Glucose Reabsorption from Bile

EVIDENCE FOR A BILIOHEPATIC CIRCULATION

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ABSTRACT Glucose is absent from human bile and present in low concentrations in bile from the rat. To study the mechanisms of this blood-bile glucose concentration difference, infusions of glucose were administered i.v. to 300–400 g male Sprague-Dawley rats with ligated renal pedicles and to two postcholecystectomy patients with indwelling t-tubes. Glucose was assayed in plasma, bile, and rat liver by a hexokinase method specific for D-glucose. In man, glucose was detected in bile when plasma glucose increased above 350 mg/100 ml. In animals studies, low concentrations of bile glucose were observed at plasma levels between 100 and 300 mg/100 ml. However, when plasma concentrations increased between 400 and 900 mg/100 ml, glucose appeared more rapidly in bile, defining by extrapolation an apparent plasma glucose threshold of 280 mg/100 ml. Intraportal phlorizin, a competitive inhibitor of glucose transport, significantly increased bile glucose concentrations. Plasma-bile concentration differences were also observed in rats after i.v. [3-^{14}C]*O*-methyl glucose (3-*O*-MG) but not after [^{3}H]mannitol. Hepatic glucose levels were never lower than plasma levels and liver-plasma 3-*O*-MG ratios were 0.92±0.22 indicating that entry of glucose and 3-*O*-MG into hepatocyte water was not limiting. Furthermore, when sodium dehydrocholate augmented canalicular secretion, biliary glucose excretion increased proportionally suggesting that glucose entry into bile was not impeded. When estimates of hepatic glucose secretion were compared with biliary glucose excretion, the latter increased progressively when estimated secretion rates exceeded 50 μg/min or when phlorizin was given. Finally, during bile stop-flow experiments, [3-^{14}C]*O*-MG and [^{14}C]glucose were selectively removed from bile compared with [^{3}H]mannitol. The findings suggest that glucose and 3-*O*-MG are reabsorbed from bile after entry at the hepatocyte, accounting for their low bile-plasma ratio. The biliary glucose transport process may be described by Michaelis-Menten kinetics and is analogous to recently defined kinetics for renal tubular reabsorption of glucose. These studies provide evidence that certain products of bile secretion may undergo a "biliohepatic" circulation.

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INTRODUCTION

Hepatic bile is a complex secretion which is iso-osmotic with respect to plasma but contains a diversity of solutes whose biliary concentrations are either greater than (bilirubin, bile salts), similar to (Na^+, K^+, Cl^-, HCO_3^-), or less than (phosphate, lecithin, cholesterol) their respective concentrations in plasma. Glucose is also an example of a solute that is found in bile in concentrations less than that of plasma, although variable bile-plasma ratios have been reported (1–3). Woodyatt (4) measured only negligible reducing sugars in human bile while Segato and Rosa (5) reported biliary concentrations that ranged from 25–180 mg/100 ml. Schein, Zumoff, Kream, Cassouto, and Hellman (6) found no glucose in human bile while detecting substantial amounts in bile from rat. These conflicting findings may be due in part to differences in glucose assay procedures which vary in specificity for reducing substances and sensitivity in the presence of bile, or differences in the range of plasma glucose concentrations at which the bile was assayed. Nevertheless, it is surprising that glucose is not found in bile in concentrations comparable to plasma since the bile-plasma ratios of other solutes of comparable molecular size such as mannitol or erythritol are generally very close to 1.0 (7–11). Either glucose entry

into bile is limited by diffusion or metabolism, or transport sites exist for removal of glucose prior to the site of bile collection.

In the present study we explored the possible mechanisms for low biliary concentrations of glucose in rat and man utilizing an enzymatic method specific for D-glucose.

METHODS

A. Animal preparation

Non-fasted male Sprague-Dawley rats, (350–400 g) were maintained on Purina Lab Chow and water *ad. lib.* On the day of study, they were anesthetized with i.p. nembutal (5 mg/100 g body wt), the common bile duct was cannulated with Clay-Adams PE 10 tubing (Clay-Adams, Inc., Parsippany, N. J.) and infusions of glucose were administered at various concentrations (0–25%) in Ringer's solutions through a no. 27 lymphangiogram needle placed in an exposed femoral vein. Infusions were maintained in all experiments at 0.06 ml/min by a Harvard no. 1100 pump (Harvard Apparatus Co., Inc., Millis, Mass.) while blood samples were obtained from an exposed jugular vein. In experiments where high plasma glucose levels were desired (greater than 250 mg/100 ml), the renal vascular pedicles were also ligated bilaterally. The temperature of each rat was maintained at 37°C by heat lamp and regulated through a rectal probe by a constant temperature monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). Bile was collected at 5–15-min intervals while blood samples were removed midway during each bile collection period. The volume of removed blood approximately equaled the volume of the infusate.

B. Glucose assay procedure

A modification of the method of Sachtor and Wormser-Shavit (12) was used, consisting of a coupled enzymatic assay in which β-D-glucopyronoside is converted to glucose-6-phosphate by Mg^{++}-ATP dependent hexokinase. Glucose-6-PO_4 is then converted to 6-phosphogluconolactone by the enzyme glucose-6-PO_4 dehydrogenase, reducing NADP to NADPH, which is measured spectrophotometrically at 340 nm. The method is specific for β-D-glucopyronoside and is sensitive to 1 mg/100 ml in rat bile and 3 mg/100 ml in human bile.

