Bile formation in the rat: the role of the paracellular shunt pathway.

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Research Article

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Bile Formation in the Rat

THE ROLE OF THE PARACELLULAR SHUNT PATHWAY

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ABSTRACT Transepithelial movement of water and solute occurs both through the cell membrane as well as across the intercellular junctional complex (paracellular shunt pathways). Permeability of paracellular shunt pathways is increased by transmucosal osmotic gradients, and in certain epithelia these changes are associated with bullous-like deformations (blisters) of the zonula occludens and localization of lanthanum within junctional complexes. Although bile acids increase biliary secretion by osmotic forces, the source of this water movement into bile is not known. In the present studies we examined whether a choleretic infusion of sodium dehydrocholic acid (DHC) or its taurine conjugate, taurodehydrocholate, altered the solute permeability characteristics and morphologic appearance of the junctional complexes of rat hepatocytes. Animals were continuously infused for 1 h with 1% albumin–0.9% NaCl alone or 120 μmol of DHC and bile flow and biliary clearance of [^{14}C]sucrose, an indirect marker of biliary permeability were measured. The number of intercellular blisters adjacent to the bile canaliculus were counted in an unbiased manner from photographs obtained with scanning electron microscopy. Bile flow and the biliary sucrose clearance remained unchanged in control animals whereas DHC infusions resulted in a progressive increase in the biliary clearance of [^{14}C]-sucrose during the 60 min of infusion even though the choleretic response to DHC was stable during the final 30 min of infusion. DHC infusions produced surface invaginations, or blisters, (0.1–0.7 μm in diameter) which were located immediately adjacent to the hemi-bile canaliculus and occurred with a frequency of 1.62±0.08 per hepatocyte surface, which was fivefold greater than observed in controls. In separate groups of animals 5 mM ionic lanthanum chloride was perfused intraportally after taurodehydrocholate infusions, and the number of junctional complexes that contained the electron dense marker were quantitated by transmission electron microscopy. Localization of lanthanum in the junctional complexes of fasted control animals was not observed, whereas ≅50% of the zonula occludens in DHC-infused animals contained lanthanum which was also occasionally identified within the lumen of the bile canaliculus. These results indicate that infusions of DHC cause blisters adjacent to the junctional complex of rat hepatocytes in association with changes in solute conductivity of the zonula occludens to cations such as ionic lanthanum chloride, and presumably to larger solutes such as sucrose. Qualitatively similar morphologic findings were also observed during the infusion of sodium taurocholate at physiologic rates (40 μmol/h). These studies suggest that the paracellular shunt pathway in the liver is an important site for bile acid-induced water and solute movement into bile.

INTRODUCTION

Bile secretion is a major hepatic function of all vertebrate species. Although the transport processes that regulate this complex isotonic secretion are still incompletely understood, the bile canaliculus of the hepatocyte is the primary source (1–3). The lumen of the canaliculus is located at the apical portion of the cell where it is formed from only a small portion (13% of the surface membrane) of two adjacent hepatocytes (4). Tight junctions (zonula occludens) demarcate the canalicular space and serve as structural barriers between the secreted bile and the intercellular space and

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sinusoidal blood (5). Freeze fracture replicas of tight junctions in the liver and in epithelial tissues indicate that these junctional contacts are formed by a complex lattice network of filamentous particles rather than a single point of contact (6–9). In epithelia with high transepithelial electrical resistance, such as the toad urinary bladder (10), these junctions are normally impermeable to marker solutes, such as ionic lanthanum chloride, and are thought to be tight (11). Low resistance epithelia such as the rat jejunum and renal proximal tubule permit the passage of fluid and solute through the junctional complex and are generally described as leaky (10). Although morphologic criteria are not always a reliable indicator of junctional permeability (12–13), several ultrastructural studies suggest that the tightness of these epithelial junctions may be correlated in some tissues with the depths and number of oppositional filaments which form the junctional contacts between cells (14, 15). Hepatocyte junctions seem to be intermediate between classical tight and leaky epithelia by these morphologic criteria (15).

Nevertheless, despite a large number of investigations directed at defining the permeability characteristics of the junctional complexes which join adjacent liver cells (7, 15–17), and a number of physiologic studies which suggest that paracellular flux between hepatocytes might occur (18, 19), doubt still remains whether fluid and solute move from the intercellular space into bile across the junctional barriers during the normal course of bile formation (20). For example, whereas bile acids are known to be a major determinant of bile secretion, presumably by generating an osmotic force within the bile canalicular lumen (21–23), it is not known whether the fluid and electrolytes that follow the biliary transport of this choleretic come from within the cell by crossing the surface membrane of the canaliculus or enter bile from the extracellular space.

