSH) responsible for most BS-independent H_2O transport. All H_2O and many inorganic cations and anions flow passively across the "leaky" tight junctions (paracellular spaces) by osmotic, solvent drag and Donnan forces. Na^+ and H_2O are concentrated in paracellular spaces by Na^+/K^+-ATPase on the basolateral membranes. 80% of total bile water is canalicular in origin, 60% of which is BS-dependent.

4. Ductular H_2O transport:

   Both secretion and absorption occur. Via secretion of HCO_3^- in exchange for Cl^- and utlizing the CFTR ion-channel for Cl^-; both stimulated by the hormone secretin. Resorption of H_2O secondary to electrolyte (NaCl and $NaHCO_3$), amino acid, and glucose absorption.

5. Gallbladder H_2O transport:

   Major site of H_2O absorption and hence concentration of bile lipids secondary to electrolyte absorption (NaCl and $NaHCO_3$). Gallbladder also acidifies bile by secreting protons (H^+) in exchange for Na^+.

D. Basolateral Lipid Transporters on Hepatocytes

- NTCP: the Na^+-BS-symporter utilizing Na^+/K^+ ATPase on the same membrane; 80% of bile salt recapture from blood.

- OATP: the Na^+-independent BS transporter – also transports many organic anions (OA), bulky cations and certain neutral compounds.

- OAT2 is a related organic anion pump (low level of expression in humans)
- OCT1, an organic cation (OC) transporter, includes choline for PC synthesis.
- MRP (multiple drug resistance related protein) 1 and 6: these are on the lateral membrane for the exit of highly polar conjugated organic anions, i.e., bile salts and bilirubin conjugates (only become important in cholestasis).

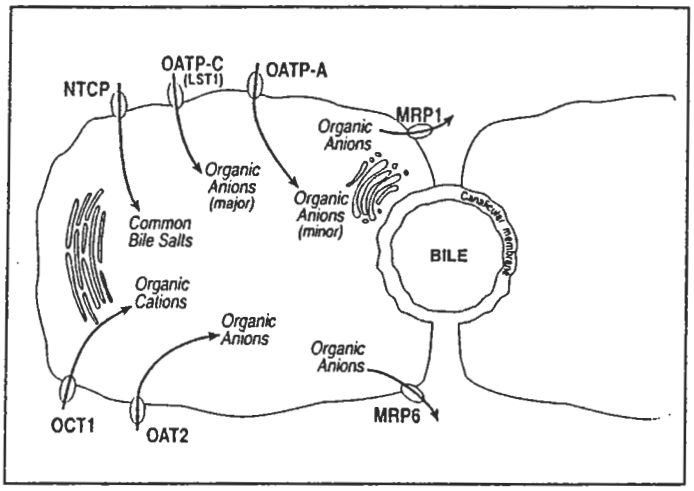

FIGURE 3: Basolateral lipid transporters of the hepatocyte

E. Canalicular Lipid Transporters

- All members of the ATP-Binding Cassette and are known as ABC transporters; they are therefore primary active exit pumps.
- MRP2 (also known as cMOAT) transports a wide range of divalent organic anions including those shown in Figure 4.
- BSEP is the common BS exit pump.
- MDR3, which is incorrectly labelled a multiple drug resistance protein (MDR2 in rodents), is in fact the lecithin "flippase" (trans-membrane PC transporter).
- MDR1 transports organic cations.
- ABCG5 and ABCG8 are the twinned half-transporters that transport cholesterol (and phytosterols) into bile.
- FIC1 is an aminophospholipid "flippase." It's on the canalicular membrane and apical membrane of the large cholangiocytes. How It's dysfunction impairs bile salt secretion unknown. Defective in Byler's disease (Progressive Familial Intrahepatic Cholestasis (PFIC) Type 1).