The assay system consisted of: (*a*) 0.10 ml of a reaction mixture comprised of 48 mg NADP, 2.5 ml of 0.5 M K_2HPO_4 at pH 7.6; 0.6 ml 0.1 M disodium ATP (Sigma Chemical Co., St. Louis, Mo.) at pH 7.0; and 0.6 ml 0.1 M $MgCl_2$, all diluted to a total volume of 10 ml; (*b*) 0.01 ml hexokinase (Sigma Chemical Co.) diluted 1:10 in 400 mg/100 ml solution bovine albumin; (*c*) 0.01 ml glucose-6-PO_4 dehydrogenase (Sigma Chemical Co.) diluted 1:5 in 400 mg/100 ml solutions of bovine albumin; (*d*) a 0.01 ml–0.05 ml sample of plasma, bile, or liver cell supernate; (*e*) and sufficient water to equal a total volume of 0.62 ml in a 1.0 ml cuvette. Plasma or bile samples (usually 0.1 ml) were diluted 1:10–1:20 in water so that the amount of glucose in each cuvette varied between 0.01 and 1.0 μmol. Initial readings of optical density were made in a Zeiss PMQ II spectrophotometer (Carl Zeiss, Inc., New York) at 340 nm and 25°C, before adding hexokinase. A reagent blank, in which water was substituted for sample, was also obtained. Hexokinase was then added and the resulting production of NADPH was measured by subtracting the final from initial optical density (OD) after correcting for absorbance in the blank (usually 0.003 OD). Since the molar extinction coefficient for NADPH is 6.22×10^6 cm^2 the OD ÷ 10 gave the total micromoles glucose in each sample. Dividing this value by the sample volume gave the glucose concentrations which was expressed in milligrams per 100 milliliter. Bile volume was measured in a tuberculin syringe. The bile and plasma samples were usually assayed directly but could be frozen for 5–10 days without loss of activity.

Assay of glucose standards gave the expected values within 5% on repeated determinations whether added to bile or to water. The addition of decholin, xylose, and α-methyl glucose had no effect on the assay. Phlorizin increased the initial OD but did not alter the recovery of glucose standards. As a further control, 0.024 μmol of glucose standard was added to each cuvette after the final OD had been determined and the increase in absorbance measured to assure that inhibitors were not present and that the reactants were in excess.

Plasma glucose values increased or decreased slowly in a linear fashion during experimental periods, changing by less than 15% and the mean plasma value for a given bile collection period was extrapolated from a linear plot of plasma concentrations.

C. Animal experiments

Plasma-bile glucose titration curve. Bile-plasma glucose ratios were measured over a 30 min control period and during a second 30 min period after infusions of various concentrations of D-glucose (0–25% solution) in 24 experiments.

Liver glucose determination. Simultaneous samples of liver, blood, and bile were obtained for glucose determinations in eight experiments. Two 1–2-g sections of liver were removed; one was immediately immersed in liquid nitrogen, the other weighed, dried overnight in a 100°C vacuum oven, and re-weighed to determine the percent wet weight. The frozen sample was weighed quickly and immersed in a known volume of distilled water in a pre-weighed vessel which had been placed in a boiling water bath. After 5 min the vessel was removed, allowed to cool, reweighed, and water was added to replace the amount lost during heating. The contents were then homogenized and centrifuged at 17,000 rpm for 30 min in a Sorvall (Ivan Sorvall, Inc., Newtown, Conn.) refrigerated centrifuge. The supernate was removed and assayed for glucose exactly as outlined in section B. The same standards and controls were performed and no interference with the assay occurred in the presence of the liver cell supernate. Centrifugation of liver homogenates at 100,000 *g* in a Beckman (Beckman Instruments, Inc., Fullerton, Calif.) L-2 ultracentrifuge produced no change in glucose concentration.

In calculating concentrations of glucose in liver cell water, a correction was made for extracellular water according to Dewey (13) who showed that 9% of the wet weight of rat liver is extracellular volume. The concentration of glucose (milligram per 100 milliliter) in intracellular water was therefore obtained by the following formula:

$$G_L = \frac{(G_H \times \mathrm{Vol}_H) - (W_T \times 0.09 \times G_P)}{(W_T \times 0.91) - W_D} \times 100,$$

where Vol_H is volume of homogenate; G_L, concentration of glucose in intracellular water in liver in milligrams per

100 ml; G_H, G_P, concentration of glucose in homogenate and plasma, respectively, in milligrams per milliliter; W_T, wet weight liver sample in grams; W_D, weight of dried sample in grams.

Sodium dehydrocholate experiments. In four other studies, 25 mg of sodium dehydrocholate (Mann Laboratories, New York) dissolved in 1.0 ml saline was given i.v. after control bile and plasma samples for glucose determinations were collected. Three 10 min post-decholin samples of bile and appropriate plasma samples were then obtained.

Competative inhibitors. 30 mg of phlorizin (KEK Inc., New York) was dissolved in 0.1 ml propylene glycol and slowly injected into the portal vein through a no. 27 lymphangiogram tubing that was shortened to remove dead space. 30 s of gentle pressure stopped the bleeding which occurred on withdrawal of the needle. Three 10 min collections of bile and appropriate blood samples were then obtained.

In three additional studies Ringer's solution was infused through a no. 27 lymphangiogram positioned in the portal vein. The rate was kept constant at 0.06 ml/min by a second Harvard pump. After 15–30 min, the control infusion was switched to test solutions of 33% α-methyl glucopyronoside (J. T. Baker Chemical Co., Phillipsburg, N. J.), 33% xylose (Ruger Chemical Co., New York) dissolved in Ringer's solution and injected intraportally. Three subsequent 10 min bile collections and plasma samples were then obtained.

