Liver

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MEDICAL GRAND ROUNDS

CHOLESTASIS

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DEFINITION:

In literal terms, cholestasis means the "STANDING OF BILE". By general concensus it means the interruption of bile flow or bile secretory failure. A difficulty arises, however, because the flow of bile is not a directly observable phenomenon and the diagnosis of cholestasis therefore rests on clinical, chemical and histological phenomena which are the consequences of cholestasis. Since the boundaries of these phenomena are diffuse and their correlation one with the other is imperfect, it becomes somewhat difficult, at times, to say whether cholestasis is present and even more difficult to say that it is not. Even in purely physiologic terms there is a difficulty in defining the process. Murray Fisher responded to this frustration in noting that "an adequate definition of cholestasis is elusive because the physiochemical processes involved in the secretion of bile are numerous, complex, interdependent and, at the present time, poorly understood".

WHAT IS BILE?

Bile is a fluid which is produced by the hepatic parenchymal cells and bile ducts at the rate of approximately 600 ml per day. It contains the following solutes in approximately these concentrations:

COMPOSITION OF HEPATIC BILE

H_2O - 97-98%
pH - 5.7-8.6
Osmolarity - isoosmotic with plasma
Bile salts (30-50 mM)
Lecithin and other phospholipids (10-15 mM)
Cholesterol (2-4 mM)
Bilirubin (0.25-1.25 mM)
Small cations (Na^+, K^+, Ca^{++}, Mg^{++}, Ca^{++}, Fe^{++})
Small anions (Cl^-, HCO_3^-, PO_4^-, $SO_4^=$)
Protein (albumin, alkaline phosphatase)
Hormones (thyroxine, estrogens)
Porphyrins

Figure 1

The major function of bile is to rid the body of bilirubin, cholesterol, and other lipid-soluble organic metabolites and drugs which cannot be readily eliminated through the kidney.

HOW IS BILE SECRETED?

Each of the various constituents of bile have a separate mechanism by which they end up in bile. Many of these mechanisms are interdependent. In the end, the essential question becomes how is the water of bile generated? The answer to this is inextricably linked with how bile salts are secreted and this, of course, is inextricably linked with the excretion of other organic anion (e.g. bilirubin) and with lipid secretion.

The hepatic excretion of osmotically active bile acids is the major determinant of bile water and solute excretion. There is a close relationship between canalicular bile formation and bile acid excretion rate. This is a linear relationship when bile acid excretion rates result in bile salt concentrations that are above the critical micellar concentration of the bile salt concerned (Figure 2). At very low rates of bile salt excretion (<CMC) the relationship is curvilinear, and because bile salts are more osmotically active in these concentrations, there is a greater relative bile flow. The bile flow that can be directly attributed to bile acid excretion has been termed Bile Acid Dependent Flow (BADF).

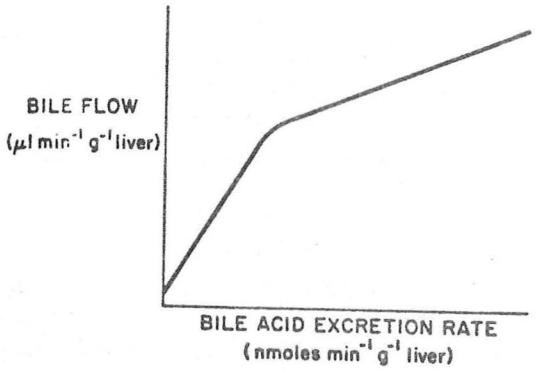


Figure 2

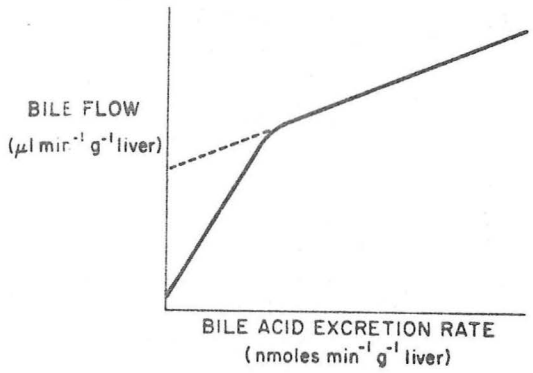


Figure 3

Extrapolation of the line relating bile flow and bile acid excretion rates to the ordinate led investigators to believe that a certain amount of bile flow occurred at a time when bile salt excretion was zero. The concept then of a bile acid independent flow (BAIF) arose and gained support from studies which showed an increase in this fraction following therapy with agents such as phenobarbital (i.e. an increase in canalicular bile flow without change in bile salt excretion). The fact of this BAIF remains somewhat controversial as does the proposed pathogenesis of its development.

The liver parenchymal cells synthesize bile acids (from cholesterol) at a rate of approximately 0.5 g/day. The liver excretes approximately 15-30 g/day of bile acids. The bulk of this comes from the entero-hepatic circulation of the approximately 2-4 g of bile salts which constitute the total body pool. The essential first step in creating bile flow is the uptake of these bile acids into the parenchymal cells from the plasma. This uptake is a Na^+ coupled carrier-mediated active

process which derives its energy indirectly from Na^+/K^+ ATPase. Na^+/K^+ ATPase is a membrane-bound enzyme which is heavily localized to the basolateral plasma membrane of the hepatocyte.

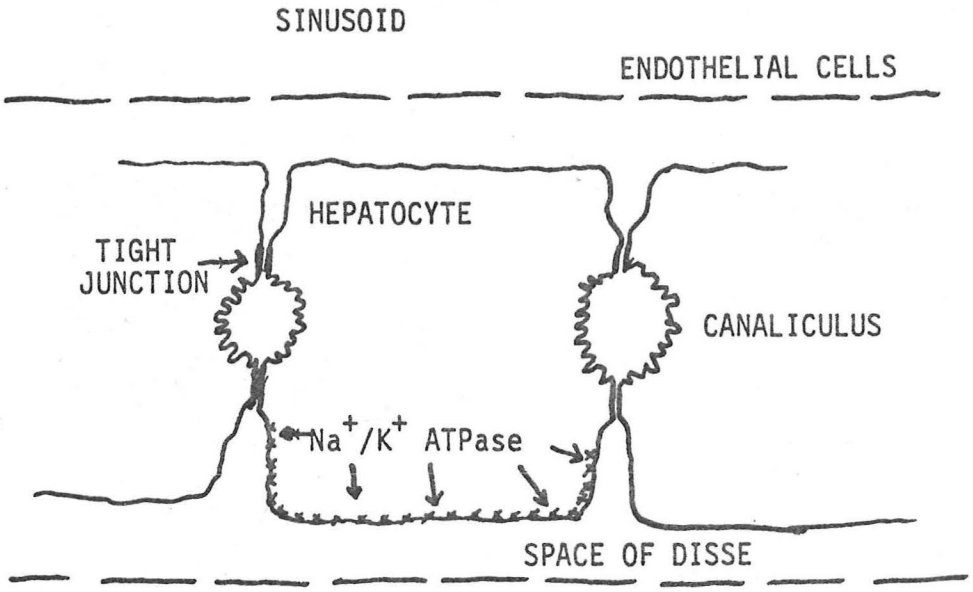
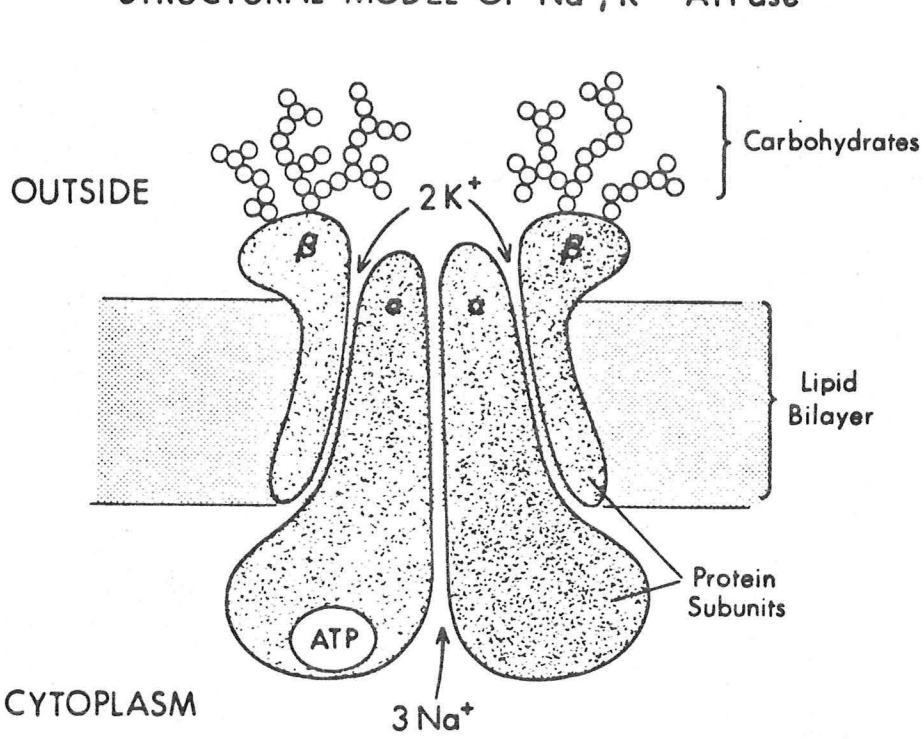