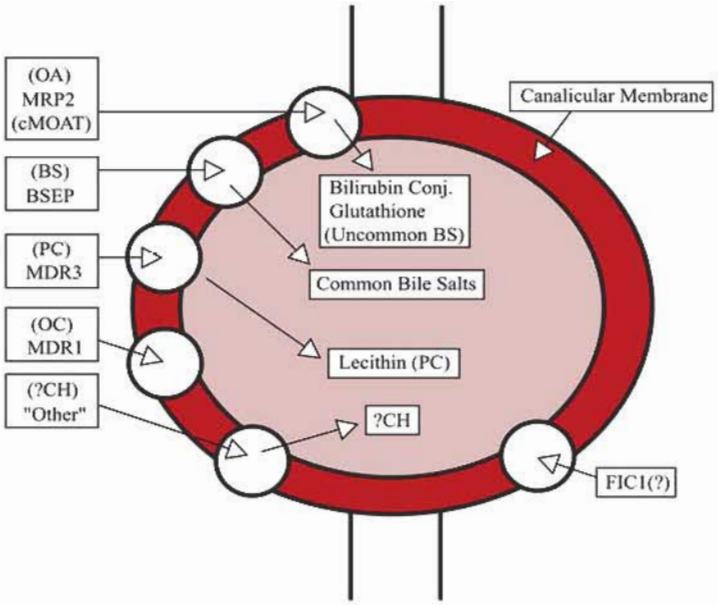

FIGURE 4: Canalicular membrane transporters

F. Transporters on Biliary Ductules

- FIC1: Familial Intrahepatic Cholestasis gene product isoform 1.
- ASBT: the apical sodium-dependent BS transporter on large cholangiocytes – possibly responsible for "cholehepatic shunting" of BS.
- CFTR: Cystic Fibrosis transmembrane conductance regulator, a Cl^-channel on large cholangiocytes – <u>not</u> on canalicular membrane.
- MRP3: multiple drug resistance related protein, isoform 3 – a basolateral BS exit pump.
- AE2: anionic exchanger isoform 2 mediating HCO_3^-/Cl^- exchange on apical membranes of large cholangiocytes.

On the large cholangiocytes the functions of CFTR and AE2 are upregulated by <u>SECRETIN</u>. Both combine to produce a highly alkaline ductular bile.

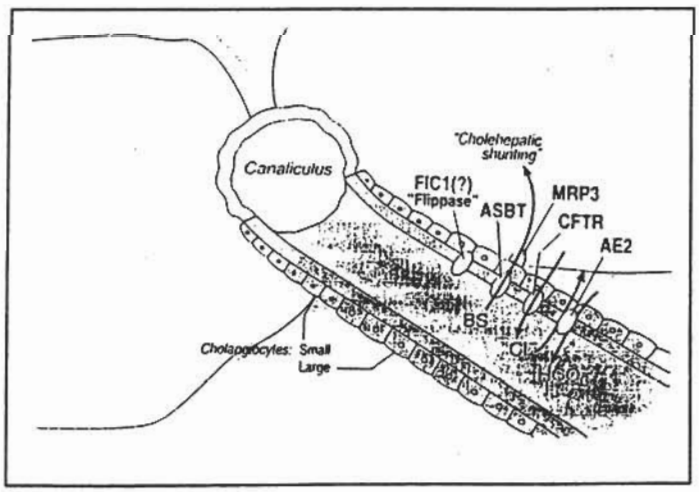

FIGURE 5: Hepatobiliary ductular functions

G. Physical Chemistry of Biliary Lipid Secretion

- Vesicles of PC+CH form on the luminal face of the canalicular membrane (MDR3 function).
- BS^- are pumped out as monomers (BSEP) and form simple micelles when [BS]>CMC.
- Bilirubin conjugates (BDG) are pumped out as monomers (MRP2). They bind with high affinity to hydrophilic faces of BS monomers.
- CH is pumped out by ABCG5/ABCG8 on the canalicular membrane. Only functions when solubilizing mixed micelles formed by BS and PC vesicles are present in the canalicular lumen.