Stop-flow experiments. Approximately 10 μCi of [3-^{14}C]-*O*-methyl glucose (New England Nuclear, Boston, Mass.) in 5 mg of cold solute and 5 μCi of [^{3}H]mannitol (New England Nuclear) in 5 mg of cold solute were mixed in a total volume of 0.7 ml Ringer's and given i.v. to six anesthetized rats with ligated renal pedicles. Bile and plasma samples were collected at 5-min intervals for the first 30 min. Bile flow was then abruptly terminated by raising the PE 10 cannula vertically for a "stop-flow" period of 5 min. Bile flow was then allowed to resume by lowering the cannula to its former level. Spontaneous flow resumed immediately and 20 subsequent drops were collected in separate vials of dioxane. The ^{3}H/^{14}C ratios in bile were subsequently determined by counting in a Nuclear-Chicago Isocap 300 liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) with quenching determined by external standard ratios. Plasma samples were obtained at the end of the stop-flow period and at the termination of the study when samples of liver were also removed for determination of liver-plasma ratios of both [^{3}H]mannitol and [3-^{14}C]-*O*-methyl glucose. Liver cell supernate was obtained as described above. Aliquots of plasma and liver cell supernate (20–50 μl) were also counted in dioxane.

In 15 additional stop-flow experiments, either 3 μCi [^{14}C]-glucose (New England Nuclear) or [3-^{14}C]*O*-methyl glucose (New England Nuclear) were mixed together with carrier and 3 μCi of [^{3}H]mannitol and carrier. 50 μl were slowly injected over several minutes into the biliary tree through the biliary cannula in a retrograde fashion, thus temporarily reversing the flow of bile. The flow of bile was then obstructed for 5 min by leaving a syringe attached to the cannula, and a plasma sample was obtained at the end of this period to estimate the amount of activity and the ratio of ^{14}C and ^{3}H in plasma. A volume of bile in excess of the estimated capacity of the distended tree was then collected (approximately 20 drops) by allowing drops of bile to be collected sequentially in separate vials of dioxane (1–2 drops/vial) which were then counted in a liquid scintillation counter for 10–20 min. ^{3}H/^{14}C ratios were calculated for the injected material, plasma, and the drops of bile collected in the immediate postobstructive period.

D. Human experiments

After informed consent, and several weeks after cholecystectomy, two fasting patients with t-tubes in place were given an intravenous bolus of 50 g glucose twice, after control blood and bile sampling. Sequential 10 min collections of bile and plasma were then obtained for 90 min. Bile samples were collected on ice and centrifuged at 17,000 rpm for 30 min at 10°C, and the supernate was assayed for glucose. High blank values from human bile specimens prevented accurate measurement of samples containing glucose concentrations below 3 mg/100 ml. However, glucose standards could be measured in human bile as accurately as observed in rat bile.

Bile-plasma ratios of glucose in humans studies were adjusted by correcting the bile glucose curve for the time elapsed during transit in the biliary system prior to the collection of bile. This dead space time was estimated in each patient by the method of Barber-Riley (14) using [^{14}C]mannitol. 50 μCi in 250 mg cold mannitol were administered i.v. at the beginning of the study, and bile was collected sequentially at 2-min intervals. Bile samples (100–200-μl aliquots) were added to 12 ml dioxane. Samples were then counted in a liquid scintillation counter to determine the mean appearance time for [^{14}C]mannitol in human bile as previously described for bromsulphalein (BSP) (14) and the bile glucose curve was adjusted accordingly with reference to the plasma curve.

RESULTS

A. Animal studies

Plasma-bile glucose titration curve. (*a*) Fig. 1 shows the bile glucose concentrations over a range of plasma glucose up to 900 mg/100 ml. At normal plasma levels (80–100 mg/100 ml) and prior to glucose infusion, some glucose was present in the bile but at much lower concentrations than would be observed if bile/plasma glucose ratios were 1.0. As the plasma glucose levels increased to 400 mg/100 ml (group A) the bile glucose concentration increased but only to a small extent. The slope of the regression line for these points (line A) was 0.22 by the method of least squares. However, as the plasma glucose rose from 400 to 900 mg/100 ml (group B) the bile glucose concentration increased at a rate three times greater than in group A, and the slope of the regression line was 0.68 (line B). If line B is extrapolated, the apparent threshold for this increase is 280 mg/100 ml plasma glucose. Statistical comparison of the slopes of lines A and B show them to be significantly different at the $P < 0.01$ level. Bile flow diminished progressively as the concentration of glucose in plasma increased (Fig. 2) presumably because of osmotic anticholeresis shown previously by Chenderovitch, Phocas, and Rautureau (15), or toxic effects.

(*b*) Plasma-bile [3-^{14}C]*O*-methyl glucose and [^{14}C]-mannitol ratios: As previously reported (7, 9, 10),

