Mechanisms of bile formation and cholestasis

J.L. Boyer, M.D.*

Despite the importance of bile formation to the maintenance of health, the relative inaccessibility of the liver and biliary tree have made it difficult to study this vital physiologic process. However, during the past decade, newer physiologic ultrastructural and biochemical developments have increased our understanding of the determinants of this secretion and have led to new insights into the pathophysiology of cholestasis. Some of these new concepts are summarized in this brief review. For more detailed information several recent references may be consulted1)–3).

I. Anatomical considerations

Although it is not conventional to think of the liver as an epithelium, the hepatocyte is a highly polarized epithelial-like cell whose plasma membrane is divided into three functional domains4). (a) The basal or sinusoidal portion of the cell is greatly amplified with microvilli that increase its surface area thereby facilitating processes involved with the transport of substances from portal blood into the hepatocyte. (b) A smooth surfaced lateral cell membranes line the intercellular space, and (c) an apical membrane that borders the lumen of the bile canaliculus and which is separated from the lateral surface membrane by tight junctions which join adjacent hepatocytes. Although the apical membrane consists of only 13% of the total surface area of the hepatocyte all excretory products of the liver cell must be transported across this membrane into bile. Intracellular organelles also contribute to the polarity of the hepatocyte. The Golgi apparatus is the most obvious, since it is always located at the biliary pole of the liver cell even though its role in bile secretion is still highly speculative. Lysosomes are also prominent in the apical portion of the cell, and lysosomal constituents are excreted in bile. Finally, tight junctions seal the lumen of the bile canaliculus and represent the only anatomical barrier between blood and bile since the sinusoidal endothelial cells are fenestrated. Thus the tight junctions may play an important regulatory role in the regulation of biliary secretion.

The role of each hepatocyte in the formation of bile is also partly dependent on its location within the lobule. Cells located within peri-portal areas of the lobule are primarily involved in the transport of bile acids and the generation of bile acid dependent secretion. In contrast central lobular cells are relatively bile acid deficient and are believed to form bile predominantly by bile acid independent mechanisms5). As the bile acid load to the liver increases, this lobular gradient for bile acid transport is redistributed, and central zone cells are recruited for bile acid transport and increase their contribution to bile acid dependent secretion.

Transport enzymes are also localized on different domains of the hepatocyte. For example, Na⁺,K⁺-ATPase is localized to the basolateral portion of the hepatocyte rather than the apical or canalicular pole. Thus the liver cell is similar to epithelial cells since this transport enzyme is not located on the membrane toward which the primary secretion is generated1),2). Receptors for many substances which are transported from blood into the hepatocyte are also localized on the basolateral portion of the cell including those for low density lipoproteins (LDL receptor), secretory component, (the receptor for immunoglobulin A,) and hormones such as insulin and prolactin. In contrast alkaline phosphatase, magnesium ATPase, leucine aminopeptidase, and gamma-glutamyltranspeptidase are usually localized to the apical cell membrane in the healthy liver.

II. Steps in the formation of bile

(a) Hepatic uptake of bile acids

Bile acids must be transported from portal blood against both chemical and electrical gradients into the hepatocyte. Studies utilizing liver perfusions, isolated hepatocytes suspensions and membrane vesicle preparations indicate that

* Liver Study Unit, Department of Medicine, Yale University School of Medicine, New Haven, CT. 06510 USA
taurocholate uptake is driven by a sodium coupled mechanism. This secondary active transport process drives taurocholate into the cell by coupling the bile acid to the sodium gradient that is generated by the activity of Na⁺,K⁺-ATPase. Presumably a carrier exists on the sinusoidal membrane which recognizes both taurocholate and sodium ions. A sodium independent system also exists at high extracellular concentrations of taurocholate and for certain other bile acids. However, since sodium omission or ouabain inhibit the uptake of taurocholate, the primary process appears to be a sodium coupled transport mechanism.