Figure 4

Its prime purpose is to serve as a Na^+ pump. The transport of Na^+ ions out of and K^+ ions into the cell is directly coupled to a metabolic energy source (in this case ATP hydrolysis). This ion transport is coupled such that 3 Na^+ ions are pumped out of the cell for every 2 K^+ ions pumped in. This results in a net transfer of electrical charge and makes the intracellular compartment electrically negative relative to the extracellular compartment. The fact that some K^+ ions diffuse back out of the cell down a chemical concentration gradient enhances this intracellular negativity. The ultimate effect of the activity of Na^+/K^+ ATPase is the creation of a steep electrical (as well as chemical) gradient favoring the passive entry of Na^+ into the cell (Figure 6).

Bile acids are transported into the cell across a Na^+ coupled membrane carrier. The anionic bile acids are cotransported with sodium cations across the basolateral membrane against otherwise unfavorable chemical and electrical gradients. Na^+/K^+ ATPase by establishing conditions favoring Na^+ entry into the cell is thus ultimately responsible for bile acid uptake because bile acid and Na^+ transport across the plasma membrane are linked. Other anions including certain amino acids (e.g. alanine) are taken up by the hepatocyte by a similar Na^+ linked cotransport mechanism. Similar mechanisms for sodium coupled anion transport processes have been proposed for a variety of secretory and resorptive epithelia.

The bile salts, once concentrated within the cell, are bound to intracellular proteins (e.g. ligandin) and are transported (by poorly understood mechanisms) to the apical pole of the hepatocyte where the canaliculus awaits.



(after Sweadner & Goldin, N Engl J Med 302: 777-783, 1980)

Figure 5

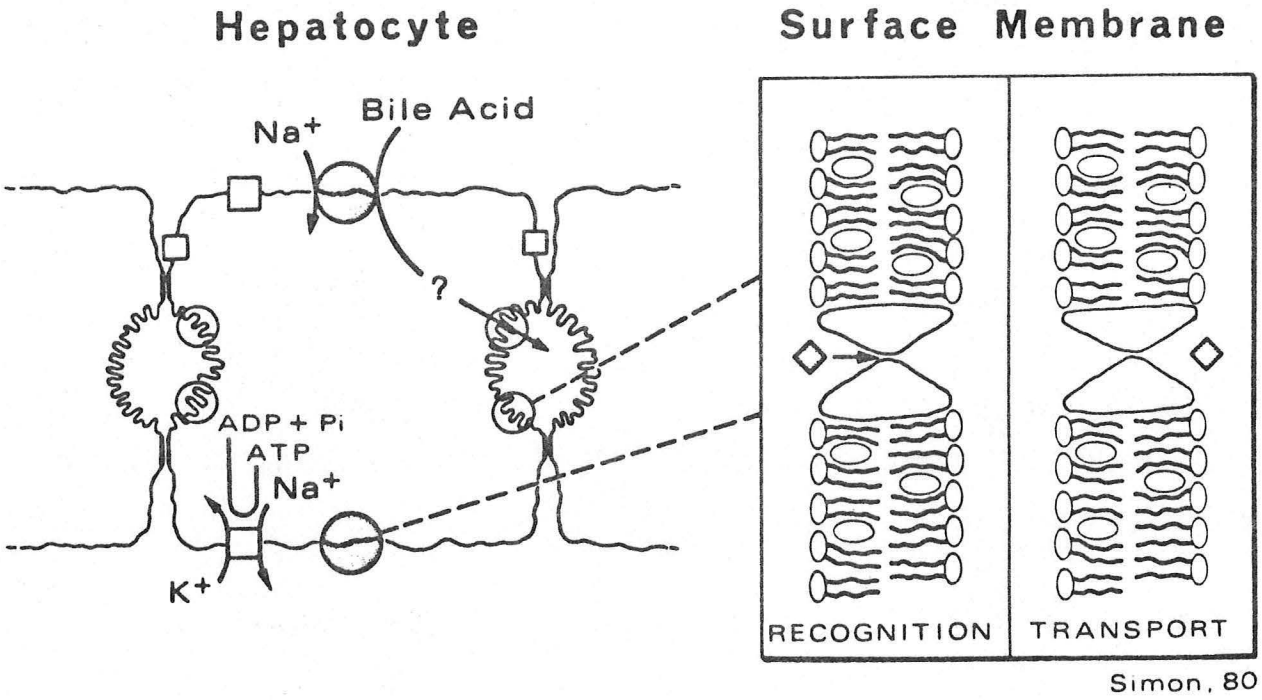


Figure 6

The means by which bile acids are excreted across the canalicular membrane have not been clearly elucidated. Three major possibilities exist and all may operate to a varying extent:

a) The intracellular transport may be vesicular - such vesicles might be excreted by exocytosis into the canaliculus.

b) The electrochemical gradient established for bile acids from the cell interior to the cell exterior would account for the passive movement of bile acids into bile, provided that the permeability of the canalicular membrane for these anions was greater than that of the basolateral membrane. The capacity of bile acids to form micelles and thereby decrease the concentration of the monomeric form of the bile acid would promote bile salt excretion by tending to maintain the gradients. On the other hand, substantial intracellular protein-binding would tend to diminish the gradients and limit the importance of this mechanism for bile acid excretion.

c) There may well be an energy-requiring carrier mediated transport mechanism that is specific for bile acids or is non-specific and available for a variety of organic anions.

The presence of anionic bile acids in the canaliculus attracts cations (sodium) and these ions then draw water (by osmosis) into the canaliculus to form the essentials of bile. The Na^+ and H_2O do not come directly from the hepatocyte but are derived instead from the sinusoid via the paracellular pathway and enter the canaliculus across the tight junctions which separate the canaliculus from the space of Disse.