In certain leaky epithelia considerable morphologic and electrophysiologic evidence has accumulated that indicates that the intercellular junctional complex is a major site of extracellular fluid movement (10, 24–26). The magnitude of transport across the intercellular junctional complex varies depending upon the epithelia examined (10, 24), the intrinsic structure of the zonula occludens (5, 14), and upon the experimental conditions that are used to examine transepithelial fluid transport (27–31). Even in tight epithelia such as the amphibian urinary bladder and skin, transepithelial osmotic gradients promote water movement from serosa (blood surface) to mucosa and cause a dramatic reduction in transepithelial electrical resistance and an increase in passive permeability to anions, cations, and other solutes that do not normally penetrate cell membranes (27–29). Wade et al. (28, 32) and DiBona and Civian (29–31) have independently demonstrated that osmotically induced changes in amphibian epithelial permeability are associated with blister-like swellings within the confines of the zonula occludens, and have shown that these osmotically induced accumulations of fluid are associated with an increase in the ionic and hydraulic conductivity of the paracellular pathway. Although osmotic gradients are not always associated with blisters when paracellular resistance is decreased (33), the presence of these intercellular dilations is generally regarded as morphologic evidence of an increase in electrical conductivity and an increase in solute permeability rather than tissue artifact. Therefore, if bile acids enhance the rate of bile flow by osmotic mechanisms that cause water and electrolytes to move between cells across the zonula occludens into bile, then analogous ultrastructural changes (blisters) within or adjacent to the junctional complex between hepatocytes might occur when bile acids stimulate secretion. To examine this hypothesis, sodium dehydrocholate (DHC)[1], or its taurine conjugate, taurodehydrocholate (TDHC), were infused in large molar quantities (120 μmol/h) to establish a significant osmotic gradient that would double the water and electrolyte flux into bile. Hepatocyte plasma membranes immediately adjacent to the margin of the bile canaliculus were examined for blisters by both scanning electron microscopy and transmission electron microscopy. These morphologic findings were then contrasted with the effect of these bile acid infusions on the biliary clearance of [^{14}C]sucrose, an indirect marker of biliary permeability, whereas more direct evidence for solute movement across the junctional complex was sought by infusion of the electron dense marker, lanthanum chloride.

METHODS

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing 225–275 g were used in all experiments. DHC was purchased from Steraloids, Inc., Wilton, N. H., and TDHC from Calbiochem, San Diego, Calif., and were greater than 99% pure by thin layer chromatography. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and the common bile duct was cannulated. Body temperature was monitored by rectal probe and maintained at 37°C by a heating lamp regulated by a constant temperature regulator (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Biliary sucrose clearance. The biliary clearance of [^{14}C]-sucrose (Amersham-Searle, Arlington Heights, Ill.) was measured in 19 animals with ligated renal pedicles after the intravenous injection of 5 μCi of [^{14}C]sucrose. [^{14}C]Sucrose was allowed to equilibrate between plasma and hepatocyte water for 60 min. Bile was subsequently collected at 10-min intervals for 20 min to determine the control rate of

[1] *Abbreviations used in this paper*: DHC, sodium dehydrocholate; SM, scanning electron microscopy; TDHC, taurodehydrocholate; TM, transmission electron microscopy.

bile flow. Animals were then infused intravenously for 60 min at a rate of 2.6 ml/h with either 1% albumin (bovine Fraction V, Armour Pharmaceutical Co., Kankakee, Ill.)–0.9% NaCl alone [7], or 120 μmol of DHC [12] dissolved in diluent while bile was collected at 10-min intervals. Duplicate samples of bile (50 μl) from each preinfusion and infusion collection period were added to 10 ml of Triton-X-toluene scintillar (Rohm and Haas Co., Philadelphia, Pa.) and counted in a Packard Tri-carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.) to an accuracy of >97%. Samples of blood (0.3 cm) were withdrawn at 70, 115, and 140 min into the experiment, and duplicate samples of plasma (25 μl) were counted for radioactivity. The biliary clearance of [^{14}C]-sucrose was measured according to the formula: microliters of bile per minute per gram of liver × disintegrations per minute of bile divided by disintegrations per minute of plasma (19, 23). Statistical differences were determined by Student's *t* test.

Scanning electron microscopy (SM). Bile was collected for 30 min to establish the control rate of bile flow. Animals (three or four in each group) were then infused intravenously for 1 h with either 1% albumin–0.9% NaCl alone or 120 μmol of DHC as described above. The abdomen was reopened during the final 5 min of the infusion and the portal vein was cannulated with a 16-gauge intracatheter while the infusion was continued. The liver was then perfused consecutively for 30 s at 15 cm H_2O pressure with Ringer's lactate (4°C containing 10 U heparin/ml) and with cold 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) (35, 36). The median lobe of the liver was removed, fractured by gentle digital pressure, and small blocks of tissue were postfixed in 2.5% glutaraldehyde–0.1 M sodium cacodylate buffer (pH 7.4) for 3 h. Specimens were prepared for SM as previously described (36).

All specimens were examined and photographed in a Coates-Welter scanning electron microscope (Coates & Walter Instrument Corp., Sunnyvale, Calif.) without the knowledge of treatment. Photographs were routinely taken at ×2,000 magnification in peri-portal regions of the hepatic lobule. These photographs were subsequently reviewed blindly and the number of invaginations, or blisters, of the hepatocyte cell surface membrane adjacent to the margin of the hemi-bile canaliculus were counted. These invaginations of the intercellular surface membrane were only recorded as blisters if they were located within 2 μm from the margin of the hemi-bile canaliculus. The surface of 70–120 hepatocytes were examined in each treatment group.