(b) Transcellular bile acid transport

Once bile acids enter the cell it is still unclear how they are directed toward the small portion of the plasma membrane that lines the canalicular lumen. Microfilaments and microtubules may play some role since the hepatic clearance of these anions is impaired by inhibitors of cytoskeletal elements. Also since infusions of large doses of bile acids result in accumulation of >1000Å vesicles in the region of the peri-canalicular ectoplasm, it is that bile acids move across the cell together with vesicles. However, to date vesicle transport has been implicated only in movement of proteins such as IgA, insulin, or horseradish peroxidase.

(c) Excretion of bile acids in bile

Canalicular excretion is the rate limiting step in the hepatic transport of bile acids and other organic anions. Several different mechanisms are postulated but none has been definitively established or rejected. For example the electrical potential inside the hepatocyte ranges between −30 and −40 MeV, and could drive bile acids into bile against a chemical gradient of approximately 2–3 nM. However, the activity of bile acids are probably reduced by intracellular binding proteins so that it is unlikely that the electrical potential is the primary driving force. Exocytosis is also an attractive but as yet unsubstantiated mechanism for the canalicular excretion of bile acids. Active transport from hepatocyte across the canalicular membrane is a third possibility.

(d) Bile acid independent secretion

Other active transport mechanisms undoubtedly exist and contribute to the osmotic activity of bile and the generation of biliary flow. Candidates include the secretion of bicarbonate, since the elimination of this anion from isolated rat liver perfusates inhibits bile acid independent bile flow. Other candidates include the amino acids, glutamic acid, glycine and the tripeptide, glutathione which exist in bile in concentrations that are higher than observed in plasma.

(e) Cytoskeletal elements

Ultrastructural studies demonstrate cytoplasmic filaments beneath the plasma membranes of hepatocytes that are particularly abundant within the region of the bile canaliculus. Microfilaments form a mesh of interconnecting fibers that insert into microvilli and the base of the junctional complexes. Intermediate filaments and microtubules can also be identified and inhibitors of microfilament and microtubule function alter hepatocyte structure in these areas. For example microfilament poisons such as cytochalasins or phalloidin both reduce secretion and result in a loss of canalicular microvilli. Cytochalasins also inhibit canalicular contractility as observed by time-lapse cinemicrophotography of hepatocytes in cell culture. These studies suggest that the canalicular lumen undergoes periodic focal contractions that propels bile forward toward the portal regions of the lobule. Phalloidin also disrupts the junctional complex and increases biliary permeability.

(f) The paracellular pathway

Bile is an iso-osmotic secretion and most epithelial tissues that secrete isotonic fluids equilibrate across leaky junctional complexes. Accordingly, bile acids and other osmotically active solutes should draw water and other electrolytes into the lumen of the canaliculus both from the cell as well as the intercellular space and across the junctional complex. Surface-volume relationships suggest that this osmotic equilibration should be optimal at the level of bile canaliculus since the surface volume ratio collapses substantially on moving from the bile canaliculus to the level of the biliary ductules and ducts. The rapid cholestatic affect of hyperosmolar solutions further suggests that bile is capable of rapid osmotic equilibration with plasma.

Indirect evidence suggests that paracellular movement of water and other small solutes may occur during stimulation of bile formation. For example when bile flow is stimulated by a potent choleretic like dehydrocholate, focal areas of blistering occur in the intercellular space adjacent to the junctional complex and ionic lanthanum can be found penetrating the junction in a number of areas. This suggests that an osmotic choleresis stimulates the movement of both water and solutes across this paracellular barrier into bile. In the iso-
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I. Introduction

II. Mechanisms of bile formation

III. Mechanisms of cholestasis

It should be obvious from this brief review that multiple steps in the process of bile formation might be impaired during liver injury. Thus with few exceptions the pathogenesis of cholestasis is also complex. Much of our information about cholestasis has been developed from studies of various experimental models including bile duct ligation, or the administration of ethinyl estradiol, lithocholic acid, phalloidin, manganese, chlorpromazine norethandrolone or protoporphyrin. Many of these agents inhibit plasma membrane enzymes such as Na+, K+-ATPase (ethinyl estradiol, chlorpromazine and protoporphyrins). Other plasma membrane enzymes are often also effected and so the effect on membrane function is probably more generalized and may involve changes in lipid composition and microviscosity. Agents such cytochalasin, phalloidin or chlorpromazine impair microfilament function and effect transcellular transport of bile acids and other choleretics. Canalicular morphology is altered by many of these agents producing loss of microvilli and an increase in canalicular ectoplasm.