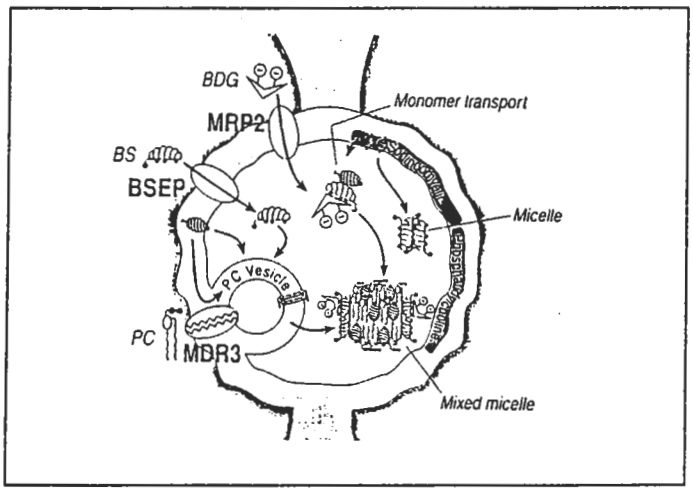

FIGURE 6: Physical-chemical reactions occurring in the canalicular lumen

H. The Enterohepatic Circulation of Bile Salts

Following biliary secretion and after playing a key role in the efficient absorption of dietary fat, 95-98% of BS are re-absorbed by the ASBT of the distal small intestine and passively by the colon, returned to the liver bound to HDL and albumin in portal blood, efficiently extracted by the liver by NTCP, and then rapidly resecreted into bile to complete an enterohepatic circulation. Note the following characteristics:

- Primary BS (made in liver from CH) are cholate, a trihydroxy species, and chenodeoxycholate, a dihydroxy species. The rate limiting enzymes in the pathways are microsomal CH 7α hydroxylase and oxysterol 7α hydroxylase.

- Secondary BS: made by anaerobic colonic flora via removal or epimerization of 7αOH group. This occurs only after hydrolysis of the amide linkage with glycine or taurine by bacterial cholylglycine amidase.

  Cholates (3α,7α,12α OH) → Deoxycholates (3α,12α OH)

  Chenodeoxycholates (3α,7α OH) → Lithocholates (3α OH) *or*

  → Ursodeoxycholates (3α,7β OH)

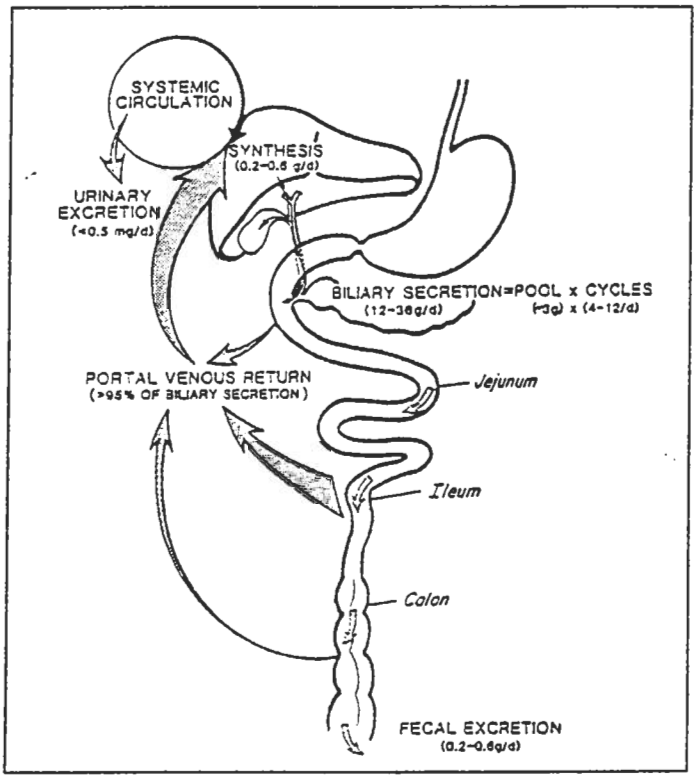

FIGURE 7: Enterohepatic circulation of BS in normal human beings. Arrows indicate BS movement within the biliary tree, intestine, portal venous system, and systemic circulation.

- Intestinal absorption is by passive nonionic and ionic diffusion from duodenal, jejunal and colonic sites, but quantitative active absorption occurs in the ileum where a specific (Na^+ coupled) BS transporter ($2Na^+$ with 1 BS) (ASBT) is present on the apical membranes of absorptive cells. This ASBT is ~100 kD and is identical to the ASBT on large cholangiocytes as well as proximal tubules of the kidney.