Transmission electron microscopy (TM). Specimens from an additional group of experiments were also examined for blisters by TM after glutaraldehyde perfusion of portal vein as described for SM. Blocks of tissue were postfixed in 2.5% glutaraldehyde–0.1 M Na^+ phosphate buffer (pH 7.4). Specimens were then postfixed in 2% osmium tetroxide–0.1 M Na^+ phosphate buffer (pH 7.4). Water was removed with acetone and the tissue was embedded in eponepoxy resin. Specimens were stained with uranyl acetate and lead citrate and examined in a RCA-EMU-4 transmission electron microscope (RCA Solid State, Somerville, N. J.).

Ionic lanthanum chloride infusion. After an overnight fast, bile was collected for an initial period of 20 min. Then either 1% albumin (four controls) or 1% albumin in combination with TDHC sodium (120 μmol) (four studies) was infused into the jugular vein by a Harvard pump (Harvard Apparatus Co., Millis, Mass.) at a rate of 2.6 ml for 1 h. At the end of the 1-h infusion the animal was heparinized by injection of heparin (1,000 U per 100 g body wt) into the vena cava. The portal vein was cannulated with a 16-gauge needle and the liver was perfused for 3 min with a 310-mosmol solution containing 5 mM lanthanum ($LaCl_3 6H_2O$, Fisher Scientific Co., St. Louis, Mo.) in 5 mM Tris pH 7.4 containing Na^+ (133 mM), K^+ (3.6 mM), Cl^- (156 mM), Ca^{++} (4.5 mM) at 15 cm H_2O pressure (37, 38). At the end of 3 min the perfusate was changed to 2.5% glutaraldehyde in 0.1 M cacodylate pH 7.4 at 4°C for liver fixation. Sections of the liver were stained with uranyl acetate and lead citrate and viewed with a Siemens Elmskop 1A electron microscope (Siemens Corp., Iselin, N. J.).

15–30 bile canaliculi were examined from each liver and the number of intercellular spaces and junctional complexes penetrated by lanthanum were counted and representative areas photographed in each animal.

Physiologic controls. To determine if the effects of DHC and TDHC were also observed during more physiologic conditions, taurocholate, the naturally occurring primary bile acid in the rat, was infused at 40 μmol for 1 h in 4 animals. The formation of blisters and the localization of ionic lanthanum in hepatocyte junctional complexes were then quantitated in a manner similar to the DHC and TDHC studies.

RESULTS

Bile flow and the biliary clearance of [^{14}C]-*sucrose.* Bile flow, bile:plasma [^{14}C]sucrose ratios, and biliary clearance of [^{14}C]sucrose remained unchanged in controls during the 1 h of 1% albumin–0.9% NaCl infusions. However, in DHC-infused animals, bile flow increased within 25 min from a preinfusion mean of 2.2±0.4 μl/min per g liver to 4.6±0.8 μl/min per g liver, and flow remained stable in the subsequent 35 min of infusion. Bile: plasma ratios of [^{14}C]sucrose decreased in the initial 25 min of DHC infusions from the preinfusion mean of 0.150±0.024 to 0.107±0.03. However, during the next 35 min bile: plasma [^{14}C]sucrose ratios progressively rose, and at the end of the 1-h infusion, bile:plasma ratios had increased to 0.157±0.04, a value which is greater but not significantly different than the preinfusion mean. The relationship between DHC-induced changes in bile flow and [^{14}C]sucrose clearance is illustrated in Fig. 1. Although bile flow was stable during the final 30 min of DHC infusion, [^{14}C]sucrose progressively increased with time, and within the final 30 min there was a 47% increase in [^{14}C]sucrose clearance. This increase in [^{14}C]sucrose clearance without a concomitant increase in bile flow suggests that the diffusion permeability of the biliary system to [^{14}C]-sucrose had changed (19, 39).

Morphologic studies. In control animals infused with 1% albumin–0.9% NaCl, small invaginations, or blisters, (<0.2 μm in diameter) were observed on the smooth surface of the hepatocyte plasma membrane facing the intercellular space with a frequency of 0.3 ±0.02 per hepatocyte surface (Fig. 2). Although there was variability from specimen to specimen in animals infused with 120 μmol of DHC, blisters were observed with a greater frequency and averaged 1.62±0.20 per