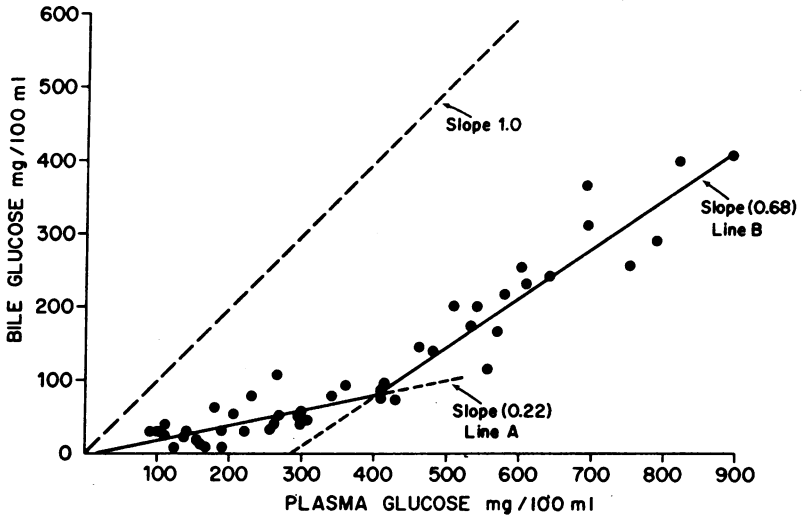

FIGURE 1 Plasma-bile glucose titration curve (milligrams per 100 ml). Line A represents the regression for values of plasma glucose ranging from 0 to 300 mg/100 ml (group A); while line B represents the regression for plasma glucose values between 400 and 900 mg/100 ml (group B). The dotted line with a slope of 1 represents the theoretical regression for solutes such as mannitol or erythritol. The data is obtained from 1 to 4 collection periods in 24 separate experiments.

mannitol achieved bile-plasma ratios of approximately one. Despite similarity in molecular size and liver-plasma ratios that also approximated 1.0, 3-*O*-methyl glucose, behaved like glucose and achieved significantly lower concentrations in bile resulting in bile-plasma ratios that averaged 0.30±0.05 (Table I).

Liver glucose. In five control experiments and three with phlorizin, simultaneous glucose concentrations in plasma, bile, and liver water indicated that glucose concentration in liver water was substantially greater in each instance than the corresponding plasma value (Table II). Since plasma and liver water glucose concentration paralleled each other over a ninefold range in plasma glucose, glucose entry into the hepatocyte was not limiting in these experiments. Furthermore, (Table I), liver-plasma ratios of the glucose analogue, [3-^{14}C]*O*-methyl glucose, were close to one, suggesting that these sugars are not limited in their entry into hepatic water.

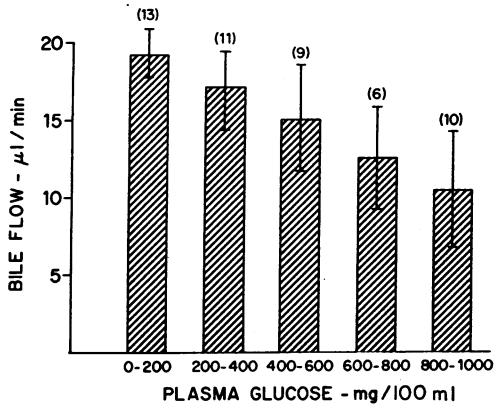

FIGURE 2 Bile flow (expressed as the mean and standard deviation) as a function of increasing concentrations of plasma glucose. The number of determinations within each range of plasma glucose concentration is given in parenthesis.

Competative inhibitors. Phlorizin caused a marked increase in bile glucose concentration when injected slowly into the portal vein, although plasma levels did not change significantly. Fig. 3 shows that the increase in bile glucose varied from 2 to 10 times that of the preinjection level. Bile glucose remained relatively constant during this time if phlorizin was not injected. Contrary

TABLE I

Bile-Plasma (B/P) and Liver-Plasma (L/P) Ratios after i.v. Mannitol and 3-O-Methyl Glucose

i.v.	B/P	L/P
D-[1-^{3}H]mannitol (6)	0.81 ±0.10	1.13 ±0.18
[3-^{14}C]*O*-methyl glucose (6)	0.30 ±0.05	0.92 ±0.22
P	<0.001	NS

The mean ±standard deviation are given. No. of experiments are in parenthesis.

TABLE II

Simultaneous Glucose Concentrations

Studies	Plasma	Liver*	Bile
	mg/100 ml	*mg/100 ml*	*mg/100 ml*
Controls			
1	191	295	38
2	335	498	65
3	790	1,151	290
4	1,043	2,059	455
5	1,152	1,799	471
Phlorizin			
6	235	416	(55)‡ 116
7	251	594	(20) 103
8	283	538	(9) 68

Glucose concentration in milligrams per 100 ml in plasma, liver, and bile in five control rats (1–5) and three treated with phlorizin (6–8).

* Milligrams glucose per 100 ml intracellular water.

‡ Pre-phlorizin value given in parenthesis.

to findings in dogs, phlorizin had no effect on bile flow in these studies.

The portal vein infusion of α-MG resulted in a two- to fourfold increase in bile glucose concentrations in two experiments with similar but not as large effects as phlorizin. In contrast, when xylose was infused in one study no change in bile glucose concentrations occurred. Portal vein and jugular vein glucose concentrations were essentially equal when sampled simultaneously during the infusion.

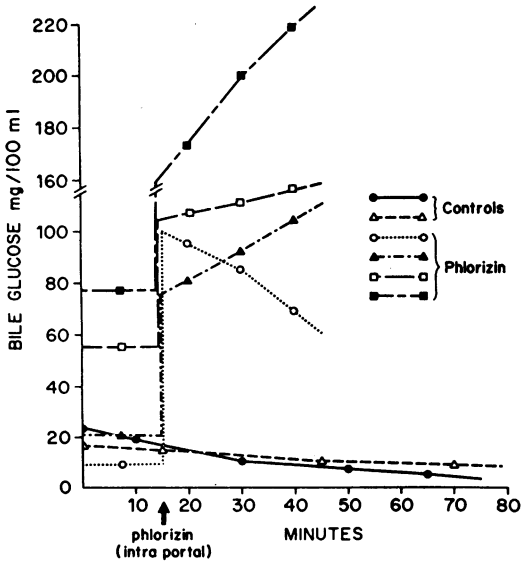

FIGURE 3 Phlorizin's effect on bile glucose concentrations after intraportal injections in four experiments compared with controls.