- After uptake into the liver cell from portal blood (>85% in a single pass) – see Figure 7 – reflux is prevented by intracellular protein binding and CoA thioester formation followed by conjugation with taurine or glycine in amide linkage (amidation) and sulfation (e.g. Lithocholic acid).

- Transcriptional control of bile salt homeostasis is via the nuclear receptor FXR. Hydrophobic bile salts bind to this nuclear receptor in hepatocytes. It forms a heterodimer with RXR and down regulates bile salt synthesis from cholesterol. This occurs via decreased transcription of the gene for cholesterol 7α hydroxylase (CYP7A), the rate limiting enzyme in the classical pathway. Binding of bile acids to FXR also upregulates the ileal bile salt binding protein, an intracellular cytosolic transport protein (ILLBP), whose precise function is presently unknown.

I. The Hepatobiliary Transporters in Cholestasis (Table 1)

- Much progress has been made during the last five years in defining hereditary and acquired defects at the molecular (gene, mRNA and transporter) levels.

- Three subclasses of PFIC (1,2,3) defined as involving mutations in FIC1, BSEP and MDR3 genes respectively – heterozygotes for mutations in MDR3 are at risk for recurrent cholestasis of pregnancy.

- The molecular changes in the acquired forms are being refined. Functioning MDR3 (PC "flippase") is required for formation of Lipoprotein X.

J. References (Biliary Secretion and Cholestasis)

1. Carey MC, Duane WC. The enterohepatic circulation. In: IM Arias, *et al.*, (eds). The Liver: Biology and Pathobiology, 3rd Ed. New York: Raven Press, 1994, pp 719-767.

2. Müller M, Jansen PLM. Molecular aspects of hepatobiliary transport. *Am J Physiol* 272, G1285-G1303, 1997.

3. Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. *N Engl J Med* 339:1217-27; 1998.

4. Boyer JL. Molecular mechanisms of cholestasis. In Hepatology into the next millenium: Lessons from the past – Issues for the future, AASLD postgraduate course 1999, pp. 111-116.

5. Meier PJ, Steiger B. Molecular mechanisms in bile formation. *News Physiol Sci* 15:89-93, 2000.

6. Meier PJ, Steiger B. Bile salt transporters. Annu. Rev. Physiol 2002; 64:635-661.

7. Thompson R, Strautnieks S. Inherited disorders of transport in the liver. Curr. Opin. Genet. Dev 2002; 10:310-13.

8. Trauner M, Boyer JL. Cholestatic syndromes. Curr. Opin. Gastroenterol 2001; 17:242-256.

9. Wittenburg H, Carey MC. Biliary cholesterol secretion by the twinned sterol half-transporters ABCG5 and ABCG8. J. Clin Invest 2002; 110:605-609.

TABLE 1: Membrane transporter defects in hereditary and acquired cholestatic disorders

Disease	Molecular Change
HEREDITARY	
Progressive familial intrahepatic cholestasis (PFIC)	
PFIC1 (low γ-GT)	Mutation of FIC1 gene (chromosome 18q21-22)
PFIC2 (low γ-GT)	Mutation of BSEP gene (chromosome 2q24); Canalicular BSEP protein absent
PFIC3 (high γ-GT)	Mutation of MDR3 gene (chromosome 7q21); Canalicular MDR3 protein absent
Benign recurrent intrahepatic cholestasis (BRIC)	Mutation of FIC1 gene (chromosome 18q21-22)
Dubin-Johnson syndrome	Mutation of MRP2 gene (10q23-24); Canalicular MRP2 protein absent
ACQUIRED	
Primary biliary cirrhosis	AE2 mRNA and protein reduced; hepatocytes and cholangiocytes are affected; MDR3 mRNA levels unchanged
Extrahepatic biliary atresia	NTCP mRNA reduced; inverse correlation with serum bilirubin levels; increase after successful Kasai procedure
Primary sclerosing cholangitis	OATP mRNA increased, possibly diminishing hepatic retention of organic anions
Extrahepatic biliary obstruction	MDR1 and MDR3 mRNA increased; direct correlation with serum bilirubin levels