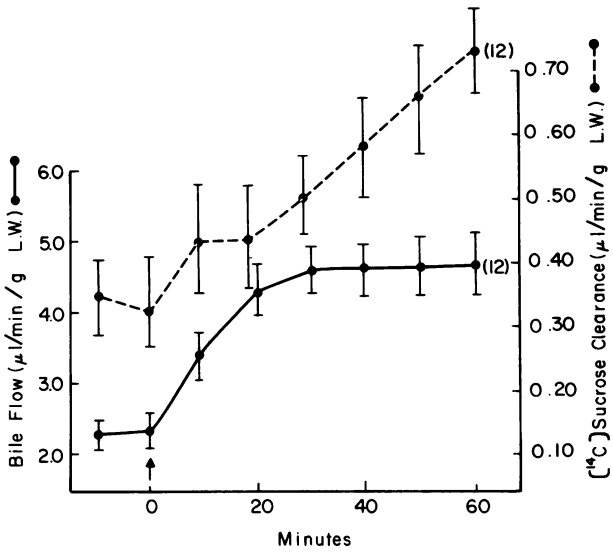

FIGURE 1 The effect of dehydrocholate (DHC) infusions (120 μmol/h) on bile flow and [^{14}C]sucrose biliary clearance is compared as a function of time. DHC infusions (indicated by the arrow) increased bile flow within 10 min, and by 30 min the choleretic response to DHC was stable. In contrast, [^{14}C]sucrose clearance increased progressively during the final 30 min of DHC infusion despite stable biliary secretion. Values equal the mean±SD of 12 experiments.

hepatocyte surface (Figs. 3 and 4). The diameter of these blisters was noticeably larger (0.2–0.8 μm) than those observed in control animals. Although all blisters were located between 0.1 and 2.0 μm from the margin of the hemi-bile canaliculus (Fig. 4), the majority were at least a distance of 0.5 μm. The surface of the blister was generally smooth except for a few small interior microvillous projections that were usually observed. Stud-like projections from the hepatocyte plasma membrane were rarely encountered near the junctional complex so that it is highly unlikely that these invaginations represent stud holes which have been previously described as interlocking attachments between cells (36, 40–42). Blisters were also identified by TM in control and DHC-infused animals (Fig. 5). These dilations of the intercellular space were observed immediately adjacent to the lateral portion of the zonula occludens and resembled the bullae or blisters that DiBona, Wade, and colleagues first described in the toad urinary bladder (28–30). Membranous material was occasionally observed within the blisters (Fig. 5) as previously described (30). Definite bullous deformation within the interior of the zonula occludens was not demonstrated.

Lanthanum infusions. In the present studies, 5 mM ionic lanthanum was perfused via the portal vein at physiologic pressures for 3 min. In control animals lanthanum was present in abundance lining the sinusoidal endothelium and Disse's spaces. However, penetration of intercellular spaces was infrequent and in only extremely rare instances was lanthanum visible within the junctional complex adjacent to bile canaliculi. Findings in animals infused with 120 μmol DHC for

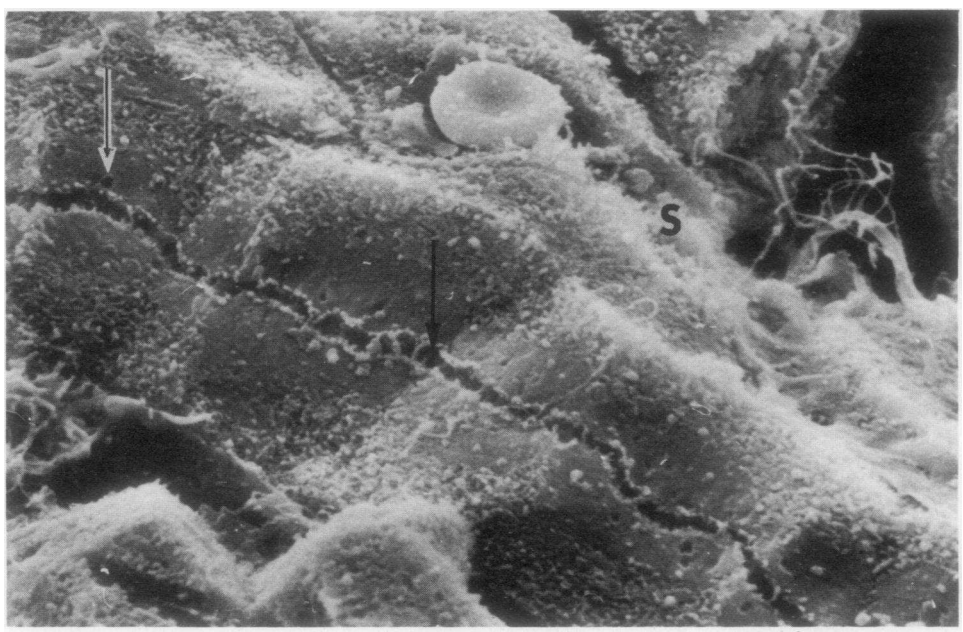

FIGURE 2 The SM appearance of sinusoids (S), hepatocytes, and hemi-bile canaliculus (arrow) is illustrated in an animal infused with 1% albumin–0.9% NaCl. Note that the hepatocyte surface lateral to the hemi-bile canaliculus (lateral surface) is relatively smooth and free of microvilli compared to the sinusoidal surface. Only a few small surface invaginations can be observed on the lateral cell surface (white arrow) (original magnification ×2,000).

FIGURE 3 In contrast to Fig. 2, many invaginations, blisters (arrows), can be appreciated on the lateral surface immediately adjacent to the bile canaliculus in an animal infused with DHC (120 μmol/h). Although the diameter of the blisters is variable, some blisters are nearly as large as the diameter of the hemi-bile canaliculus. Intercellular connections (studs) are notably lacking (original magnification ×2,000).

