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Intrahepatic bile ducts transport water in response to absorbed glucose

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Masyuk, Anatoly I., Tatyana V. Masyuk, Pamela S. Tietz, Patrick L. Splinter, and Nicholas F. LaRusso. Intrahepatic bile ducts transport water in response to absorbed glucose. *Am J Physiol Cell Physiol* 283: C785–C791, 2002. First published May 15, 2002; 10.1152/ajpcell.00118.2002.—The physiological relevance of the absorption of glucose from bile by cholangiocytes remains unclear. The aim of this study was to test the hypothesis that absorbed glucose drives aquaporin (AQP)-mediated water transport by biliary epithelia and is thus involved in ductal bile formation. Glucose absorption and water transport by biliary epithelia were studied in vitro by microperfusing intrahepatic bile duct units (IBDUs) isolated from rat liver. In a separate set of in vivo experiments, bile flow and absorption of biliary glucose were measured after intraportal infusion of D-glucose or phlorizin. IBDUs absorbed D-glucose in a dose- and phlorizin-dependent manner with an absorption maximum of 92.8 ± 6.2 pmol·min^{-1}·mm^{-1}. Absorption of D-glucose by microperfused IBDUs resulted in an increase of water absorption (J_v = 3−10 nl·min^{-1}·mm^{-1}, P_f = 40 × 10^{-3} cm/sec). Glucose-driven water absorption by IBDUs was inhibited by $HgCl_2$, suggesting that water passively follows absorbed D-glucose mainly transcellularly via mercury-sensitive AQPs. In vivo studies showed that as the amount of absorbed biliary glucose increased after intraportal infusion of D-glucose, bile flow decreased. In contrast, as the absorption of biliary glucose decreased after phlorizin, bile flow increased. Results support the hypothesis that the physiological significance of the absorption of biliary glucose by cholangiocytes is likely related to regulation of ductal bile formation.

liver; cholangiocytes; absorption; secretion; aquaporins; phlorizin; microperfusion; intrahepatic bile duct units

IT HAS BEEN KNOWN FOR YEARS (12) that the basal concentration of glucose in hepatic bile is extremely low (i.e., ≥0, <1.0 mM) compared with plasma (i.e., >5.0 mM). Earlier elegant studies explored blood-bile glucose concentration differences and provided convincing evidence that glucose enters canalicular bile in equal concentration to plasma and then is subsequently reabsorbed from bile by the intrahepatic bile ducts by sodium-dependent and sodium-independent transport mechanisms (12, 16, 25). Recently, we extended these studies employing confluent, polarized monolayers of rat cholangiocytes and demonstrated that cholangiocytes express a sodium-dependent glucose transporter, SGLT1, at their apical plasma membrane and a facilitative glucose transporter, GLUT1, at their basolateral plasma membrane domain. We proposed that these two proteins accounted for the vectorial (i.e., from bile to blood) transport of glucose and that their coordinated activities provided a plausible explanation for the low concentration of glucose in bile (15).

Nevertheless, the physiological relevance of glucose absorption from bile by cholangiocytes remains unclear, although at least two possibilities have been offered. It was suggested that absorption of biliary glucose contributes to the sterility of bile by removing a potential microbial nutrient (16). Alternatively, we proposed that absorption of biliary glucose by cholangiocytes would induce water absorption by biliary epithelia because glucose would act as an osmolyte in the biliary tree (15). However, direct exploration of this second hypothesis was not possible because an appropriate experimental approach was unavailable until now. Recently, we developed and characterized a novel experimental model, the microperfused rat intrahepatic bile duct unit (IBDU); this model allows direct measurement of ion, solute, and water transport across biliary epithelia (19). In the study described here, we applied this model to complement in vivo experiments to test the hypothesis that glucose absorbed from bile by cholangiocytes drives water absorption by the biliary epithelia and, thus, is involved in ductal bile formation.

MATERIALS AND METHODS

Materials. All chemicals were of the highest purity commercially available and were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated. Fluorescein sulfonate [FS, fluorescein-5(6)-sulfonic acid, trisodium salt] was obtained from Molecular Probes (Eugene, OR).

Animals. Male Fisher 344 rats (225–250 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN), housed in a

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temperature-controlled room (22°C) with 12:12-h light-dark cycles, and maintained on a standard diet with free access to water. All experimental procedures were approved by the Animal Use and Care Committee of the Mayo Foundation.

Solutions. The composition of standard Ringer-HCO_3 buffer (KRB) was (in mM) 120.0 NaCl, 5.9 KCl, 1.2 Na_2HPO_4, 1.0 $MgSO_4$, 25.0 $NaHCO_3$, 1.25 $CaCl_2$, and 5.0 D-glucose. The concentration of D-glucose in KRB perfused through the lumen of IBDUs was varied from 0 to 30 mM; the bathing KRB contained corresponding concentrations of sucrose. The precise osmolality of KRB solutions was determined with a freezing point osmometer (Advanced Micro-Osmometer, model 3300; Advanced Instruments, Norwood, MA).