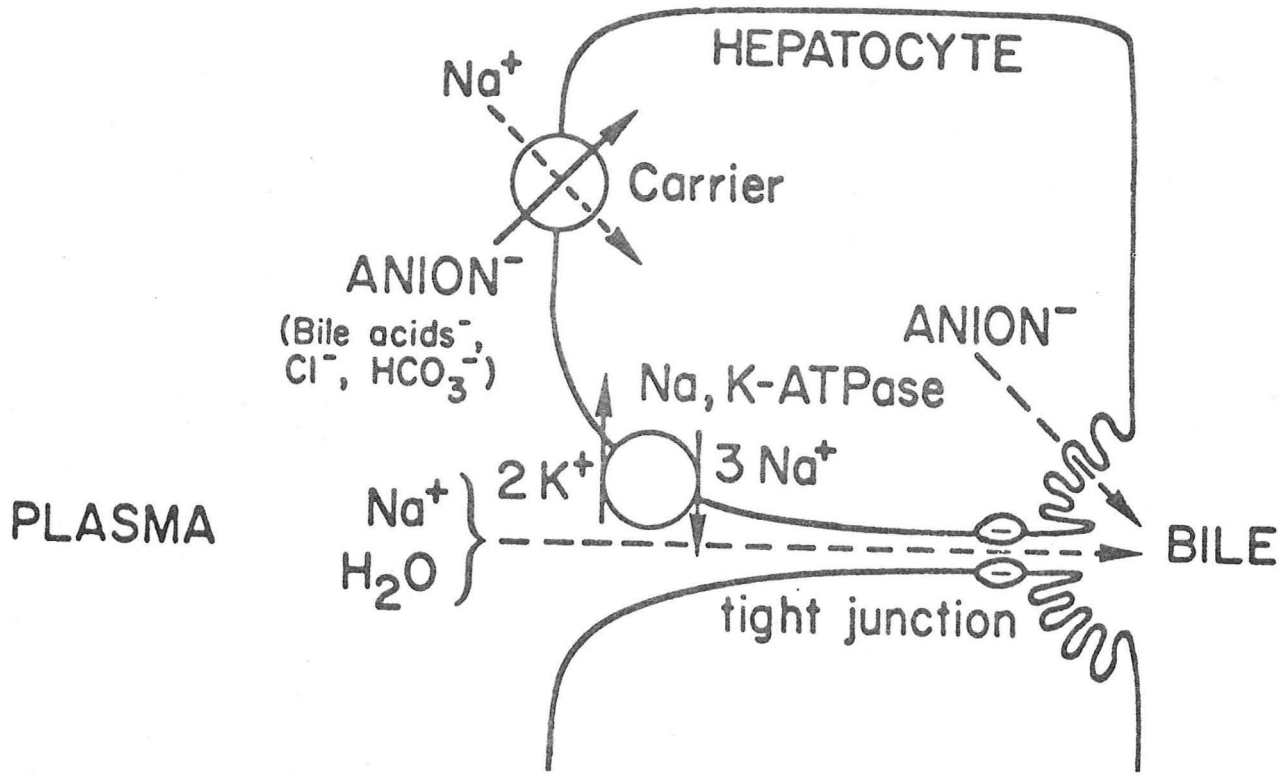


Figure 7

These "tight junctions" are in fact a negatively charged electrical barrier that favor the movement of small cations while retarding the passage of negatively charged anions. Thus the movement of Na^+ is possible from plasma to bile but there is inhibition of movement of bile acids from bile back to plasma despite the electrochemical gradient established.

In summary, it is the transcellular concentrative secretion of bile acids into the canaliculus that is the main driving force for bile production. This produces osmotic and electrical gradients between the canaliculus and the intercellular interface (in continuity with the space of Disse and hence the plasma). "Leaky" tight junctions allow the passage of Na^+ and H_2O down the osmotic and electrical gradients through the paracellular pathway to achieve osmotic and electrical neutrality. The negatively charged bio-electric barrier of the tight junction prevents egress of anionic bile salt monomers back into the intercellular space while larger bile salt/cholesterol/lecithin micelles are retained by the small pore-size of the tight junctions.

BILE ACID INDEPENDENT FLOW:

There are two basic mechanisms proposed to explain the occurrence of bile flow that is independent of bile acids. One of these proposes that other anions are excreted into bile and that these also generate osmotic force and draw Na^+ and H_2O through the paracellular pathway. No such anions have been identified but it is argued that the relationship between the apparent BAIF and the activity of Na^+/K^+ ATPase speaks to a Na^+ linked co-transport mechanism as being essential to the genesis of this fraction of bile flow. This then speaks to an anionic species as being important.

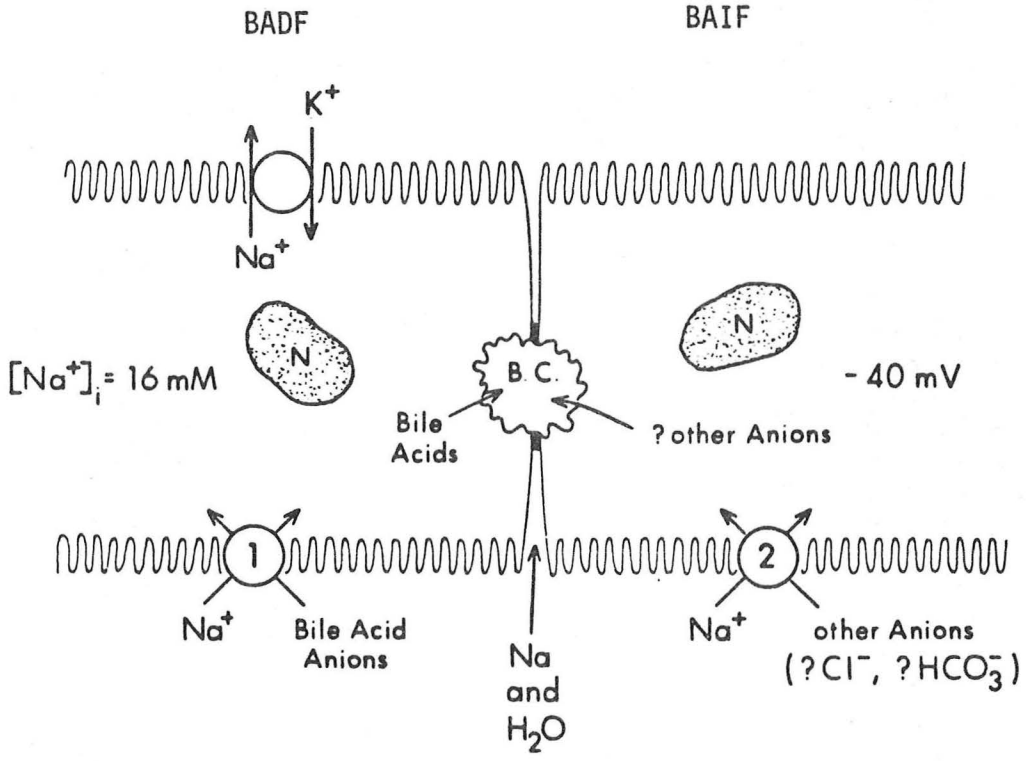
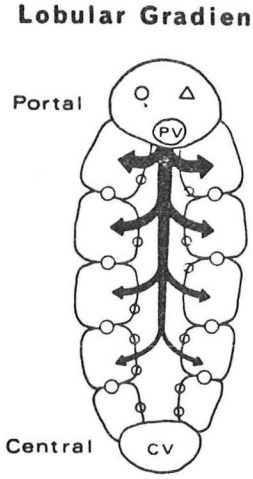


Figure 8

Chloride is one candidate ion but its dependence on Na^+ co-transport has not been proven. The other major candidate anion is HCO_3'. The extent to which bile acid independent bile flow contributes to bile volume is unclear. It probably varies from time to time during the day (as the bile salt load passing through the liver varies with meals) and also appears to vary within the liver lobule. The periportal hepatocytes are largely responsible for bile salt uptake and thus for BADG. It appears that centrilobular cells are more involved in producing BAIF.



Bile acid uptake progressively diminishes down the lobular gradient. Bile flow generated in the pericentral zones is largely BAIF.

Figure 9

MODIFICATION BY BILE DUCTS:

The volume of bile formed at the canaliculus is approximately 450 cc/day and this is isotonic fluid. A further volume of approximately 150 cc/day is secreted by the bile duct epithelium. The ducts and gallbladder may also resorb water and electrolytes. The secretion of H_2O in this system depends mainly on the active transport of HCO_3' and is under direct control of secretin and other G.I. hormones. Bile duct bile may be rendered hypertonic by the secretion of HCO_3'-rich fluid from the ducts.

HOW DOES BILE FLOW?

The traditional view of bile flow ascribes its movement through ductules and ducts which have no demonstrable smooth muscle or contractibility to the osmotic force generated by the secretion of solutes. The recent demonstration that non-muscle cells (including hepatocytes) contain contractile elements has caused some

revision of this concept. There are two separate filament systems in hepatocytes. One, composed of microfilaments, contain actin and are 5 to 7 nm in width. The other, microtubules, are 24 nm structures and are composed of tubulin. There is also a small amount of myosin present in the hepatocytes.