Biliary glucose excretion as a function of the estimated secreted glucose load. Assuming that glucose concentrations in canalicular bile initially equaled plasma concentrations, estimates of the secreted load of glucose (plasma glucose concentration × bile volume) are shown in Fig. 4. Below glucose levels of 50 μg/min, there was little increase in the biliary excretion of glucose as the glucose load increased. Indeed the correlation coefficient was only 0.39 indicating no significant relationship. However, above a glucose load of 50 μg/min, there was a progressive and highly significant linear increase ($r = 0.93$) in biliary glucose excretion as the rate of hepatic glucose excretion increased. During phlorizin administration biliary glucose excretion increased above control values.

Sodium dehydrocholate. Following i.v. sodium dehydrocholate, bile flow increased in each experiment. However, bile-plasma glucose concentration ratios did not change from the expected values in Fig. 1. When estimates of hepatic glucose excretion were correlated with biliary glucose excretion over a wide range in plasma glucose concentration, biliary glucose excretion increased in a linear fashion identical to the control observations obtained at lower flow rates. (Fig. 5).

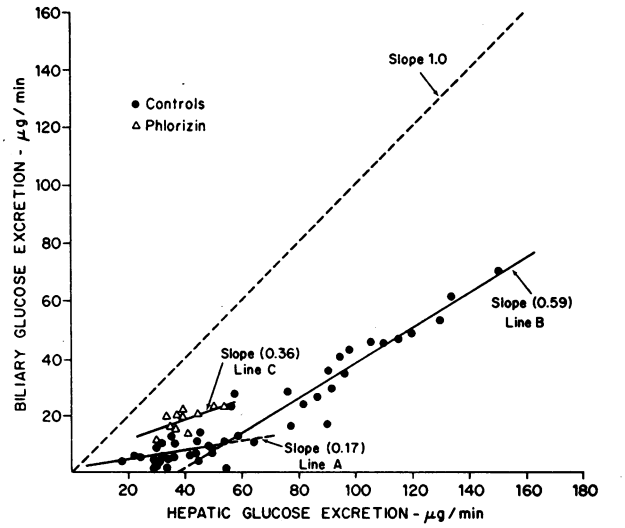

FIGURE 4 Biliary glucose excretion ($B_G \times$ bile volume) as a function of hepatic glucose excretion (the estimated secreted glucose load) ($P_G \times$ bile volume) where B_G and P_G = bile and plasma glucose concentrations, respectively. The solid dots are determinations obtained from 24 control experiments during glucose infusions where plasma glucose ranged from 100 to 1,150 mg/100 ml. The open triangles represent four studies during intraportal phlorizin administration. Line A ($y = 0.92 + 0.17\ x$, $r = 0.39$, $p < 0.1$), line B ($y = -20.4 + 0.59\ x$, $r = 0.93$, $P < 0.001$), and line C ($y = 4.36 + 0.36\ x$, $r = 0.63$, $P < 0.01$) were obtained by the method of least squares. The slopes of the lines are given in parenthesis.

Stop-flow experiments. After intravenous administration of [^{3}H]mannitol and [3-^{14}C]*O*-methyl glucose ([3-^{14}C]*O*-MG),[1] both substances entered hepatic cell water in ratios that roughly equaled plasma (Table I).

Significantly higher concentration of [^{3}H]mannitol were observed in bile compared with [3-^{14}C]*O*-MG. Furthermore, bile ^{3}H/^{14}C ratios increased further in all experiments in drops of bile collected immediately following a 5 min period of stop-flow, and returned to control ratios when the biliary dead space volume (approximately 0.012 ml/g of bile obstructed liver according to Barber-Riley) (14) was voided (Table III). A larger increase in ^{3}H/^{14}C ratios was observed in other experiments when mixtures of either [^{14}C]glucose of [3-^{14}C]*O*-methyl glucose and [^{3}H]mannitol were injected slowly in a retrograde fashion into the biliary tree through the PE 10 cannula and bile flow was temporarily stopped for 5 min (Fig. 6).

B. Human experiments

Patient no. 1 was studied 6 wk after cholecystectomy and common duct exploration at a time when routine liver function tests were normal. There was no history of diabetes and an i.v. glucose tolerance test was normal. During the control period bile glucose could hardly be detected at plasma glucose levels of 90 mg/100 ml. Following two i.v. injections of 50 g of glucose, bile levels of glucose were detected at low concentrations that paralleled changes in plasma values (Fig. 7). The apparent threshold for glucose appearance in bile was above 350 mg/100 ml plasma glucose. Bile flow did not change significantly during the period of hyperglycemia. Patient no. 2 was studied 2 wk after common duct exploration and gave entirely similar results.