SOURCE: Boyer, 1999

K. Cholesterol Gallstone Formation

1. Cholesterol (CH) supersaturation of gallbladder bile is necessary but not sufficient – requires GB hypomotility and accumulation of GB mucin gel (a nucleation matrix).

   a) CH supersaturation is generally from hypersecretion of biliary cholesterol by the liver. Causes likely to be both genetic (many *Lith* genes discovered in mice and in humans ethnicity and familial preponderance) and environmental factors (diet, pregnancy, obesity, drugs, hyperlipidimea and certain rare diseases somatostatinoma). Principal independent associations: i) family history, ii) high body mass index, iii) parity, iv) high serum triglyceride, v) low plasma cholesterol.

   b) GB hypomotility — from cholecystokinin (CCK) – transduction decoupling at level of gallbladder smooth muscle cells from toxicity of absorbed cholesterol molecules.

   c) Mucin gel accumulation: true hypersecretion and deficient clearance secondary to GB hypomotility. Stimulus unknown, but, probably, CH molecules absorbed by GB mucosa. Possibly, a defense reaction, which is also characterized by acute and chronic <u>sterile</u> inflammation of the gallbladder wall.

2. CH-rich vesicles present in GB bile. These nucleate solid $CH.H_2O$ crystals. The cause is high cholesterol saturation due to excess secreted CH plus PC molecules out of proportion to the solubilizing capacity of BS secreted by the liver.

3. $CH.H_2O$ crystals agglomerate within biliary mucin gel, usually on the mucosa or in the most dependant part of the GB to form mature CH stones.

4. Once the diathesis is set in motion (the trigging factor is unknown, since supersaturated-lithogenic bile can persist for years without a solid phase separating) a larger proportion of hepatic bile bypasses the dysfunctional gallbladder to be exposed to colonic microorganisms. The result is the increased conversion of primary bile salts to secondary species (see figure 4), particularly, deoxycholate accumulates and nearly doubles in most subjects. This BS is pro-lithogenic, since when it accumulates in the enterohepatic circulation it a) promotes further hepatic cholesterol hypersecretion into bile, b) accerates solid phase separation in the GB, and c) prologes intestinal transit time – all constituting a vicious cycle of events.

L. Pigment Gallstone Formation

1. <u>Subclass</u>: "Black" stones (GB only)

a) Calcium hydrogen (unconjugated) bilirubinate supersaturation of GB bile i.e. $Ca(HUCB)_2$ ion product is in excess of solubility product of the salt in that bile. There is also accumulation of mucin gel, possibly secondary to UCB absorption by GB mucosa. There is some GB hypomotility, and bile is sterile.

b) The cause is hepatic hypersecretion of bilirubin conjugates, which provide an additional substrate for endogenous ß-glucuronidase and non-enzymatic hydrolysis in GB. Sources of excess bilirubin conjugates delivered to bile are:

i) chronic or paroxysmal hemolysis from any cause, congenital or acquired.
ii) "shunt" hyperbilirubinbilia – mainly due to severe Vitamin B_{12} or folic acid deficiency (rare).
iii) enterohepatic cycling of bilirubin from ileal disease, resection, bypass, alcoholism and alcoholic cirrhosis, chronic parenteral nutrition, esp. total parenteal nutrition (TPN), ABST dysfunction from genetic causes and inhibitors, cystic fibrosis and possibly high starch – polymer diets.
iv) phototherapy for severe neonatal jaundice.

c) Because of the increased bilirubin load to the liver, the BDG/BMG ratio (normally 4.1) can become inverted. Thereby, BMG is the substrate that is easier to hydrolyze to HUCB.

d) $Ca(HUCB)_2$ precipitates in sterile bile invariably accompanied by $CaCO_3$ and possibly $Ca_3(PO_4)_2$. The reason for supersaturation with inorganic salts is unknown, but pigment – rich bile is deficient in vesicular binders for Ca_2^+, because of BS-PC uncoupling by excess anions in the canalicular space and bile may be more alkaline, because of buffering of secreted protons by the sialic acids of hypersecreted mucin.

e) $Ca(HUCB)_2$ becomes polymerized and degraded in the solid state, possibly from free radicals secreted by the liver. With passage of time, a black vinyl polymer is formed which is insoluble in all organic or polar solvents.