Microperfusion of isolated rat IBDUs in vitro. IBDUs, which are pieces of intrahepatic bile ducts ranging in luminal diameter from 100 to 125 μm and in length from 1.0 to 1.5 mm, were isolated from normal rat liver and perfused as previously described in detail (19). Briefly, individual isolated IBDUs were placed in a specially designed, temperature-controlled chamber mounted on the stage of an inverted fluorescence microscope (Nikon, eclipse, TE300). Concentric glass pipettes were used to position and perfuse the IBDUs at a rate of 40 nl/min. The system was checked for potential leakage by using trypan blue dye or FS. The bath solution was changed during the experiment with an exchange rate of 1.0–1.5 ml/min. The perfused IBDUs were allowed to equilibrate for 30 min before the experimental protocol was started; their viability was assessed by trypan blue exclusion.

Measurement of D-glucose absorption. IBDUs were perfused with KRB containing physiologically relevant concentrations of D-glucose (i.e., 5, 10, and 15 mM). In addition, IBDUs were perfused with 30 mM D-glucose to determine a biliary glucose absorption maximum, Bm_G. At 30-min intervals, the collected fluid was removed by a sampling pipette, and D-glucose concentration in timed samples was determined with a biochemistry analyzer (YSI 2700 Select; Yellow Springs Instrument, Yellow Springs, OH). Absorption of D-glucose by microperfused IBDUs was calculated from the concentrations of D-glucose in the perfused and collected solutions, the time of perfusion, and the length of IBDUs and was given in picomoles per minute per millimeter. To study the effects on D-glucose absorption by cholangiocytes of a specific inhibitor of SGLT1, phlorizin, and a sulfydryl reagent, $HgCl_2$, that blocks water channels, IBDUs were perfused with KRB containing 15 mM D-glucose and 0.5 mM phlorizin or with KRB containing 15 mM D-glucose and 0.3 mM $HgCl_2$, respectively.

Measurement of water movement across intrahepatic biliary epithelia. IBDUs were perfused with KRB containing both the impermeable fluorescent volume marker FS (1 mM) and different concentrations (0, 5, 10, 15, and 30 mM) of D-glucose. FS fluorescence was detected from 50-μm-diameter circular spots at the proximal and distal ends of IBDUs by a photosensor module (H5784; Hamamatsu Phototonics, Bridgewater, NJ), as previously described in detail (19). Net water movement (J_v) was calculated from the perfusion rate and the initial (C_O) and collected (C_L) osmolalities of the perfusate: $J_v = V_O/L(C_L/C_O - 1)$, where V_O is the perfusion rate (in nl/min); L is the length of bile duct unit studied (in mm); C_L is the osmolality of collected fluid; and C_O is the osmolality of the perfusate. C_L was determined from the product of C_O and the ratio of fluorescence intensities at the proximal and distal ends of microperfused IBDUs, as described previously (14, 19). The transepithelial osmotic water permeability coefficient, P_f (cm/s), was calculated from the relation $P_f = J_v/V_m \cdot A \cdot \Delta C$, where J_v is net water movement (in cm^3/s); V_m is partial molar volume of water (18 cm^3/mol); A is the surface area of the lumen of IBDUs (in cm^2); and ΔC is the osmotic gradient (in mol/cm^3) (7). J_v, C_O, and C_L were calculated as described above. The osmotic gradient was calculated as $\Delta C = C_L - C_O$. The luminal surface area A was calculated as $A = \pi/DL$, where the inner diameter D and the length L of IBDUs were determined by using a calibrated eyepiece micrometer. To study the effects of phlorizin and $HgCl_2$ on transepithelial water transport, IBDUs were perfused through their lumen with KRB containing 15 mM D-glucose and 0.5 mM phlorizin or with KRB containing 15 mM D-glucose and 0.3 mM $HgCl_2$, respectively.