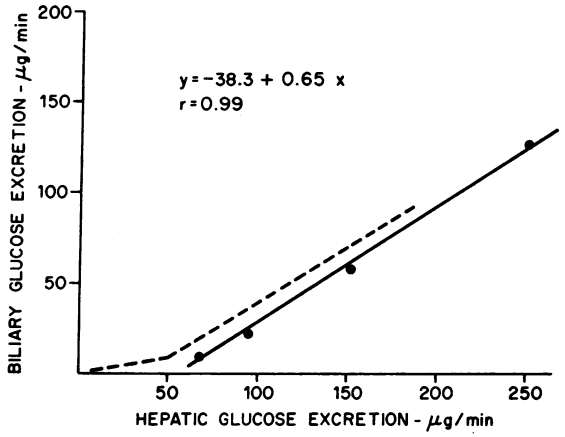

FIGURE 5 The effect of dehydrocholate on biliary glucose excretion in four studies obtained at plasma glucose concentrations ranging from 300 to 1,250 mg/100 ml. The regression line ($y = -38.3 + 0.65\ x$) had a correlation coefficient of 0.99, $P < 0.001$, and was nearly identical to line B in Fig. 4, represented by the dotted line. Post-dehydrocholate choleresis increased flows from 16 to 31, 17 to 22, 12 to 26, and 8 to 20 μl/min.

[1] *Abbreviations used in this paper:* B_G, bile glucose concentration; BGR, bile glucose reabsorption; HGE, hepatic glucose excretion; MG, methyl glucose; P_G, plasma glucose concentration.

TABLE III
*^{3}H/^{14}C Ratios in Bile (6)**

Pre	Stop-flow	Post
1.0*	1.18 ±0.06	0.95 ±0.06
	$P < 0.001$‡	

* All pre-stop flow ratios normalized to 1.0. The mean ±standard deviation is given.
‡ Stop-flow values were significantly different from both pre- and post-obstruction values.

DISCUSSION

These studies demonstrate that at normal plasma glucose concentrations, glucose is virtually absent from human bile but is present in low concentrations in bile from the rat. Following intravenous glucose infusion, small amounts of biliary glucose can be detected in man, whereas large increases in concentration occur in the rat. How glucose enters the bile is not known, however, the mechanism of entry of other small lipid insoluble molecules such as mannitol or erythritol is thought to be

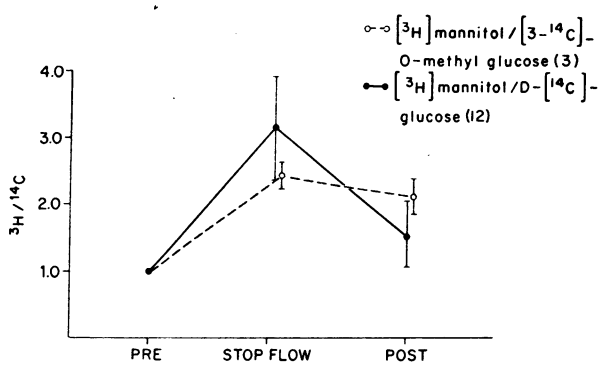

FIGURE 6 [^{3}H]mannitol and [^{14}C]glucose (12 studies) or [3-^{14}C]*O*-methyl glucose (three studies) ratios in bile obtained during a 5 min period of "stop-flow" after retrograde injection. The (pre) values were normalized to 1, and were obtained from bile which remained in the biliary cannula during stop-flow. Values during (stop-flow) were obtained from bile which was calculated to be within the biliary tree (14) while (post) values were obtained after resumption of flow when estimated biliary volume had been collected. The bars represent the standard deviations from the mean.

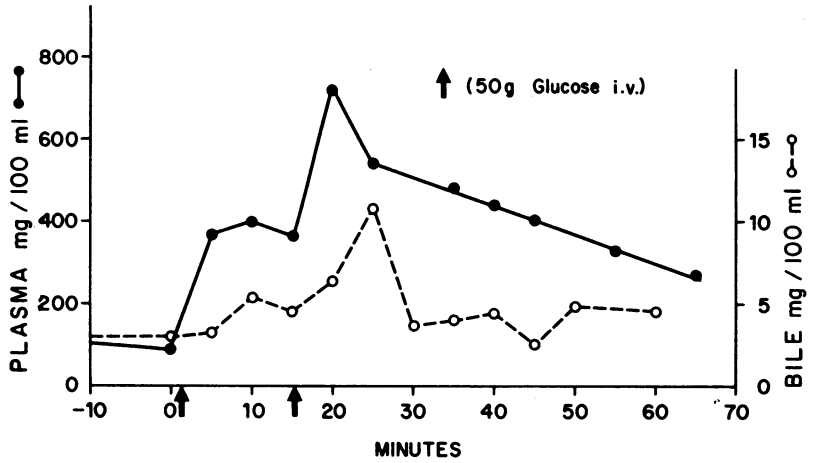

FIGURE 7 Glucose in bile following i.v. glucose infusions (arrows) in man. Note the difference in scale for plasma and bile glucose concentration.

a combination of diffusion and osmotic filtration across the canaliculus (9, 10). Various investigators have shown that bile-plasma [^{14}C]mannitol ratios in the rat are close to one over a wide range in canalicular flow (7, 10) and similar results have been obtained with [^{14}C]erythritol (8). These solutes appear to equilibrate rapidly in hepatocyte water within minutes (16) and enter bile at that level rather than through or between bile ductular cells. Therefore, glucose which is similar in molecular size might also be expected to enter bile in similar concentrations and its markedly diminished concentration in bile is unexplained, although several possibilites could account for this phenomenon.

First, glucose entry into the hepatocyte might be limited. Secondly, glucose entering the liver might be rapidly metabolized, converted to glycogen or otherwise consumed so that a reduced amount is available for diffusion into bile. Third, there could be a selective barrier for glucose entry from the hepatocyte into bile which is less effective at higher glucose concentrations.