Actin filaments are present throughout the cell but are especially numerous beneath the cell membranes in the region of bile canaliculi. These filaments are constantly undergoing active polymerization and depolymerization in the cell. There are cross-linkages between actin filaments which influence the gelation of actin. A number of proteins and other substances have been found to influence actin filament assembly, filament-filament interaction or filament-membrane interaction.

Actin containing microfilaments provide the means for a range of subcellular motility events including endocytosis, exocytosis, secretion and the movement of vesicles. There is evidence, from time-lapse cinephotomicrographs of hepatocytes in early monolayer formation, that actin filaments also provide a contractile function to the canaliculus. These studies have demonstrated a repetitive cycle of slow dilatation of the canaliculi followed by a rapid and forceful contraction. These studies suggest that these canalicular contractions may act as a motor which by their pumping action facilitates bile flow in the canalicular system. It is of interest in this respect that two agents which interfere with microfilaments have a profound influence on bile flow.

Phalloidin prevents actin depolymerization. Its effect on hepatocytes is to induce a marked increase in pericanalicular actin filament formation such that the canaliculus becomes encased in fibers. There is an associated marked decrease in bile flow. The mechanism for this cholestasis is not clear but alterations in the tight junction permeability or a loss of canalicular contractibility may be involved.

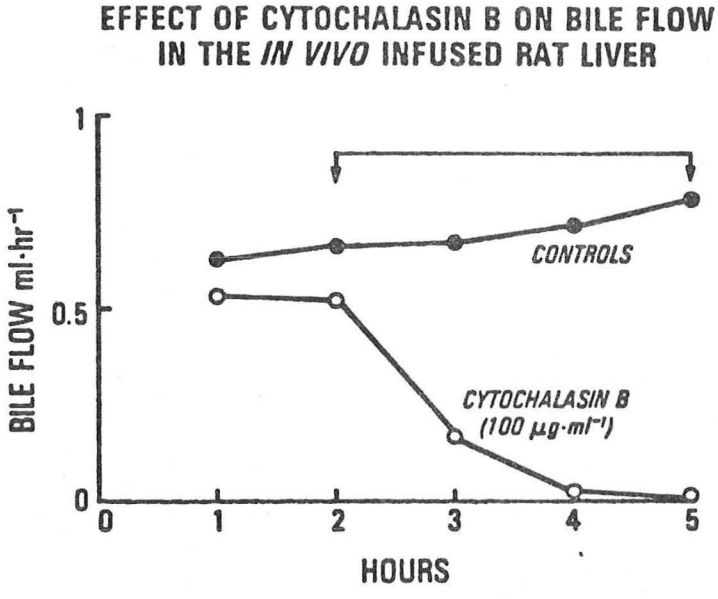


Figure 10

Cytochalasin B is a well-known inhibitor of actin-mediated motility (e.g. clot retraction, endocytosis, etc.). In the hepatocyte cytochalasin disrupts the microfilament-membrane attachments and causes a marked decrease in bile flow. Again the precise cause for the cholestasis has not been elucidated. One common consequence of cholestasis, no matter what the etiology, is dilatation of canaliculi with loss of the usual microvilli seen on electron-microscopy. It is tempting to attribute these histological changes to a loss or dysfunction of the normal support and contractile functions provided by the actin microfilaments.

WHAT ARE THE CAUSES OF CHOLESTASIS?

Theoretically it is clear that deficits at a number of locations could lead to a state of diminished bile flow. For example, inhibition of Na^+/K^+ ATPase could abolish the gradients favoring Na^+ entry into the cell and thus interfere with bile salt uptake and excretion. A membrane defect which distorted the receptors mediating the Na^+-bile acid co-transport would have a similar effect. Changes affecting the canalicular membrane could limit the transport of bile acids out of the cell while disturbances of the tight junctions could dissipate the plasma/bile electrochemical gradients and diminish the influx of Na^+ and H_2O from the paracellular pathway. Interference with the microfilaments abolishing the presumptive contractile property of the canaliculi might be expected to cause bile stasis as does the mechanical obstruction of bile flow from lesions involving the larger ducts.

MECHANISMS FOR INTRAHEPATIC CHOLESTASIS

Na^+/K^+ ATPase inhibition

Canalicular membrane modification

Tight junction permeability changes

Alteration of the cytoskeleton

Figure 11

There are many different agents and disease processes that are accompanied by cholestasis. In most instances it is likely that a number of the above effects contribute to the bile secretory failure. The fact that the process of bile secretion is rather complex and involves so many different cell organelles and functions helps to explain why this excretory function is so vulnerable to disruption and why cholestasis is such a common manifestation of liver injury.

EXCRETION OF ORGANIC ANIONS:

The most biologically important example of this class of compounds is bilirubin. The uptake of organic anions is carrier-mediated, saturable and competitive. There is recent evidence that the membrane receptor binds the bilirubin-albumin complex and is specific for the albumin moiety rather than the organic anion component. Organic anions in the hepatocyte are either passed directly to the canaliculus for excretion

or undergo a prior conjugation step (with glucuronide in the case of bilirubin). There is a strong probability that excretion of organic anions into the canaliculus is also carrier mediated, saturable and competitive. The selective excretory defect seen in patients with Dubin-Johnson syndrome argues for the existence of two different carrier mechanisms for organic anions and bile acids. The maximum excretory rate for some organic anions (e.g. bilirubin and BSP) is enhanced by bile salt infusions. The mechanism whereby this facilitation of excretion is effected has not been elucidated but it is possible that it reflects an enhanced association in bile between the organic anion and bile salt micelles.

EXCRETION OF BILIARY LIPIDS:

The liver plays a major role in the metabolism of lipoproteins and their constituent lipids.

ROLE OF LIVER IN LIPID AND LIPOPROTEIN METABOLISM

SYNTHESIS	
LIPIDS	TRIGLYCERIDES, CHOLESTEROL PHOSPHOLIPID
APOPROTEINS	B, C-I, II, III, E, A-I, II
LIPOPROTEINS	VLDL, NASCENT HDL
ENZYMES	LCAT
BILIARY EXCRETION	CHOLESTEROL, PHOSPHOLIPIDS
CATABOLISM	CHYLOMICRON REMNANTS, HDL

Figure 12

Bile acid secretion appears to be the major driving force of biliary lipid secretion. Biliary lipid output increases non-linearly with bile acid dependent bile flow. Thus the greatest excretion of phospholipid and cholesterol occurs during states of high bile salt excretion but the greatest biliary concentration of the lipids occurs at low bile salt excretion rates.

EXCRETION OF OTHER SUBSTANCES:

Small amounts of a variety of proteins (e.g. IgA), amino acid, metals (e.g. copper), vitamins (e.g. 25-hydroxy vitamin D) and other relatively lipid-soluble materials are excreted in bile. A number of mechanisms including vesicular transport and lysosomal exocytosis are involved.

CLASSIFICATION OF CHOLESTATIC DISORDERS:

Virtually any disorder affecting the liver will manifest itself with some degree of cholestasis. In practical terms the diagnosis is applied to those circumstances where the phenomena associated with bile secretory failure predominate in the clinical picture. Thus, most patients with acute viral hepatitis are not considered to have a cholestatic disorder because the evidence of hepatocellular necrosis predominates over the cholestasis that is always present in such patients. Some patients with acute viral hepatitis have more evident cholestasis and at times these features will be the predominant ones. In such circumstances, viral hepatitis can reasonably be considered to be a cholestatic disorder. In some conditions the cholestasis appears to be rather "pure" and, even when prolonged, is not associated with any progressive liver injury. Other disorders cause cholestasis but also cause a destructive hepatic injury which in time can lead to substantial structural abnormalities.