In vivo experiments. Rats were anesthetized with intraperitoneally injected nembutal (5 mg/100 g body wt); the common bile duct was cannulated above the pancreas with PE 10 intramedic polyethylene tubing (Clay-Adams, Parsippany, NJ), and glucose or phlorizin was infused into the portal vein through a 16-gauge intraveneous catheter (Becton Dickinson Infusion Therapy Systems, Sandy, UT) by using a microliter syringe pump (model 2274; Harvard Apparatus, Holliston, MA) with a Hamilton 5-ml gas-tight syringe. Infusions were maintained in all experiments at a rate of 60 μl/min to produce in portal blood the required final concentration of D-glucose (15 mM) or phlorizin (0.5 mM). The temperature of each rat was maintained at 37°C by Vetko thermal barrier and regulated through a rectal probe by Tele Thermometer YSI (Yellow Springs Instrument). Bile

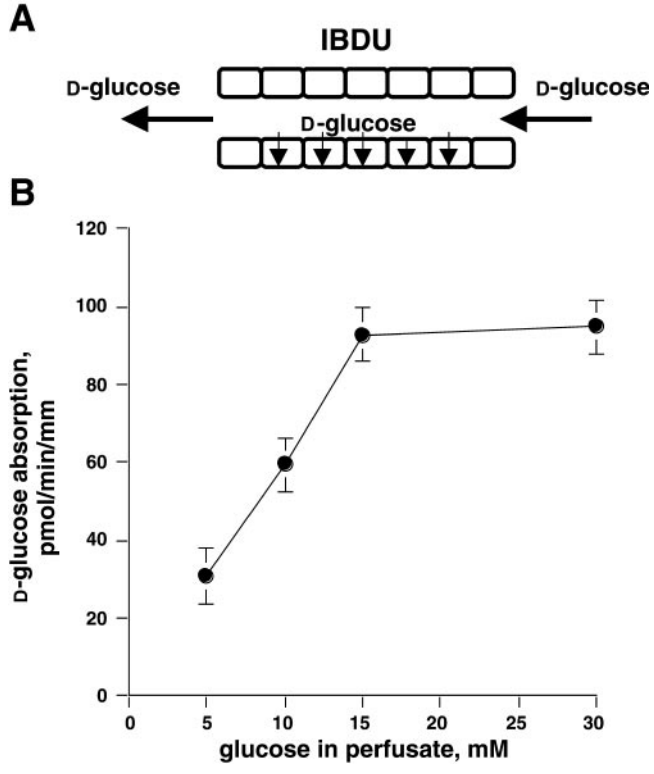

Fig. 1. Absorption of D-glucose by rat microperfused intrahepatic bile duct units (IBDUs). *A*: a model of microperfused rat IBDU to study D-glucose absorption by the biliary epithelium. Individual IBDUs were perfused through their lumen with standard Ringer-HCO_3 buffer (KRB) containing from 5 to 30 mM of D-glucose. Perfusate was then collected, and glucose concentration and glucose absorption were determined. See MATERIALS AND METHODS for details. *B*: microperfused IBDUs absorbed D-glucose in a dose-dependent manner with a biliary maximum for D-glucose absorption (Bm_G) of 92.8 ± 6.2 $pmol \cdot min^{-1} \cdot mm^{-1}$. Values are means ± SE of 4–6 microperfused IBDUs in each group.

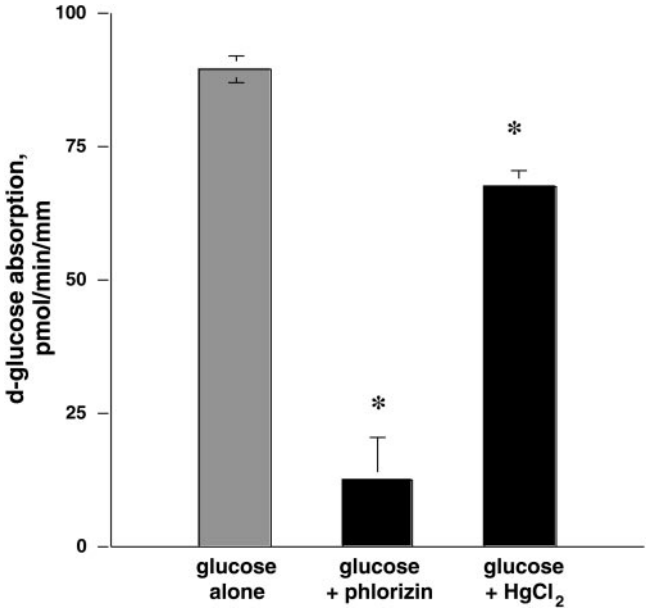

Fig. 2. Microperfused IBDUs absorb D-glucose in a phlorizin-dependent manner. A specific inhibitor of the sodium-dependent glucose transporter SGLT1, phlorizin, perfused through the lumen of IBDUs blocked absorption of D-glucose by cholangiocytes by 85.6%. A sulfydryl reagent, $HgCl_2$, blocked absorption of D-glucose by IBDUs by 24.5%. Values are means ± SE of 5–10 microperfused IBDUs in each group. *$P < 0.0001$ for phlorizin and $HgCl_2$ effect.

was collected every 10 min, and volume was determined gravimetrically by assuming a bile density of 1.0 g/ml. Bile flow was given in microliters per minute per gram of liver. The concentration (in mM) of glucose in plasma and bile and bile osmolality (mosM) were measured as described above. The amount of glucose absorbed (in mM) by intrahepatic bile ducts was estimated from the concentration of glucose in plasma and bile before and after intraportal infusion of D-glucose and phlorizin.