1 h, who had experienced a >100% increase in bile flow, were in marked contrast to controls (Fig. 6). Penetration of lanthanum between intercellular spaces was extremely widespread and lanthanum was frequently seen within the junctional complex (Table I). Lanthanum was usually seen extending to the canaliculus and could also be identified within the lumen of the canaliculus on rare occasions (Fig. 7). Bile canaliculi were usually devoid of any material possible because it was washed away during processing of the tissue blocks or because it was irreversibly bound to junctional tissues (25). Lanthanum was never observed within hepatocytes.

Physiologic controls. During taurocholate infusions (40 μmol for 1 h), blisters were also observed in the intercellular space adjacent to the junctional complex with a frequency that was significantly greater than controls (1.44±0.16 per cell surface). Ionic lanthanum was also observed within the junctional complex of the bile canaliculi, although both the frequency of localization and the intensity of the lanthanum was less than during TDHC infusions.

DISCUSSION

Although it has been assumed that osmotic forces are the major determinant of bile formation (21–23), uncertainty exists concerning the site of water and solute transport into the lumen of the bile canaliculus. The present observations that DHC and TDHC infusions produced blister-like swellings in the intercellular space adjacent to the bile canaliculus, increased the biliary permeability to [^{14}C]sucrose, and moved ionic lanthanum chloride into the junctional complex and into bile, provides evidence that the paracellular pathway is an important site of fluid entry into bile under experimental conditions that establish steep osmotic gradients between bile and the intercellular space. These findings do not necessarily exclude the possibility that water and solute also enter bile in

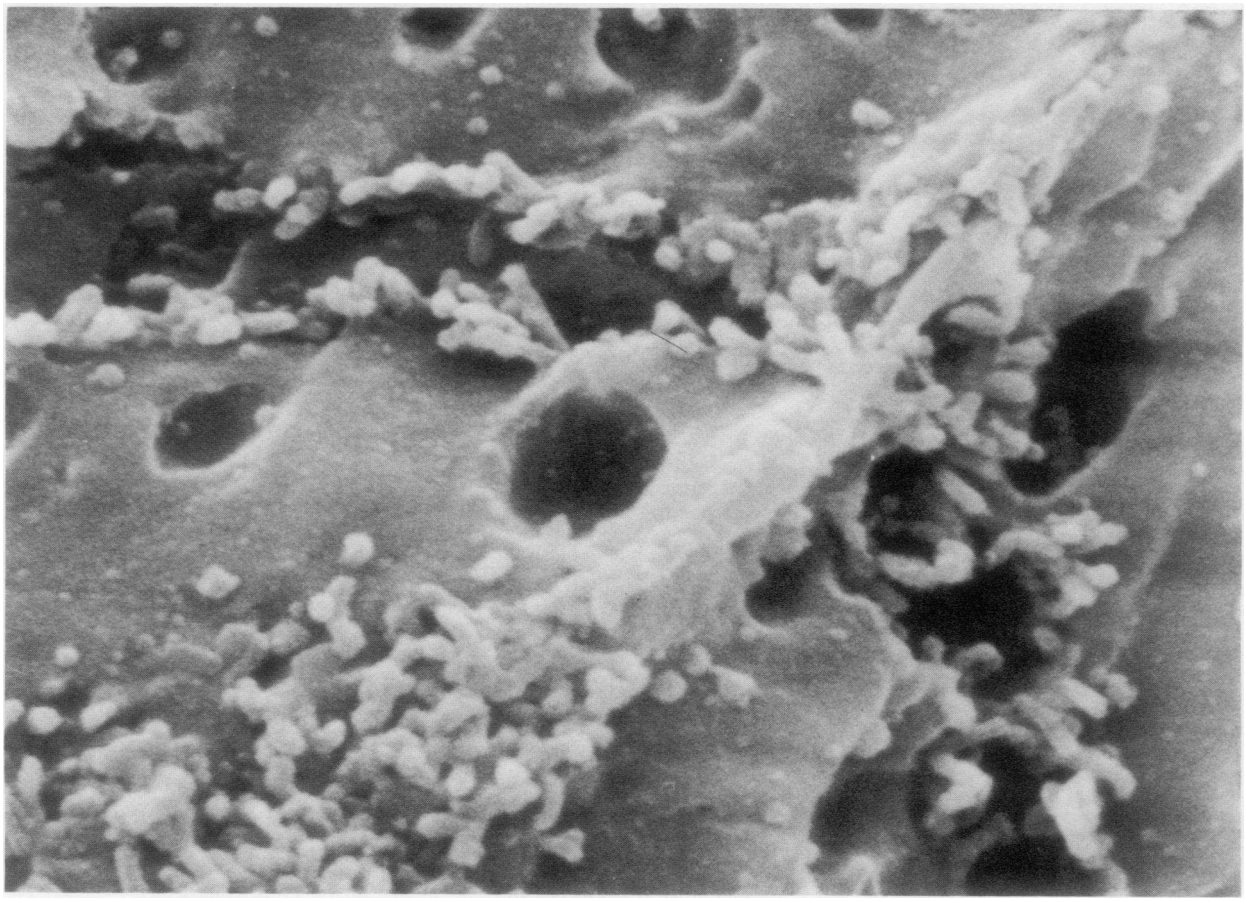

FIGURE 4 The surface membrane of the interior of blisters is illustrated at higher magnification (×10,000) from an animal infused with 120 μmol of DHC. These blisters are within 0.2–0.5 μm from the margin of the hemi-bile canaliculus.