CHOLESTATIC DISORDERS

Cholestasis with minimal structural hepatic disease
- drugs (e.g. methyltestosterone)
- benign postoperative cholestasis
- cholestasis of pregnancy and due to birth control pills
- benign recurrent cholestasis
- cholestasis of Hodgkin's disease

Cholestasis with structural hepatic disease
- cholestatic hepatitis due to virus or drug (e.g. chlorpromazine)
- primary biliary cirrhosis
- sclerosing cholangitis
- biliary hypoplasia
- others - tumor, occasionally alcoholic hepatitis, etc.

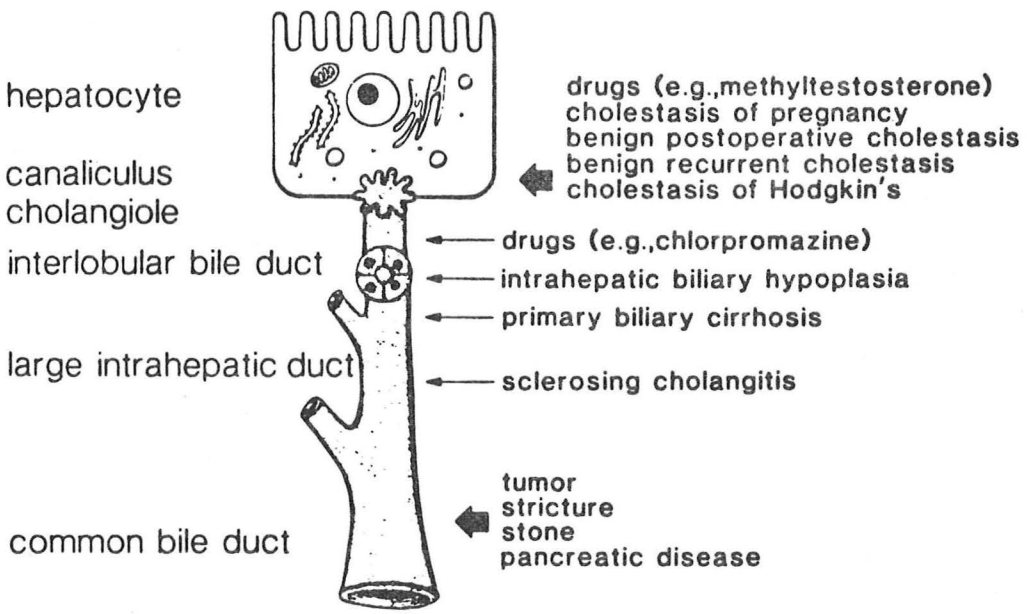
Cholestasis due to large bile duct obstruction

Figure 13

The above classification is useful in stressing these prognostic differences. There is an implication, too, that the first group of disorders are the consequence of a "functional" failure of bile secretion while the majority of the other disorders suggest a more mechanical explanation for the cholestasis (Figure 14).

BENIGN RECURRENT IDIOPATHIC CHOLESTASIS:

This rare disease is described by its name. Patients have recurrent discrete episodes of cholestasis. These normally begin in childhood. Each episode may last weeks or as long as two years. The episodes spontaneously remit and between episodes, patients may have symptom-free intervals lasting years. During these intervals, results of all liver tests are normal and liver biopsy findings are quite unremarkable. No consistent precipitating events have been identified but some epi-



Anatomic sites at which lesions that can cause cholestatic disorders occur.

Figure 14

sodes appear to be triggered by pregnancy or estrogen use. Some case reports have stressed a familial predisposition. The disorder is essentially benign in that despite numerous episodes of cholestasis (as many as 40 over a lifetime), there is no loss of liver mass, no tendency to structural liver damage and, while the symptoms may be distinctly unpleasant, no risk of liver failure. During the episodes the patients complain of intense pruritus, and often suffer substantial weight loss from the associated malabsorption. Jaundice is variable. Results of liver tests are characteristic for cholestasis, eosinophilia is not a feature and the liver biopsy shows a very bland lesion with marked centrilobular cholestasis with minimal inflammation and rare foci of necrosis.

No reasonable pathogenetic explanations to explain this disorder have been proposed. The recurrent nature of the disorder with prolonged episodes of apparent non-disease suggest that an exogenous trigger might be involved. The non-destructive nature of the lesion suggests a "functional" pathogenesis but the precise location of the disorder remains obscure. One possible hypothesis proposed that the production of an abnormal bile salt in the gut could have been responsible for the cholestatic episodes. With this in mind, some authors have suggested that prolonged (say continuous) therapy with cholestyramine might prevent the recurrence of this syndrome.

ESTROGEN-INDUCED CHOLESTASIS:

Estrogens consistently promote cholestasis in man and other animals. This is demonstrable in all people by sophisticated methods for measuring organic anion transport but reaches clinical and chemical expression in only a minority of persons exposed. These persons (both men and women) appear to have an ex-

aggerated response to the universal cholestatic effect of estrogens. This hyperresponsiveness is under genetic control and accounts for the racial predilection for cholestasis of pregnancy and oral contraceptive-induced cholestasis among Scandinavian and Chilean people.

The clinical manifestations, when they occur, usually begin within the first six cycles of oral contraceptive therapy and often during the first cycle. Pruritus is usually the first and most important symptom. There is seldom a significant G.I. disturbance and fever, arthralgias and skin rashes do not occur. Jaundice, if it develops, is usually mild. The liver and spleen size remain normal and these organs are usually non-tender. Serum bilirubin levels are often normal and are rarely greater than 10 mg/dl. Serum alkaline phosphatase levels may remain normal despite the intense cholestasis or may be variably increased. Transaminase elevations tend to be minor. The histologic findings are largely confined to the presence of canalicular bile plugs. Cell necrosis, fibrosis and inflammatory cells are trivial or absent. The disorder remits usually within a month of discontinuing the estrogen therapy and there are no chronic sequelae. Recurrence is to be expected if the patient is exposed to the same dose of the agent.

This syndrome is also likely to recur if the patient is exposed to high endogenous levels such as occur late in pregnancy. The condition, then called pruritus gravidarum or cholestasis of pregnancy, is similar in all respects to the OCP induced disease except that the serum alkaline phosphatase is always elevated (in part from the placental contribution) and the disorder persists, sometimes intolerably, until delivery. It then remits over the next four weeks. There is no associated maternal morbidity but the syndrome has been associated with an increased incidence of premature birth with all of its accompanying problems.

PHENOTHIAZINE-INDUCED CHOLESTASIS:

These drugs are generally regarded as representing the prototype of hypersensitivity mediated cholestasis. The association between chlorpromazine and acute cholestasis was recognized very soon after the drug was induced. Several studies have assessed the risk of overt jaundice from using chlorpromazine at between 1% and 2%. Prospective studies have shown that as many as 50% of recipients will develop an impairment of BSP clearance after taking this drug. The onset of jaundice is characteristically within four weeks of initiation of therapy. There is often a prodrome of fever with malaise and GI symptoms and pruritus is common. There is sometimes an associated rash and often eosinophilia is present. Liver tests show a variable degree of hyperbilirubinemia, a high SAP and a mildly elevated SGOT. Liver biopsies demonstrate cholestasis, often an eosinophilic portal infiltrate, minor evidence of individual cell necrosis and not much else. No specific therapy is required and the lesion has usually completely resolved within three months of cessation of the drug. An occasional patient has been described who appears to progress to a chronic cholestatic syndrome even after stopping the agent. A clinical and histological picture akin to biliary cirrhosis has been observed in such patients.

The nature of the pathogenesis of this drug-induced lesion is called into question by the experimental evidence that in animals chlorpromazine, in a dose-dependent universal manner, interferes with bile secretion. The effect is pre-

dominantly on the bile salt independent fraction of bile and is believed to be linked to the demonstrable inhibition of Na^+, K^+ ATPase by this drug. Chlorpromazine is also an ampipathic molecule which allows it to intercalate into lipid bilayers (e.g. membranes). Thus, it may also alter membrane function by virtue of its physical presence in the membrane. Correlating these direct effects of the drug to the clinical circumstances which suggest a hypersensitivity reaction is not easy. In truth, the clinically apparent episodes of cholestasis may indeed be mediated by hypersensitivity reactions and be quite independent of this universal dose-dependent subclinical phenomenon.