More recent evidence suggests that the permeability characteristics of the junctional complex may be altered in a number of these cholestatic models. Tight junctions consist of a series of parallel interconnecting strands or elements that average 4-5 in number and which represent a barrier to diffusion between the intercellular space and the bile. Considerable heterogeneity exists in this structure and focal areas may be found within the junctional complex where there are only 1 or 2 strands or as many as 8 or 9. Normally these junctional barriers are impermeable to large molecules such as proteins. However bile duct ligation results in an alteration of the junctional complex which allows penetration of large molecule solutes such as horseradish peroxidase (MW~60,000) into the junctional complex and bile by way of the intercellular space. This paracellular movement of these large solutes appears to be secondary to a structural alteration in the junctional barrier that permits diffusion of solutes between blood and bile.

Phalloidin administration to rats produces a marked increase in the biliary clearance of inulin and sucrose at the same time that ionic lanthanum can reflux from bile to plasma. More recent evidence suggests that the permeability characteristics of the junctional complex may be altered in a number of these cholestatic models. Tight junctions consist of a series of parallel interconnecting strands or elements that average 4-5 in number and which represent a barrier to diffusion between the intercellular space and the bile. Considerable heterogeneity exists in this structure and focal areas may be found within the junctional complex where there are only 1 or 2 strands or as many as 8 or 9. Normally these junctional barriers are impermeable to large molecules such as proteins. However bile duct ligation results in an alteration of the junctional complex which allows penetration of large molecule solutes such as horseradish peroxidase (MW~60,000) into the junctional complex and bile by way of the intercellular space. This paracellular movement of these large solutes appears to be secondary to a structural alteration in the junctional barrier that permits diffusion of solutes between blood and bile.

In all three cholestasis models, freeze fracture analysis of the junctional complexes reveals significant alterations in the junctional anatomy. After 24-46 hours of bile duct ligation, the number of parallel strands that comprise the interconnecting network of the junctional barrier are substantially reduced from an average of 4 to 5 strands to only 2 or 3. Phalloidin administration also results in a marked distortion of the junctional complex; strands extend out in an abluminal fashion from the canalicular lumen and there are focal areas...
where only one strand is left intact. Ethinyl estradiol also produces significant, although less striking reductions in the junctional complex strand density compared to control animals. All these studies suggest that back diffusion from bile to blood might occur during cholestasis and result in a reduction in biliary secretion. According to this hypothesis increases in permeability in the junctional barrier would be associated with loss of some osmotically active solutes in bile as a result of paracellular reflux; Also some water would diffuse from bile to blood as the hydrostatic secretory pressure in bile increased. The net result would be a significant reduction in both bile water flow and biliary solute excretion, such as bile acids. Both ultrastructural alterations in the junctional complex and increased paracellular permeability suggest that regurgitation of bile from the canaliculus may be important as a mechanism of cholestasis in both intrahepatic as well as extrahepatic cholestatic disorders. These observations provide experimental evidence for theories of jaundice proposed more than 50 years ago by hepatologists such as Eppinger, Pavel and Rich.

Nevertheless, many unanswered questions still remain. For example, what is the nature of the sinusoidal carrier for bile acid transport? How are bile acids transported across the cell to the bile canaliculus? Is there a role for the Golgi apparatus in bile formation? Does exocytosis play an important role in biliary secretion? What is the source of the biliary micelle, is it formed intracellularly, or does it arise from the canalicular membrane or within bile itself? Is the role of the cytoskeleton in cholestasis secondary or does impairment of these important structural elements play a primary role in cholestasis? How is junction permeability regulated? Is this a primary site of injury during cholestasis? These and many other questions, pose important problems for further detailed study. Despite the incompleteness of the story, the developments of the past decade give every promise that additional answers to many of these questions should be forthcoming in the years ahead. As the pathophysiology of bile formation and cholestasis is better understood, better treatment for cholestatic liver disease will hopefully evolve.

Key words: Bile formation Cholestasis Na⁺,K⁺-ATPase

References