Fourth, glucose might enter the canalicular bile in equal concentration to liver cell water and plasma and subsequently be removed from bile by active transport at some site along the biliary system. If glucose load exceeded the capacity for reabsorption, its concentration would increase in collected bile in a manner analogous to the mechanism of renal glycosuria. Finally, water alone might be secreted at the ductular level causing dilution of bile and diminished glucose concentration. This last possibility may be excluded in the rat since ductular secretion is minimal and other nontransportable, nonmetabolized solutes such as mannitol would be expected to be diluted as well, and this does not occur (10).

Considering the possibility of limited hepatic glucose entry or increased hepatic glucose consumption, Cahill, Earle, and Zotta have shown that glucose freely enters the liver and that glucose concentrations in rat hepatic water are roughly comparable to those in the plasma over a range in plasma concentration (17). Our own studies were similar except that hepatic glucose concentrations were somewhat higher than plasma. It is possible that gluconeogenesis or glycogen degradation to glucose might have occurred during the boiling procedure for hepatic glucose extraction. However, our technique did not reveal higher values for hepatic glucose in normal rats when compared with Cahill's method. Our animals were not fasted, however, so that it is possible that some glycogenolysis did occur. Nevertheless, the ratio of liver to plasma glucose remained fairly constant despite a 10-fold change in plasma glucose concentrations so that glucose must be available for entry into the hepatocyte over the range of plasma glucose used in this study. We can assume, therefore, on the basis of Cahill's studies and our own, that glucose should be present in liver cell water in amounts at least equal to, if not greater than that observed in plasma.

If we assume that glucose can enter bile, presumably by diffusion and solvent drag, as is the case for solutes like mannitol (9), then biliary glucose excretion may be represented as a function of the estimated secreted hepatic load. If glucose enters bile in concentrations that are equal to plasma, as shown in Fig. 4, no significant relationship is observed between load and excretion until the glucose load exceeds 50 μg/min. Thereafter, a progressive increase in biliary glucose excretion is observed for every increment in hepatic glucose secretion, a phenomenon which suggests a threshold for biliary glucose reabsorption.

Should glucose enter bile in lower concentrations than estimated, then glucose must be restricted in diffusion from the hepatocyte, unlike mannitol, a carbohydrate of similar size. In this case, dehydrocholate in-

duced canalicular secretion might be expected to lower the bile-plasma glucose concentration ratios. This did not occur. Rather, bile-plasma glucose concentration ratios remained essentially unchanged from the control titration curve observed in Fig. 1, and glucose clearance was increased proportionately to the canalicular choleresis that occurred when dehydrocholate was infused. However, bile flow diminishes as plasma glucose rises and if this hyperglycemic anticholeresis (Fig. 2) is produced by osmotic effects at the canaliculus, then glucose could not be in diffusion equilibrium between liver cell water and bile since the concentration of glucose in hepatocyte must be increasingly larger than bile as bile flow diminishes. Should this occur, our figures for hepatic glucose excretion and thus biliary glucose reabsorption would be progressively overestimated as plasma glucose concentrations increased. It is difficult to reconcile this possibility however with the failure of dehydrocholate choleresis to diminish bile-plasma glucose ratios, as discussed above. Alternatively, hyperosmolar glucose might have a toxic effect on hepatocyte bile secretion as Brauer has suggested to explain why i.v. hyperosmolar sodium chloride diminishes bile flow in the rat (18).

Could glucose entry into bile be larger than estimated? This is possible, particularly since liver cell glucose concentrations appeared to be higher than concentrations in plasma (Table II). Also, canalicular flow might be greater than total flow if water is reabsorbed from bile at the duct level as plasma glucose concentrations increase. Both of these possibilities would result in a larger than estimated secreted load of glucose, thereby lowering the slope of line B in Fig. 4, and indicating that the ductular capacity to reabsorb glucose was actually larger than suggested by the present estimates. It seems unlikely, however, that bile flow was diminished by reabsorption of water at the bile duct level during hyperosmolar anticholeresis (Fig. 2), since Chenderovitch's studies with hyperosmotic mannitol in the rabbit (19) indicate that the effects of osmotic anticholeresis occur proximally at the level of the hepatocyte rather than distally at the ducts. Furthermore, in our own studies in the rat, bile-plasma ratios of [^{14}C]erythritol were increased by only 3±3.7% when total bile flow was reduced by 52±4.6% during hyperglycemia in four experiments suggesting that hyperglycemic anticholeresis appears to predominantly affect canalicular flow in the rat as well.

That the increasing appearance of glucose in bile is not related to the rate of bile flow per se can be deduced from a comparison of line B in Fig. 4 and the post-dehydrocholate data (Fig. 5). Biliary glucose excretion is identical at a given glucose load irrespective of whether the load is produced by high plasma glucose concentrations and low bile flow rates (Fig. 2) or at low plasma glucose concentrations but high post-dehydrocholate flow rates. The determinants of biliary glucose excretion, therefore, seem to be the magnitude of the glucose load irrespective of the rate of canalicular bile secretion.

These considerations leave us with the final possibility; namely, that glucose must be transported out of bile at some point during passage from the hepatocyte to the biliary cannula, a conclusion which can be strengthened by several additional findings.

As already indicated, the pattern of the bile-glucose titration curves (Figs. 1 and 4) suggest a transport system which results in an increase of glucose in bile when the biliary ductules are presented with an increase in substrate load.