POST-OPERATIVE CHOLESTASIS:

Although the exact incidence is unknown, jaundice is a not uncommon complication of major surgery. The causes for this are manifold and include drug reactions (reactions to anesthetic agents in particular), bilirubin loads from transfused blood, liver injury from shock, heart failure, hypoxemia and sepsis. Besides these recognized causes of liver injury, an occasional patient after major surgery and general anesthesia will develop acute cholestasis. The onset is usually within one to two days of the procedure, is self-limited and benign. The mechanism is unclear. The importance of this entity lies in its recognition and the avoidance of invasive activities attempting to deal with a presumed common duct obstruction.

Many patients with sepsis, be it staphylococcal, pneumococcal, or due to gram negative organisms may also develop "cholestatic" liver chemistries. At times the exclusion of biliary sepsis in these patients can be extremely difficult. Again the mechanism for these episodes is not known. The liver lesion resolves with effective therapy of the underlying infection.

WHAT ARE THE CONSEQUENCES OF CHOLESTASIS?

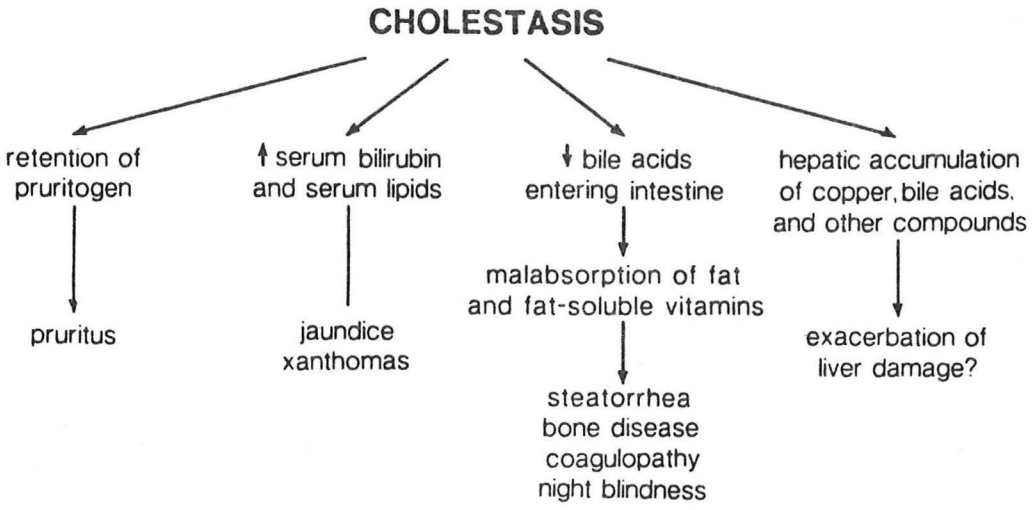
The consequences of cholestasis are of two basic sorts. Some results flow from the retention of materials normally excreted in the bile within the liver cells and then ultimately in plasma and in other tissues. Other consequences result from the failure of bile to deliver substances to the intestine (Figure 15).

CLINICAL MANIFESTATIONS OF CHOLESTASIS:

PRURITUS:

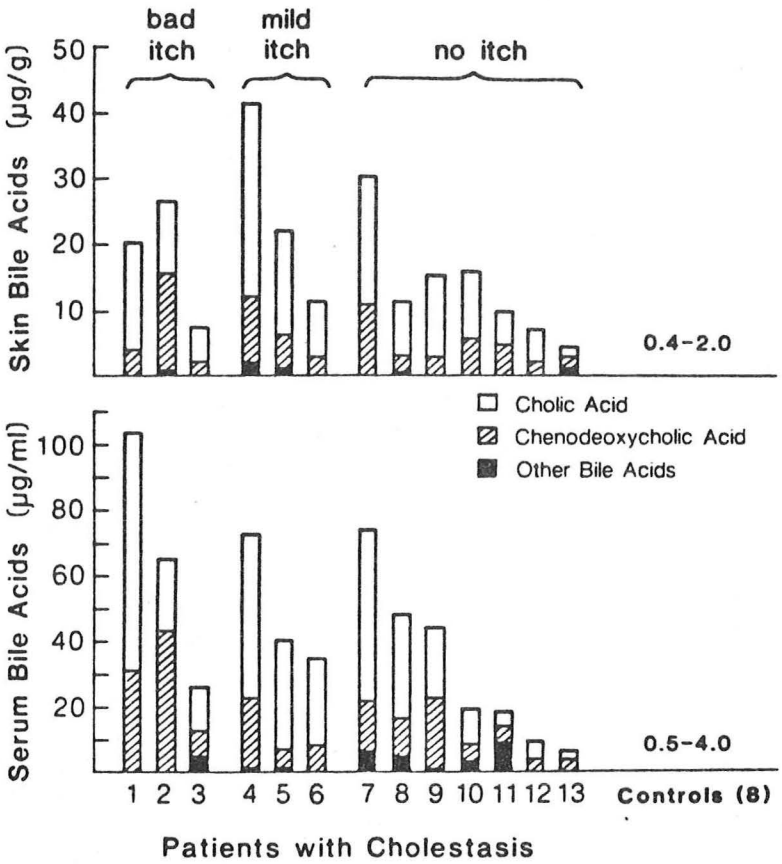
Pruritus is the single most definitive clinical consequence of cholestasis. It has been generally accepted that this symptom results from the retention of bile salts, their accumulation in the skin and their local stimulation of the appropriate nerve fibers there. Support for this concept of pathogenesis is based on the fact that

1. Serum bile salts are raised in patients with cholestasis and pruritus
2. Pruritus is often relieved by cholestyramine
3. Application of bile salts directly to dekeratinized skin results in local pruritus



Schematic representation of the effects of cholestasis.

Figure 15



Serum and full-thickness skin bile acid concentrations in 13 patients with cholestasis and 8 control patients.

Figure 16

Recently, however, this concept has been challenged because

1. there is a very poor correlation between the presence of pruritus and the serum or skin concentrations of total bile acids in patients with cholestatic disorders.
2. there is no correlation between the serum levels of any of the major individual bile acids and the occurrence of pruritus.
3. cholestyramine will bind a variety of substances other than bile salts. (Figure 16)

The current notion then is that there is certainly a pruritogen which is presumably excreted normally in bile and which accumulates in patients with cholestasis. The nature of the pruritogen is not known but it does not appear to be any of the major bile acids or the sum of these. It may be an intermediate of bile acid synthesis, a minor bile acid or an entirely different substance. The discrepancy between bile salt levels and the occurrence of the symptom may instead simply represent a variation in the sensitivity various individuals have to the stimulus that provokes the symptom.

MALABSORPTION:

Failure of delivery of bile salts in adequate concentrations to the small bowel results in failure of micellarization of the monoglycerides and fatty acids released from lipase digestion of triglycerides. This results in the subsequent malabsorption of fat as a selective absorption defect. The accompanying malabsorption of lipid-soluble vitamins such as Vitamin D, A, E and K may have clinical consequences. In clinical terms, the malabsorption of cholestasis reflects itself in weight loss with or without significant G.I. symptoms such as bloating, cramps and diarrhea. On occasion, in patients with low-grade chronic cholestasis (e.g. PBC), the initial presentation may be with such symptoms or with bone disease secondary to hypovitaminosis D.

XANTHOMATA:

In a minority of patients with chronic cholestasis, florid eruptive xanthomata develop. These may be crusted all over the body with a special predilication for the palmar creases. Xanthomatosis infiltration of peripheral nerves may result in a sensory neuropathy which can provoke very unpleasant hyperesthesiae. Red blood cell abnormalities are also frequent. These result from an increase in the unesterified cholesterol content of erythrocyte membranes and produce the target cells so common in patients with liver diseases. Such cells have an increased surface area with an unchanged cell volume. In extreme circumstances, the excessive cell membrane results in the formation of spur cells. These are quickly removed from the blood and are destroyed by the RES, leading to a mild hemolytic anemia.