response to an osmotic gradient by movement from the hepatocyte across the canalicular membrane. Rather, they indicate that the intercellular space and the junctional complex of hepatocytes respond to transcellular osmotic gradients in an analogous manner to certain epithelial tissues (28–30).

Because the bullous accumulations of fluid in hepatic intercellular spaces resemble the osmotically induced blisters described by Wade et al. (28, 32) and DiBona and Civian (29, 30), in the toad urinary bladder, it is likely that the hepatocyte blisters also reflect an increase in ionic conductivity in the paracellular pathway between hepatocytes. However, the location differs slightly in that most blisters occur immediately lateral to the zonula occludens in the region of zonula adherens and desmosomes (20). Because the length of the hepatocyte zonula occludens could not be determined with certainty by SM, the percentage of blisters within the region of the zonula occludens could only be estimated. Some blisters were clearly within the contact zones of the junction, although the majority appeared to extend laterally to involve the intercellular membrane between the zonula adherens and desmosomes. Other explanations for blister formation must also be considered. Endocytic vacuoles might look like blisters with SM. Although endocytosis with intracellular vacuole formation is a proposed mechanism for cell uptake of large molecular solutes (43, 44), bile acid hepatocyte uptake is a carrier-mediated process which is sodium dependent (45, 46). Stud holes have been observed by both TM and SM in rat liver (36, 40–42), and are usually situated along the intercellular surface. However, we rarely observed corresponding stud-like projections. The TM studies essentially exclude the possibility that the blisters are outpouchings or diverticuli of the lumen of the bile canaliculus although we have observed canalicular diverticuli after DHC infusions (47, 48). Because DHC is metabolized in the rat to bile acids that form micelles, to a limited extent it is possible that at these high rates of infusion the morphological changes resulted from the effects of micelle formation. Although

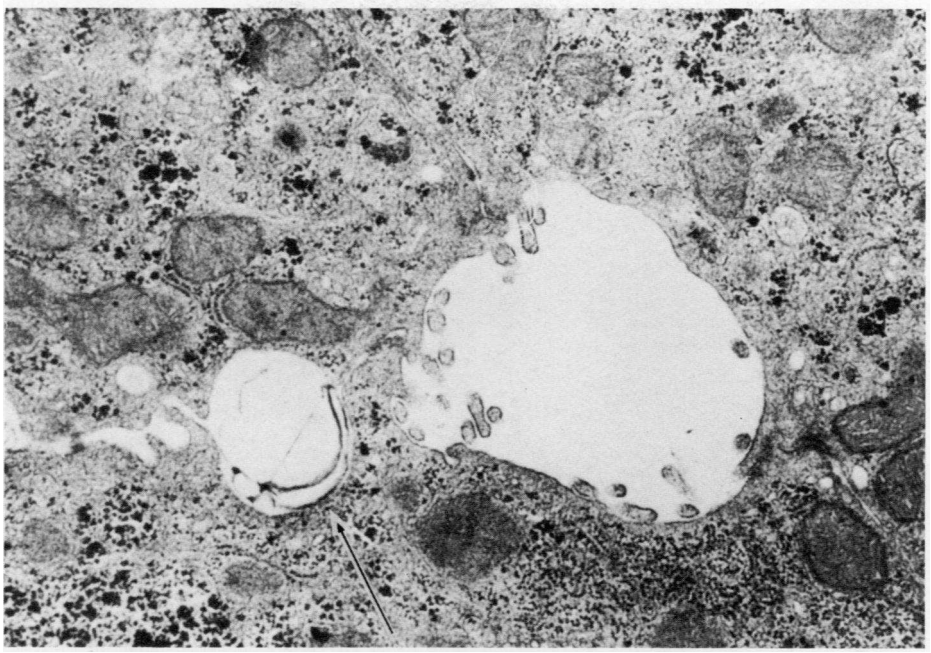

FIGURE 5 A blister (arrow), containing membranous material, is illustrated from an animal infused with 120 μmol of DHC (original magnification $\times$10,000).

this possibility has not been excluded, it seems unlikely that micellar interaction with the lateral surface membrane causes changes because biliary lipid and protein excretion are not altered by DHC, and the localization of blisters is not on the sinusoidal surface where bile acids might have the greatest contact.