Statistical analysis. All values are expressed as means ± SE. Statistical analysis was performed by the Student's *t* -test, and results were considered statistically different at $P < 0.05$.

RESULTS

Absorption of D-glucose by microperfused IBDUs. Data presented in Fig. 1 show that microperfusion of IBDUs with KRB containing different concentrations of D-glucose results in absorption of D-glucose from the perfusate with a biliary glucose absorption maximum (D-glucose Bm_G) of 92.8 ± 6.2 pmol·min^{-1}·mm^{-1}. The amount of absorbed D-glucose depended on the concentration of D-glucose in the perfusate and reached D-glucose Bm_G when IBDUs were perfused with 15 mM D-glucose.

Identification of mechanisms of D-glucose absorption by microperfused IBDUs. IBDUs were perfused with KRB containing 15 mM D-glucose and phlorizin, a specific inhibitor of the sodium-dependent glucose transporter, SGLT1, expressed on the apical plasma membrane of cholangiocytes (15). Data in Fig. 2 show that phlorizin inhibited absorption of D-glucose in microperfused IBDUs by 85.6%, indicating that intrahepatic biliary epithelia absorb D-glucose mainly via SGLT1. $HgCl_2$ also inhibited absorption of D-glucose in microperfused IBDUs; however, its inhibiting effect was limited because the absorption of D-glucose was inhibited by only 24.5%.

Water transport by microperfused IBDUs. Initially, IBDUs were perfused through their lumen with FS, the impermeable fluorescent volume marker, in glucose-free KRB. In this experiment, FS fluorescence detected at the proximal and distal ends of IBDUs was constant (Fig. 3). In contrast, when IBDUs were per-

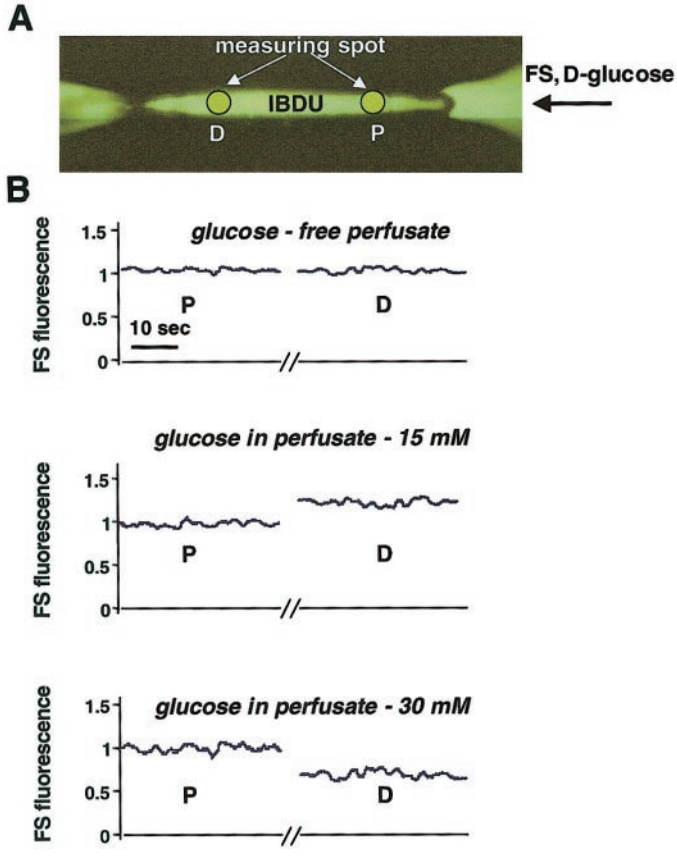

Fig. 3. Water transport by IBDUs microperfused with D-glucose. *A*: model of microperfused rat IBDUs to study water transport by the biliary epithelium. Individual IBDUs were perfused through their lumen with 1 mM of fluorescein sulfonate (FS) as a volume marker in glucose-free KRB or in KRB containing 5, 10, 15, and 30 mM D-glucose at constant (40 nl/min) rate. FS fluorescence which reflects luminal FS concentration, was detected from 50-μm-diameter measuring spots at the proximal (P) and distal (D) ends of IBDU. See MATERIALS AND METHODS for details. *B*: single tracings of luminal FS fluorescence at the proximal and distal ends of IBDU microperfused with glucose-free KRB, 15 mM glucose, and 30 mM D-glucose. The FS fluorescence at the proximal end of IBDU was adjusted to 1 unit in each experiment and served as an initial or control fluorescence. The FS fluorescence measured at the distal end of IBDU (i.e., resulted fluorescence) reflected water movement across bile duct epithelia. The FS fluorescence was constant at the proximal and distal ends when IBDUs were perfused with glucose-free KRB. In contrast, when IBDUs were perfused with 15 mM D-glucose, the FS fluorescence at the distal end of the IBDU increased, reflecting water movement from lumen to bath, i.e., absorption. When IBDUs were perfused with 30 mM D-glucose, the FS fluorescence at the distal end of IBDU decreased, reflecting water movement into the lumen of IBDUs, i.e., secretion.