The studies with classic inhibitors of glucose transport (Figs. 3 and 4) provide further support for a biliary glucose transport system since phlorizin is known to cause reversible inhibition of active transport of glucose in the intestine and kidney (20). Since phlorizin neither inhibits glucose entry into liver cells (21) nor lowers the hepatic concentration of glucose in the present study, and since phlorizin entered bile (as indicated by a rise in blank values in the glucose assay, and as previously shown by Jenner and Smyth for the dog) (22) it is likely that the rise in biliary glucose excretion was secondary to inhibition of glucose reabsorption from bile. An increase in bile glucose has also been observed in dog bile after phlorizin (23). Another competitive inhibitor of glucose transport in the intestine, α-methyl-*d*-glucopyronoside, also increased the bile glucose concentration without resulting in a parallel rise in plasma glucose concentration while xylose, which has no effect on intestinal glucose transport, did not (24). Since α-methyl-*d*-glucopyronoside freely penetrates liver cells (17) and is not metabolized, it also could have competitively inhibited glucose reabsorption from bile. It was necessary to inject both phlorizin and α-methyl-*d*-glucopyronoside into the portal vein in order to demonstrate increases in bile glucose suggesting that dilution in peripheral blood negated any effect of these compounds on biliary glucose transport.

Finally, results from the stop-flow experiments are also consistent with the glucose reabsorption hypothesis since they demonstrate that both glucose and 3-*O*-methyl glucose are selectively removed from bile during periods of stop flow when compared with mannitol, a sugar of comparable size. 3-*O*-methyl glucose, like glucose, is present in bile in concentrations that are considerably less than mannitol during free flow of bile and diminish further when bile is temporarily stopped. Selective reabsorption of either glucose or 3-*O*-methyl glucose also occurred if these solutes were injected with

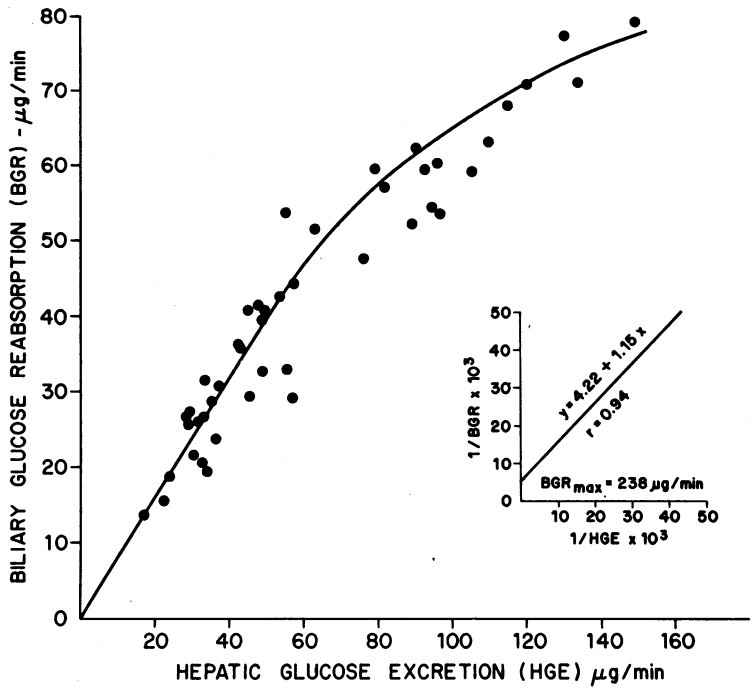

FIGURE 8 Biliary glucose reabsorption (BGR) plotted as a function of hepatic glucose excretion (HGE). The data describes an hyperbola which estimates a V_{max} for BGR of 238 µg/min when analyzed by a double reciprocal plot (see insert). r = correlation coefficient.

mannitol in a retrograde fashion into the biliary tree (Fig. 6).

Because all these experimental approaches suggested the existence of a biliary transport system for both glucose and 3-*O*-methyl glucose, this system should be analagous to tubular transport mechanisms for glucose in the kidney. If this were so, then biliary reabsorption of glucose must be a function of the hepatic excretion of glucose much in the manner that tubular reabsorption of glucose in the kidney is a function of the filtered load at the glomerulus. This relationship could be expressed mathematically again assuming that bile glucose levels were initially comparable to plasma levels prior to reabsorption of glucose from bile. Then: bile glucose reabsorption (BGR) = hepatic glucose excretion (bile flow × plasma glucose concentration) − (bile glucose concentration × bile flow). This function is represented in Fig. 8 and describes an hyperbola suggesting that glucose transport in the rat biliary tree obeys Michaelis-Menten kinetics. Although precise determinations require knowledge of the exact amount of glucose secreted from the hepatocyte into the bile, a double reciprocal plot of the data describes a straight line resulting in an estimated V_{max} for biliary glucose reabsorption of 238 µg/min.

Classic views of renal glucose transport state that when the glucose load increases above the renal tubular threshold, the increased load should be completely excreted in the urine. This did not occur in the present study. However, recent reexamination of this problem in the isolated perfused kidney, in which higher filtered loads were used than in earlier investigations, indicates that a classic transport maximum is actually not achieved, and that the transport titration curve has the characteristics of an hyperbola, exactly as demonstrated for glucose excretion in bile (25). Thus, our findings are entirely consistent with current concepts of tubular glucose transport in the kidney and suggest that analogus mechanisms are operative.

Although the significance of biliary glucose reabsorption remains to be established, the findings in the present study provide evidence that there is a biliohepatic circulation for glucose in the rat which can explain low bile-plasma glucose ratios. While the magnitude of this transport system appears to be small so that conservation of glucose by reabsorption from bile is not of major importance in glucose homeostasis, the evidence for biliary glucose transport suggests that other solutes initially secreted in bile might also undergo a biliohepatic circulation.

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