The most direct evidence that bile acids increased the ionic conductivity of the junctional complex comes from finding ionic lanthanum within the zonula occludens of many of the junctional complexes after TDHC-stimulated bile production. Previously, many studies have attempted to demonstrate junctional permeability in mouse or rat hepatocytes but the results have usually been equivocal either because the electron dense tracer penetrated only an occasional junction or because the tracers were injected in a retrograde fashion by way of the biliary ducts where excessive biliary pressures probably disrupted the integrity of these intercellular barriers and therefore the result could not be related to the normal process of bile formation (16, 17, 49–52). Many of these same tracers, including colloidal lanthanum nitrate, have been infused into the sinusoidal space and evidence sought for their passage into bile. However, colloidal lanthanum nitrate has a relatively large particle size ($\cong$25 Å) compared to sodium (3.3 Å) (24) and in contrast to ionic lanthanum. Thus Goodenough and Revel (7) found that the zonula occludens in mouse liver was penetrated by colloidal lanthanum only after treatment with 60% acetone. Schatzki (16) found lanthanum nitrate on both sides of the tight junction in rat liver but only occasional pictures suggested the presence of lanthanum within the tight junction. Shea (53) perfused colloidal lanthanum into the portal vein of rats, but it was largely confined to the sinusoids and Disse's spaces.

The use of ionic rather than colloidal lanthanum was a major advance in the morphologic study of junctional complex permeability (37). Machen et al. (25) demonstrated that ionic lanthanum penetrated consistently into regions where no reproducible penetration with colloidal lanthanum could be demonstrated. Furthermore when both electrophysiologic measurements and lanthanum permeability have been employed to estimate junctional permeability, they have given equivalent assessments (26). Thus the equivocal or negative findings with other electron dense tracers may have resulted from the use of solutes with large molecular radii which may not have easily penetrated the junctions. Also, the present studies were performed during a brisk osmotic choleresis induced by a nonmicelle forming bile acid that permits the formation of a steeper bile-plasma osmotic gradient than would occur under physiologic conditions of bile acid excretion. Third, most TM and SM studies and experiments examining the movement of electron dense tracers have been performed under experimental conditions that would reduce the bile acid gradient during the routine process of tissue fixation so that the intracellular space within or adjacent to the zonula occludens might collapse. Indeed, blisters were only

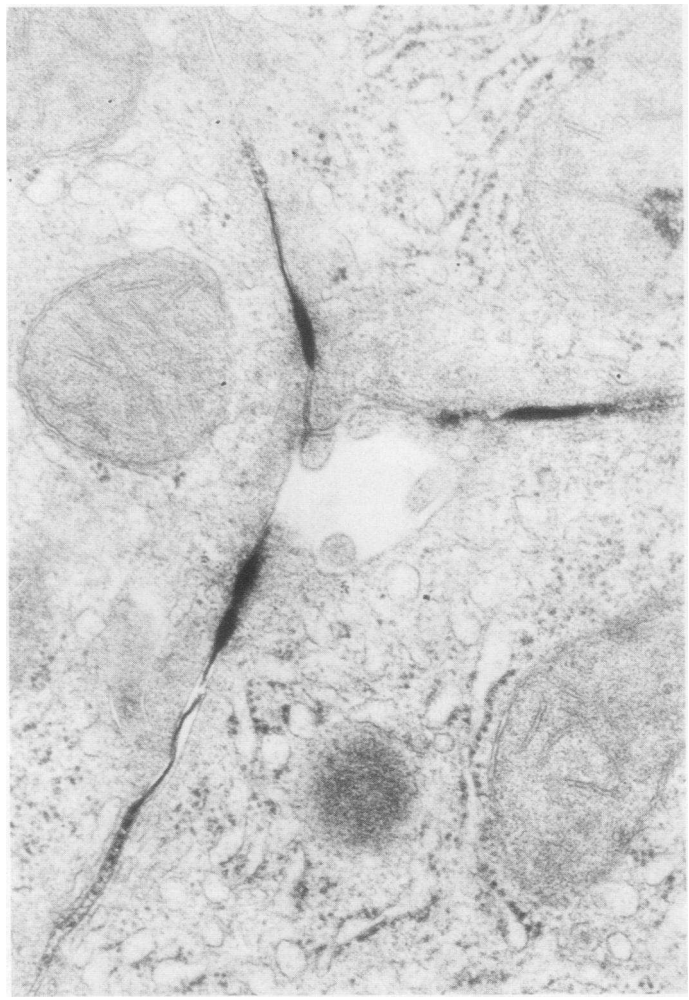

FIGURE 6 TM appearance of bile canaliculus showing presence of electron-dense lanthanum in three adjacent intercellular spaces and the junctional complexes. Animals were infused with sodium taurodehydrocholate (120 μmol/h) followed by 5 mM $LaCl_3$ in 0.1 M Tris pH 7.4 for 3 min intraportally and fixation-perfusion with 2.5% glutaraldehyde in 0.1 M cacodylate pH 7.4 (original magnification ×10,000).

seen frequently in the present studies when the liver was fixed in situ by portal vein perfusion during the infusion of bile acids.