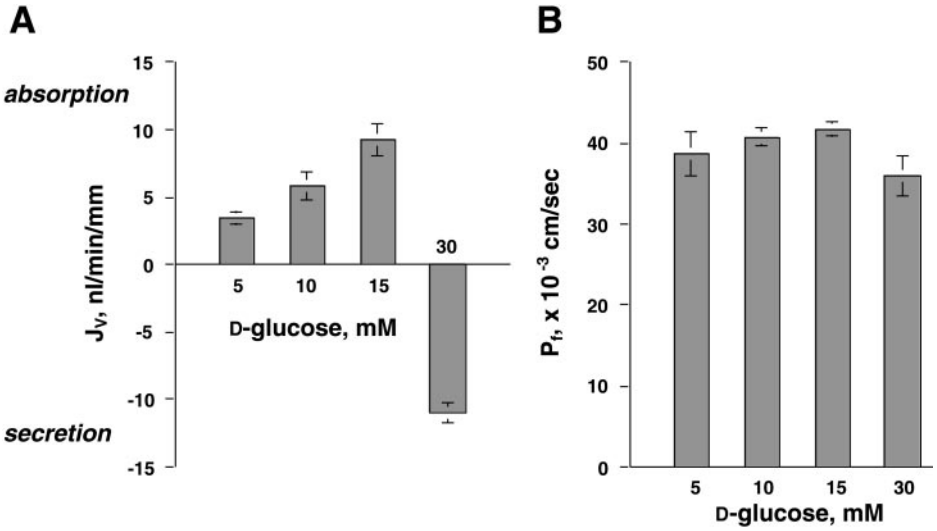

Fig. 4. Water transport by IBDUs microperfused with D-glucose. *A*: net water movement (J_v) in IBDUs microperfused with D-glucose. J_v was determined in experiments when IBDUs lumen was perfused with 5, 10, 15, and 30 mM D-glucose. See MATERIALS AND METHODS for details. During the perfusion with 5, 10, and 15 mM D-glucose, net water absorption occurs (positive value); during the perfusion with 30 mM D-glucose, net water secretion occurs (negative value). *B*: osmotic water permeability (P_f) in IBDUs microperfused with D-glucose. Calculated P_f reflects rapid water transport across the biliary epithelium. P_f does not depend on the concentrations of D-glucose in perfusate and direction of water movement. Values are means ± SE of 4–6 microperfused IBDUs in each group.

fused with 5, 10, and 15 mM D-glucose, the FS fluorescence at the distal end of the IBDU increased, reflecting water movement from lumen to bath, i.e., water absorption (as an example, the original tracing of FS fluorescence in IBDU lumen perfused with 15 mM D-glucose is shown in Fig. 3). However, increasing luminal glucose concentration above the absorptive capacity of SGLT1 (i.e., 30 mM) resulted in a decrease of luminal FS fluorescence, reflecting water movement from bath to lumen, presumably by increasing the osmotic gradient favoring secretion (Fig. 3). The calculated J_v values suggest (Fig. 4) that water is absorbed by IBDUs perfused with 5 to 15 mM of D-glucose with rates from 3 to 10 $nl \cdot min^{-1} \cdot mm^{-1}$. Calculated P_f ($\approx 40 \times 10^{-3}$ cm/s) values showed (Fig. 4) that water is absorbed by the biliary epithelia rapidly, suggesting that specific aquaporins (AQPs) may be involved in this process. The absolute values of physical characteristics (i.e., J_v and P_f) for water secretion in microperfused IBDUs were similar to those observed for water absorption (Fig. 4).

Identification of mechanisms of water transport by microperfused IBDUs. IBDUs were perfused with KRB containing 15 mM D-glucose and a specific inhibitor of SGLT1, phlorizin, or with KRB containing 15 mM D-glucose and an inhibitor of AQP, $HgCl_2$. Data in Fig. 5 show that both phlorizin and $HgCl_2$ decrease water absorption by IBDUs microperfused with 15 mM D-glucose by 75.5 and 68.9%, respectively. These data, in conjunction with data demonstrating absorption of D-glucose by IBDUs in the presence and absence of phlorizin and $HgCl_2$ (Fig. 2), suggest that water follows actively absorbed D-glucose passively mainly transcellularly with mechanisms involving mercury-sensitive AQPs expressed on the cholangiocyte apical plasma membrane.