Lipoprotein X is formed in plasma as a direct result of the regurgitation of biliary lipids. It is a large disc-shaped particle which is rich in free cholesterol and phospholipid (lecithin). It forms in plasma whenever excessive amounts of free cholesterol and phospholipid are present (e.g. during infusion of intralipid or after surgically created anastomoses between the bile duct and plasma). It is an extremely common finding in patients with all forms of cholestasis and has no diagnostic utility.

CHEMICAL MANIFESTATIONS OF CHOLESTASIS:

SERUM ALKALINE PHOSPHATASE:

This term refers to a group of enzymes with low substrate specificity that catalyse hydrolysis of phosphate esters at alkaline pH. They are present in many tissues of the body, are found principally at the absorptive or secretory surfaces of cells, and are bound to cell membranes. Serum normally contains some enzyme activity which serves no known function. It appears to be derived partly from bone and partly from the liver. Elevations of the serum activity of alkaline phosphatase are common in any form of liver disease but reach very high levels in patients with cholestasis. The origin of this increase in SAP has been the subject of some controversy. The original view that the elevation of SAP was due to a failure of biliary clearance of the enzyme has been comprehensibly refuted. The disposition of alkaline phosphatase is not by excretion in bile. It is catabolized like other plasma proteins in multiple sites throughout the body.

Any process that produces cholestasis provokes a marked increase in the synthesis of alkaline phosphatase in the hepatic parenchymal cells. This stimulus seems to be the consequence of bile acid accumulation in the cell and it can be prevented by co-administration of protein synthesis inhibitors (e.g. cycloheximide). Bile salts vary in the degree with which they stimulate the synthesis of the enzyme. Some bile salts also facilitate the release of the enzyme from cell membranes into the circulation. For example, taurocholic acid (TC) both stimulates the synthesis and facilitates release. Dehydrocholic acid (DHCA) stimulates synthesis but has no effect on release.

When there is a total obstruction to the common bile duct, complete cholestasis supervenes and all of the parenchymal cells are faced with elevated concentrations of bile salts. All of the cells are then stimulated to produce extra alkaline phosphate and there is facilitation of the enzymes' release into plasma as well. Because the obstruction is complete, there is ultimately a marked increase in serum bilirubin and bile salt levels as well. The alkaline phosphatase activity may become very high indeed in this circumstance and the patient is likely to be deeply icteric and to have pruritus.

If there is an obstruction confined to either the right hepatic duct or the left hepatic duct, the situation will be different. The cells of half of the liver drained by the obstructed duct will see a markedly increased concentration of bile salts and the serum alkaline phosphatase will rise because of the enhanced synthesis and release of the enzyme from these cells. The serum bilirubin will remain normal, however, because any bilirubin retained in serum due to the dysfunction of the obstructed cells will be cleared effectively by hepatocytes in the other (unobstructed) lobe. The functional reserve for clearance of bilirubin is much higher than that for bile salts and such a person may have some elevation of bile salts and may have pruritus because the unobstructed lobe is not adequate to deal effectively with the bile salts and pruritogen retained by the obstructed lobe.

If a single small ductule is obstructed, all of the cells drained by that duct will be stimulated to produce alkaline phosphatase but there will be no increase in serum bilirubin or bile salts and no clinical consequences because of the unimpaired capacity to clear organic anions in the rest of the liver. If a number of

ducts are so obstructed, the increased production of alkaline phosphatase may be sufficient to cause an elevation of the serum activity of the enzyme. This is the circumstance pertaining in patients who have multiple focal lesions throughout the liver (e.g. granulomata, metastases, fibrous scars). These patients are asymptomatic and have normal bilirubin and bile salt levels because there remains sufficient unobstructed parenchyma to clear these substances effectively.

Primary biliary cirrhosis provides a fine model for the understanding of the consequences of duct obstruction. This is a disease of unknown etiology characterized by the progressive destruction and obstruction of small bile ducts. It is usually a very slowly progressive disease. The initial stages of the disease are not recognized because only a few ductules are involved. There are no symptoms and the blood chemistries are normal. Eventually a sufficient number of ducts are affected to induce sufficient alkaline phosphatase synthesis for this to be reflected in an elevated serum level of this enzyme activity. The patient remains asymptomatic, however, because there is sufficient uninvolved parenchyma to clear the retained bilirubin and bile salts. With further progression of the disease the capacity to clear bile salts is exceeded and the consequences of bile salt deficiency in the gut (steatorrhea) and of bile salt excess (or another pruritogen) in the skin (pruritus) become manifest. The patient remains anicteric, however, because there is still sufficient uninvolved parenchyma to clear the normal daily load of bilirubin. If often takes many years for the disease to progress to the point where jaundice is overt and this is usually a very late event in the natural history of the disease. By this time, virtually all of the inter-lobular ducts have been destroyed and virtually all of the parenchymal cells are, in effect, obstructed.

When the cholestasis results from a lesion at the parenchymal cell level, the consequence will be determined by the number of cells affected. If every cell is involved, the patient will be deeply jaundiced, pruritic, have clay-colored stools and have a very high alkaline phosphatase. If the injury is either less complete or involves fewer cells, then the patient may have pruritus and a high serum alkaline phosphatase but may be anicteric (e.g. the pruritus gravidarum syndrome). If the lesion is even milder in severity or extent, the only manifestation may be the detection of an elevated serum alkaline phosphatase in an otherwise asymptomatic patient (e.g. some drug reactions).

SERUM BILIRUBIN:

Jaundice, or an elevated serum bilirubin level, is not a requirement for the diagnosis of cholestasis. Its presence in fact speaks to the severity of the lesion causing cholestasis. Many patients have profound cholestasis while maintaining a normal serum bilirubin. When the serum bilirubin level does rise, there is elevation of both the conjugated and unconjugated fraction of serum bilirubin. This is true independent of the cause of the disorder and independent of the level in the biliary system that the lesion is expressed.

An elevation in conjugated bilirubin is not difficult to understand. The cellular processes of uptake and conjugation of bilirubin are unimpaired (at least initially) so that conjugated bilirubin is formed in the cell. Failure of excretion leads to a rise in the intracellular concentration of conjugated bilirubin and ultimately the diffusion of same down a concentration gradient back into plasma.

Within the cell, conjugated bilirubin molecules occupy the binding sites on the ligands (4+2 proteins). This accounts for the elevation of the unconjugated fraction of serum bilirubin. Availability of binding sites on these proteins is essential for the uptake of unconjugated bilirubin. Thus, a defect in uptake of unconjugated bilirubin is superimposed and may be compounded by competition for carriers in the plasma membrane by conjugated bilirubin and bile salt molecules. The consequence is an elevation of both fractions of bilirubin. In practice the serum of patients with cholestasis usually contains about 50% conjugated and 50% unconjugated bilirubin. This ratio is maintained, in part, by the kidney's ability to excrete conjugated bilirubin and thus offer an alternate means of excretion for this fraction of the pigment load.

SERUM TRANSAMINASE ACTIVITIES:

In most instances the SGOT and SGPT activities will be only mildly increased. The elevation of these enzymes in extrahepatic obstruction and in rather "pure" forms of intrahepatic cholestasis probably reflect hepatocellular damage and necrosis resulting from retained bile salts. In its mildest pathological expression, this is seen as "feathery degeneration" and in more extreme cases results in "bile infarcts".

SERUM PROTEINS:

Most patients with cholestasis, even when it is prolonged, retain good hepatic synthetic function and hence generally maintain their serum albumin levels near normal. Certain specific disorders may have specific abnormalities in the globulin-fraction (e.g. high levels of IgM in PBC).

PROTHROMBIN TIME:

Classic teaching indicates that cholestasis depletes Vitamin K levels and produces a reversible (by parenteral Vitamin K administration) prolongation of the prothrombin time. This is essentially true but is also quite unusual. There is a secondary source of Vitamin K (from intestinal bacteria) and in general a reversible prolongation of protime is much more likely to be seen in a patient with cholestasis if the patient has also been treated with broad spectrum antibiotics.