Although the present observations suggest that cationic solutes such as sodium, which are also similar to ionic lanthanum in size, are also likely to readily enter bile via the paracellular pathways, it is not entirely clear whether or not larger solutes, such as sucrose, which are normally restricted in their entry into bile, also pass through these barriers (19). However, the present studies provide indirect evidence to support this concept. Solutes such as sucrose or inulin are felt to cross other epithelia predominantly through paracellular pathways and not through cell membranes (10). Although the cell membrane of some epithelia act as an effective barrier to movement of inert solutes such as sucrose, the liver cell membrane does allow entry of sucrose and inulin into cell water (19, 54, 55). However, solutes such as sucrose or inulin, which are normally restricted in their entry into bile, come into equilibrium with bile water at a time when their concentrations in hepatic cell water are still rising (55). Furthermore, if the membrane of the bile canaliculus behaves like other epithelial membranes, intrahepatic sucrose should also be restricted by the membrane of the bile canaliculus so that it is likely that solutes such as sucrose would enter bile more readily by the paracellular shunt pathway. Transport of sucrose into hepatic bile is dependent on solvent drag (bulk flow), diffusion, and the inherent restriction to solute movement that is provided by the membrane (Staverman reflection factor) (19, 39). At high rates of bile secretion,

TABLE I
Frequency of Intrajunctional Localization of Ionic Lanthanum

Treatment	Canaliculi counted in each specimen	No. with lanthanum in junctional complex	Average
			%
1% Albumin-saline	20	0	0
	18	0	0
	15	0	0
	30	0	0
Total	83	0	0
Taurodehydrocholate, 120 μmol/h	25	5	25
	22	11	50
	30	21	70
	20	10	50
Total	97	47	48.5
Taurocholate, 40 μmol/h	30	5	17
	32	6	19
	35	8	23
	30	20	66
	30	7	23
Total	157	46	29

which would occur during bile acid choleresis, solvent drag is the predominant mechanism for solute transport into bile (19, 39). Thus, the extent of movement (clearance) of [^{14}C]sucrose during DHC infusions should be proportional to the rate of bile flow (solvent drag) and the extent to which the membrane restricts sucrose movement. The observation that [^{14}C]sucrose clearance progressively increased during DHC infusions despite a stable bile flow (solvent drag) indicates that the permeability of the biliary system to [^{14}C]sucrose was altered during the DHC infusion, perhaps by a structural change in the paracellular shunt pathway.

Finally, the observations that physiologic infusions of taurocholate also result in blister formation and the localization of ionic lanthanum in bile provide preliminary evidence that the paracellular pathway may be a site for water and solute transport into bile during the normal process of bile formation. The DHC and TDHC effects on paracellular ion and fluid movement into bile, thus, are likely to represent merely an augmentation of a normal physiologic process.

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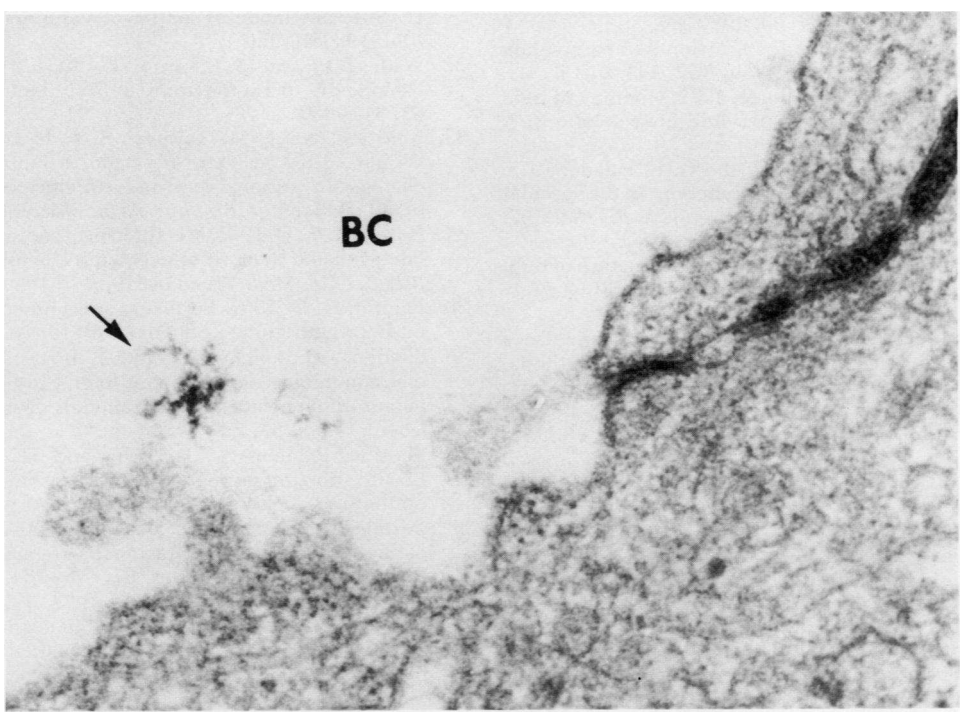

FIGURE 7 TM appearance (×20,000) of bile canaliculus (BC) in taurodehydrocholate-infused animal. Lanthanum is clearly visible within the zonula occludens and within the lumen of the canaliculus (arrow).

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