Biliary water absorption as a function of D-glucose absorption in microperfused IBDUs. Data presented in the Fig. 6 show that absorption of D-glucose by microperfused IBDUs is associated with a proportionate increase in water absorption. These data, taken together with data demonstrating inhibition of D-glucose and water absorption by phlorizin and $HgCl_2$ (Figs. 2 and 5), suggest that D-glucose absorbed by cholangiocytes drives water absorption by biliary epithelia.

Bile flow as a function of glucose absorption by intrahepatic bile ducts in vivo. D-Glucose and a specific inhibitor of SGLT1, phlorizin, were infused into the portal vein to examine the effects of biliary glucose on bile secretion in vivo. Assuming that glucose enters canalicular bile in concentrations equal to blood concentrations, we estimated the amount of D-glucose absorbed by intrahepatic bile ducts after intraportal in-

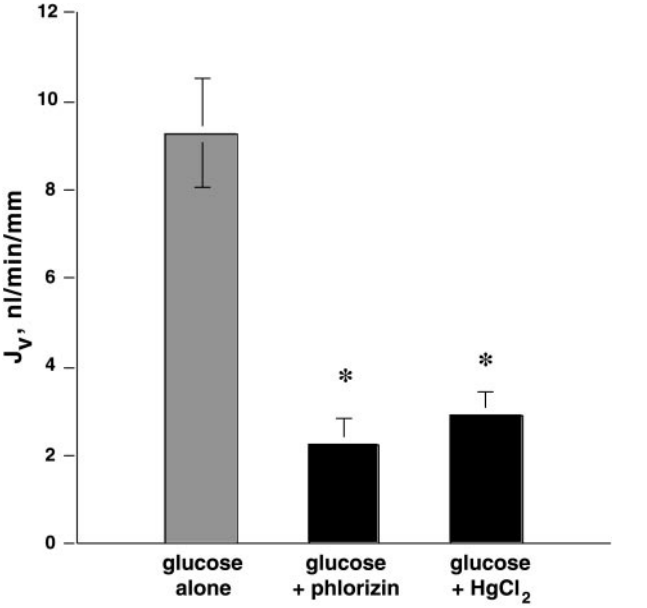

Fig. 5. Microperfused IBDUs absorb water in a phlorizin- and $HgCl_2$-dependent manner. Water absorption in microperfused rat IBDUs in response to absorbed D-glucose was inhibited by 75.5% by a specific inhibitor of SGLT1, phlorizin, and by 68.9% by an inhibitor of aquaporins (AQPs), $HgCl_2$. Values are means ± SE of 4–8 microperfused IBDUs in each group. *$P < 0.001$ for phlorizin and $HgCl_2$ effect.

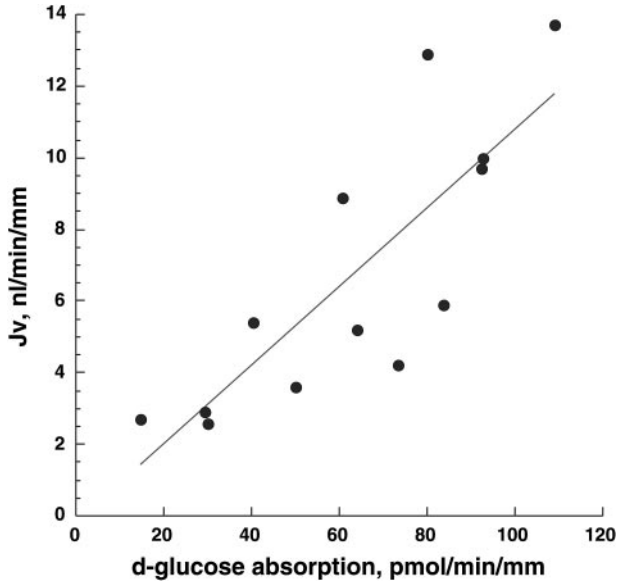

Fig. 6. Biliary water absorption as a function of D-glucose absorption in microperfused IBDUs. Each point represents a pair of IBDUs perfused at constant perfusion rate (40 nl/min), with KRB containing the same concentration of D-glucose. In one IBDU, absorption of D-glucose in pmol·min^{-1}·mm^{-1} was measured, while in another IBDU absorption of water in nl^{-1}·min·mm^{-1} was measured as described in MATERIALS AND METHODS ($y = -0.18969 + 0.11x$; $R = 0.81708$; $P < 0.001$).

fusion of D-glucose and phlorizin and analyzed these data in conjunction with bile flow (Table 1). These data, taken together, show that as the amount of absorbed biliary glucose increased 2.6-fold after intraportal infusion of 15 mM D-glucose, bile flow decreased 9.3%. In contrast, as the absorption of biliary glucose decreased by 3.6-fold after phlorizin, bile flow increased by 11.7%. Bile osmolality did not change after infusion of D-glucose but increased after phlorizin (Table 1).