2. Subclass: "Brown" stones (invariably Bile Duct System only)

a) Caused by chronic infection of the biliary tree with mixed colonic organisms, capable of producing enzymes that hydrolyze not only bilirubin conjugates (ß-glucuronides), but biliary PC (phospholipase A_1) and bile salt conjugates (cholyl-glycine amidase) – all three can precipitate with biliary calcium as $Ca(HUCB)_2$, Ca Soaps (palmitate/stearate) and calcium unconjugated bile salts. Because all the solubilizers for CH are destroyed, "brown" stones commonly contain a lot of CH.

b) There must be biliary tree stasis, i.e. a lack of normal flow for chronic infection to be initiated and persist. The causes of biliary stasis in order of decreasing frequency are:

i) A migratory GB stone
ii) Congenital anomalies of the biliary tree
iii) Duodenal diverticulae
iv) Foreign bodies – sutures, ova, clips, stents
v) Infestation with worms (Ascaris lumbricoides and Clonorchis sinensis), especially in underdeveloped countries
vi) Advanced AIDS, including AIDS papilitis

c) Characteristic features of brown stones are:

i) lots of mucin from bile duct wall
ii) stone shape molded by the biliary tree
iii) soft feculent smelling stones
iv) skeletons of dead bacteria within the stones structure.

d) As stones grow, they become attached to the duct wall, further incurring stasis – vicious cycle is set up.

M. Clinical Manifestations

a) 20-50% of patients with stones experience:

i) Biliary pain (females much more then males): Sudden onset in right upper quadrant or elsewhere in abdominal/chest area. Usually, nocturnal within 1-2 hours of going to bed. It is unremitting and, therefore, not a colic and lasts more than 30 minutes. The cause is believed to be a stone wedged in the neck of the gallbladder which then drops back into the fundus or migrates into the bile duct system. No laboratory changes. Patient is very uncomfortable; unable to lie still; surprisingly, little tenderness over the gallbladder. Much more frequent than the principal complications of stones, which are
ii) Acute cholecystitis: Starts as the above; continues to worsen; pain very severe; very tender over the gallbladder; systematic signs with fever and leucocytosis.
iii) Acute pancreatitis (see Dr. Apstein's lecture notes).
iv) Acute cholangitis: this occurs from a stone lodged in the duct system (choledocholithiasis). It begins with severe biliary pain, but rapidly the patient becomes jaundiced, febrile with leucocytosis and laboratory evidence of cholestasis. There is rapid progression with systemic disturbances, This constitutes a medical emergency, particularly, if invasion with pus-forming organisms occurs, and therefore, requiring emergent ERCP for drainage.
v) Other complications of stones are empyema, perforation, fistulae, ileus, "porcelain" GB, hydrops, limy bile, cancer, etc. all of which are rare.

N. Treatment

a) Painless stones in the gallbladder → expect management – no treatment.

b) Symptomatic of complicated GB stones → laparoscopic cholecystectomy.

c) Hepatic or bile duct stones (symptomatic or asymptomatic) – ERCP with sphincterotomy and extraction.

a) and b) are so effective that stone dissolution with bile acids (Chenodiol, CDCA or Ursodiol, UDCA) have now no role – very inefficient and slow. Furthermore, extracorporeal shock lithotripsy was never FDA approved in the United States.

O. References (Gallstones)

1. Please see reference list in JN Donovan's lecture notes (AASLD, Fall Course, 1999) attached.

2. Excellent chapters on gallstones may be found in all the major textbooks of Internal Medicine: In Stein's Internal Medicine (4th edition) there is a separate chapter on the physiology and pathophysiology of bile.

3. Heuman DM, Mills AS, and McGuire HH. Gastroenterology. WB Saunders Co., Philadelphia, 1997. (Outstanding on bile, cholestasis and gallstones.)