DIAGNOSIS OF CHOLESTASIS AND ITS CAUSES:

As I have already indicated, a minimal definition of cholestasis is not possible because to some extent cholestasis is present in all forms of liver disease. When the predominant clinical flavor of a disorder is pruritus, steatorrhea, and jaundice, and the chemical profile is one of high serum alkaline phosphatase activity with moderate transaminase elevations and hypercholesterolemia, the categorization of the lesion as "cholestatic" is not difficult. Some combination of some of these features or the presence of just one (e.g. pruritus, high SAP) may be all that is present in certain patients with cholestasis. The non-specificity of each of these phenomena, however, precludes their being used to define the process.

Once the disorder has been recognized as being cholestatic, the next important distinction is whether or not the lesion is extrahepatic or intrahepatic. Ultimately, this rests on the demonstration of dilatation of the biliary system either by the palpability of a distended gallbladder, or indirect or direct radiographic visualization of the duct system.

The technical means available to evaluate the biliary system are multiple. Ultrasonography, C.T. scans, HIDA scans, endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography (PTC) or even intravenous cholangiography (IVC) are techniques available to us. It is conceivable, too, that on occasion one might have to resort to more than one of these diagnostic techniques. It is clear, however, that all techniques do not have to be employed in all patients and that clinical methods (i.e. history and physical exam) remain the most valuable means of making the correct diagnosis. The choice of diagnostic methods to supplement and confirm clinical suspicions is one which will be determined in large measure by the availability and reliability of the local options.

COMPARISON OF CLINICAL EVALUATION, ULTRASOUND, CAT SCAN, AND BILIARY SCANS IN THE DIAGNOSIS OF EXTRAHEPATIC OBSTRUCTIVE JAUNDICE

	NO.	SENSITIVITY	SPECIFICITY	OVERALL ACCURACY
CE	(50)	95%	76%	84%
US	(49)	55%	93%	78%
CT	(47)	63%	93%	81%
BS	(41)	41%	88%	68%
US&CT	(46)	72%	86%	80%

(modified from Lumeng et al)

Figure 17

The extent to which one investigates a patient should be determined by the clinical suspicion one holds. All patients with cholestasis should have either a sonogram or a C.T. scan. If this is normal, if the disorder is acute and if the clinical suspicion is that the patient has a parenchymal form of cholestasis, direct visualization of the biliary tract is unnecessary unless and until the course of the disease subsequently casts doubt on that clinical suspicion. If on clinical grounds, however, one strongly suspects an extrahepatic lesion, a normal sonogram or C.T. would not discourage further evaluation with ERCP or PCT. If cholestasis is chronic, even if the diagnosis of an intrahepatic process is secure (e.g. PBC), it is reasonable and appropriate to establish the non-involvement of the extrahepatic biliary system by direct visualization irrespective of the findings on sonography or C.T. scan.

The choice between ERCP and PCT has often provoked somewhat heated arguments. Both have advantages and disadvantages and the value of each is very much influenced by the skill and experience of the performer. If both procedures are available with equal expertise, there are times when one has value over the other. Again, the choice may be determined by clinical suspicion (e.g. ERCP is more appropriate in a patient with PBC, PCT more useful in a patient with a bifurcation tumor). On occasion, both procedures will be needed to define the extent and nature of the lesion.

SUCCESS RATE OF DEMONSTRATING BILE DUCTS BY PERCUTANEOUS TRANSHEPATIC CHOLANGIOGRAPHY

	NO.	DILATED DUCTS	NON-DILATED DUCTS	OVERALL RATE
KREEK and BALINT	322	89%	52%	81%
OTHER REPORTED SERIES (11)	905	100%	60%	85%

(from Kreek and Balint)

Figure 18

SUCCESS RATE OF DEMONSTRATING BILE DUCTS BY ENDOSCOPIC RETROGRADE CHOLANGIOGRAPHY

	NO.	OVERALL RATE
UP TO 25 STUDIES (93 EXAMINERS)	1,185	38%
200 OR MORE STUDIES (12 EXAMINERS)	4,535	85%
TOTAL	10,435	70%

(from Bilbao et al)

Figure 19

MAJOR COMPLICATIONS OF PTC

	OTHER REPORTS (905 STUDIES)	KREEK AND BALINT (322 STUDIES)
SEPSIS	3.1%	3.1%
BLEEDING	0.4%	4.0%
BILE PERITONITIS OR LEAKAGE	2.6%	1.9%
EMERGENCY SURGERY	2.8%	3.4%
FATALITY	0.1%	0.9%
OVERALL INCIDENCE	5.4%	10.2%

(from Kreek and Balint)

Figure 20

COMPLICATIONS OF ERCP

(Based on 10,435 studies from Bilbao et al)

INJECTION PANCREATITIS	10.0%
CHOLANGITIC SEPSIS	0.8%
DRUG REACTIONS	0.6%
PANCREATIC PSEUDOCYST	0.3%
FATALITIES	0.2%
OVERALL	9.3%
UP TO 25 STUDIES (93 EXAMINERS) 1,185 STUDIES	15.0%
200 OR MORE (12 EXAMINERS) 4,335 STUDIES	3.5%

Figure 21

THERAPY OF CHOLESTASIS:

THERAPY OF CAUSE:

Most instances of extrahepatic biliary obstruction can be alleviated by surgical or endoscopic means. Many of the intrahepatic disorders that result in cholestasis are self-limited or resolve with removal of the offending agent. Most of these disorders, however, require symptomatic care in the interim and some are chronic diseases for which there is no specific treatment.

SYMPTOMATIC THERAPY:

Pruritus: The major therapeutic weapon available for this distressing symptom is the exchange resin cholestyramine. Bile salts (+ ?other pruritogenic substances) that reach the intestine are bound to the resin and are not then reabsorbed. Over time there is a gradual reduction in the skin and plasma levels of these substances and the symptom of pruritus remits. There is a requirement that some bile salts reach the intestine for cholestyramine to be effective. In any case, symptomatic improvement does not occur for several days. Doses of 16 g/day are usual but can be increased. The resin, in powdered form, is given mixed with flavored drinks in divided doses. It is generally rather poorly tolerated. It may cause nausea and vomiting. Constipation may be a substantial problem for some patients while occasionally a patient will develop diarrhea with its use.

Cholestyramine therapy should be initiated in all patients with pruritus as a major symptom of their liver disease. It has sometimes been useful in treating the pruritus of chronic renal failure but has no value in treating pruritus associated with skin diseases.

Other agents which are used in patients with pruritus have very little value. Antihistamines have not proven useful. Phenobarbital, which might theoretically increase bile flow, causes drowsiness but has little effect on the symptoms. Nonetheless, if it is considered useful to sedate the pruritic patient, it makes more sense to use phenobarbital than any other drug.

In situations of prolonged pruritus, plasmapheresis offers effective relief at least for a period of days. Phototherapy does not appear to offer much to patients with pruritus on the basis of liver disease. Therapy with 17α-alkyl androgens (e.g. methyl testosterone) does relieve pruritus at times. This is at the cost of deepening jaundice and masculinization and hence this approach is seldom employed.

Xanthomata: Reduction of the bile salt level with cholestyramine will eventually decrease the serum cholesterol as well and will lead to regression of xanthomata. When the bile salts are depleted, there is utilization of cholesterol in bile salt synthesis. This appears to be the explanation for the benefit seen with cholestyramine therapy in patients with xanthomata. Clofibrate causes a paradoxical increase in serum cholesterol levels and is not useful in these patients.

Malabsorption: If weight loss is important, medium chain triglycerides will provide extra calories. Since MCT does not require micellarization, its absorption is not affected by bile salt deficiency. The diarrhea associated with steatorrhea can usually be controlled by limiting the dietary fat intake to 40 g per day or less.

Parenteral administration of the fat soluble vitamins A, K and E should be given prophylactically in patients with prolonged cholestasis. Vitamin D should also be administered. Oral preparations of 25-hydroxy vitamin D are now available and may be preferred.

It is important to recognize that therapy with cholestyramine can be expected to aggravate the symptoms of malabsorption by sequestering whatever bile salts do reach the intestinal lumen and thus promote the malabsorption of fat.

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