DISCUSSION

The primary objective of this study was to define the physiological significance of the absorption of glucose from bile by intrahepatic bile ducts. Whereas a considerable body of evidence indicates that glucose stimulates absorption of water in a variety of transporting epithelia (2, 4, 10, 13, 23, 32), direct evidence for a specific effect of glucose on water transport in intrahepatic biliary epithelia is lacking.

We used both a novel experimental model, the microperfused rat IBDU, which we recently developed (19), and a conventional in vivo model to address this issue. The microperfused IBDU allowed us to directly quantitate both glucose and water transport by the biliary epithelia by perfusing the intrahepatic bile ducts with physiological concentrations of D-glucose at rates that correspond to basal intrahepatic bile flow in vivo (20). The basal concentration of glucose in rat blood is 5–6 mM and might be increased up to 20 mM under physiological and pathological conditions (2, 11, 12). In contrast, concentrations of biliary glucose never exceed 1–2 mM even in rats with experimental diabetes (11, 12). These observations suggest that glucose which enters canalicular bile at concentrations 5–20 mM is effectively absorbed by cholangiocytes under normal and pathological conditions. Using a microperfused IBDU model, we demonstrated that, under physiological conditions, biliary epithelia absorb D-glucose with a Bm_G of 92.8 ± 6.2 pmol·min^{-1}·mm^{-1}, a number comparable to the glucose absorption maximum measured in the microperfused proximal convoluted tubule (Tm_G) of the rat (51.8 ± 2.0 pmol·min^{-1}·mm^{-1}) and rabbit (78.5 ± 5.1 and 83.2 ± 5.1 pmol·min^{-1}·mm^{-1}) kidney (3, 8, 29).

Table 1. *Effects of absorbed biliary glucose on bile flow and bile osmolality*

	Absorbed D-Glucose, mM	Bile Flow, μl·min^{-1}·g liver^{-1}	Bile Osmolality, mosM
Controls	5.2 ± 0.4	2.05 ± 0.04	229 ± 1
D-Glucose (15 mM)	13.6 ± 0.5*	1.86 ± 0.04*	230 ± 2
Phlorizin (0.3 mM)	1.4 ± 0.6*	2.29 ± 0.03*	235 ± 1*

Data represent means ± SE of 27 separate measurements of collected samples of bile from 3 separate experiments. *$P < 0.001$ for glucose and phlorizin effects vs. control.

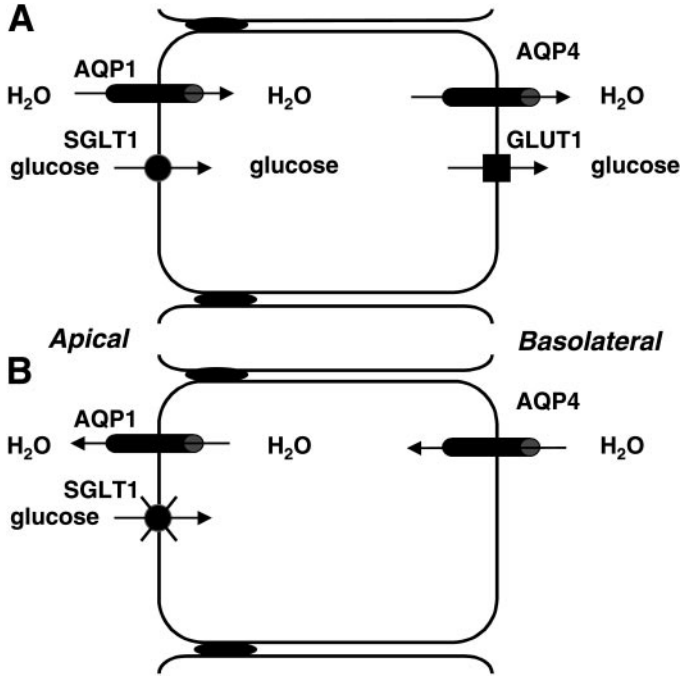

Fig. 7. Working model of glucose and water transport by intrahepatic bile ducts. *A*: glucose is actively absorbed from bile by cholangiocytes via a sodium-dependent glucose transporter, SGLT1, expressed on the apical plasma membrane domain. Absorbed glucose generates osmotic gradients between the bile duct lumen and intracellular milieu, which drives passive entry of water into the cells via a water channel (AQP1) expressed on the apical plasma membrane of cholangiocytes. Nonmetabolized glucose is released from the cholangiocytes at the basolateral domain through a facilitative glucose transporter, GLUT1. Water leaves cholangiocytes via water channel AQP4 expressed on the basolateral plasma membrane domain. *B*: when SGLT1 is saturated or inhibited, glucose accumulates in the lumen of intrahepatic bile ducts, generating local osmotic gradients that drive AQP-mediated water secretion.

The absorption of D-glucose by microperfused IBDUs was inhibited by phlorizin, suggesting that cholangiocytes absorb D-glucose mainly via SGLT1. This finding is consistent with our previous observation that isolated cholangiocytes absorb glucose mainly by this transporter (15) and with the observation that phlorizin also effectively inhibits glucose absorption by microperfused proximal convoluted kidney tubules (32). Absorption of D-glucose by microperfused IBDUs was also somewhat inhibited by $HgCl_2$, possibly due to binding of $HgCl_2$ to cysteine residues on SGLT1. However, that inhibition was not specific and was more limited than phlorizin inhibition of SGLT1.

A specific role for D-glucose on water transport by intrahepatic bile ducts is supported by our studies. The close correlation between glucose and water absorption by microperfused IBDUs and our finding that biliary absorption of water is inhibited by phlorizin suggest that absorbed D-glucose drives water transport in cholangiocytes.

Thus our findings here, taken together with previous observations regarding the expression of SGLT1 and AQP1 in both the proximal tubule of the kidney (24, 33) and in cholangiocytes (15, 27), suggest that analogous mechanisms of glucose-driven water absorption are operative in both the kidney and liver. The high value of P_f we observed, as well as the inhibition of biliary water absorption by $HgCl_2$, provides further evidence that glucose-driven movement of water in cholangiocytes is AQP mediated. This conclusion is consistent with our previous observations that cholangiocytes express a number of AQPs, which are presumably responsible for the large amount of water transported by the biliary epithelia (6, 21), and with recent studies demonstrating that the osmotic water permeability of perfused proximal kidney tubules, which actively absorb D-glucose by SGLT1, was reduced in AQP1-knockout mice by 80% (28). Indeed, net fluid absorption by this segment of the nephron determined in both perfused tubules and in vivo micropuncture studies was reduced in AQP1-knockout mice by 50% (30). The importance of AQP1 in water movement across cell membranes in response to transported glucose was also demonstrated in experiments employing expression of AQP1 and SGLT1 in *Xenopus* oocytes (31). The results suggested that, although SGLT1 has several different roles in water transport (9, 17, 22), it is principally involved in generating an osmotic driving force for aquaporins; this observation is in complete agreement with our findings and interpretations.

Our complementary in vivo studies showed that perturbations affecting the absorption of glucose from bile by cholangiocytes also affect ductal bile secretion. Infusion of D-glucose into the portal vein resulted in an increase of D-glucose absorption from bile by intrahepatic bile ducts and a decrease in bile flow. When absorption of glucose from bile was inhibited by phlorizin, the concentrations of biliary glucose increased, as did bile secretion. These results are consistent with previous observations that bile flow diminished progressively in rats as the concentration of glucose in plasma increased after infusion of D-glucose into the femoral vein (12) and that the rate of bile secretion decreased by about one-fifth when isolated rat livers were perfused with solutions containing 15 mM D-glucose (16). Our data are also consistent with the previous observation that phlorizin caused a marked increase in bile glucose concentration when infused into the portal vein, although plasma levels did not change significantly (12). However, contrary to findings in earlier studies that phlorizin had no effect on bile flow in rats (12), we found that bile flow increased in phlorizin-treated rats by 11.7%. The changes in total bile flow after phlorizin were moderate but quantitatively important because, in the rat, intrahepatic bile ducts contribute a relatively small amount to total bile secretion. Studies employing the segmented retrograde intrabiliary injection technique in rats also demonstrated that phlorizin inhibited absorption of biliary glucose and moderately increased bile flow (25). Thus the changes in total bile flow that we observed in our in vivo experiments may reflect a physiological response of intrahepatic bile ducts to changes in biliary glucose.

Our results and our interpretation of them may have pathophysiological relevance. Decreased bile flow in diabetic rats (5, 11, 18), in rats maintained on parenteral glucose (26), and in patients receiving total parenteral alimentation (1) has been reported; these data suggest that absorption of large amounts of D-glucose from bile by cholangiocytes may lead to increased water absorption and thus contribute to the cholestasis seen in these conditions (1, 11, 26).

In summary, we have directly demonstrated that D-glucose absorbed from the lumen of intrahepatic bile ducts drives water absorption by biliary epithelia and have proposed a model describing these observations (Fig. 7). From both in vitro microperfusion studies and in vivo studies, we conclude that glucose transport by cholangiocytes is intimately involved with cholangiocyte water movement and, hence, ductal bile formation.

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