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Research Article

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Role of the Hepatic Artery in Canalicular Bile Formation by the Perfused Rat Liver

A Multiple Indicator Dilution Study

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Abstract

The role of the hepatic artery in tracer water exchange and regulation of permeation of small solutes during canalicular bile formation was studied in the rat using a system that permitted perfusion of both hepatic artery and portal vein. Hepatic vein and biliary multiple indicator dilution curves were obtained after injection of indicators into either vessel. The main difference in hepatic venous dilution curves was a 3.1-fold longer t_0 (time spent in nonexchanging vessels) and a 5% larger equivalent water space after injection into the hepatic artery. Biliary tracer recovery of water was markedly higher after arterial injection than after portal vein injection. Both taurocholate and taurodehydrocholate stimulated bile flow and increased biliary tracer recovery after injection into either vessel. The biliary recovery of sucrose relative to that of water, which is a measure of biliary sucrose permeation, was much lower when given into the hepatic artery than when given into the portal vein. During taurocholate infusion, it decreased by 33% in the hepatic artery but increased 36% in the portal vein. Taurodehydrocholate, by contrast, did not affect permeation of sucrose given into the portal vein. Our studies demonstrate marked exchange of tracer water in the biliary epithelium. Taurocholate, but not taurodehydrocholate, increases permeation of sucrose into bile in the portal vein bed while both bile salts decrease it in the arterial bed.

Introduction

Canalicular bile formation is a process of osmotic filtration (1). The main determinants of canalicular bile formation are thought to be the active secretion of bile salts, the activity of different ion pumps, and the permeability of the biliary tree (reviewed in reference 2). The perfused rat liver has been a popular tool by which the mechanisms of bile formation have been elucidated (3–7). One of the main disadvantages of this model system is its failure to take into account the influence of the hepatic artery although it is well known that this vessel is permeable to water and many inert solutes used in probing biliary permeability (8). This difficulty has been overcome by the recent development of different systems to perfuse livers through both the hepatic artery and the portal vein (9–10).

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We used our own approach to dual perfusion and a recently described biliary multiple indicator dilution technique (11) to characterize the influence of taurocholate and taurodehydrocholate, a micelle- and a non–micelle-forming bile salt, respectively, on biliary water exchange and permeability to sucrose in the domains of both the hepatic artery and the portal vein.

Methods

Materials. Male Sprague-Dawley rats were obtained from the Süddeutsche Versuchstierfarm, Tuttlingen, FRG, and maintained on a standard rat diet and tap water ad lib. The animals were housed in climatized animal quarters under a 12-h light-dark cycle. At the time of study, body and liver weights averaged (mean±1 SD) 546±117 and 17.1±3.9 g, respectively.

^3H_2O (sp act 1 mCi/ml), [^{14}C]sucrose (sp act 673 mCi/mmol), and [^{14}C]taurocholate (sp act 61 mCi/mmol) were obtained from New England Nuclear, Boston, MA. Bovine erythrocytes, obtained from the local slaughterhouse, were labeled with ^{51}Cr as previously described (5). BSA was obtained from Calbiochem-Behring Diagnostics, American Hoechst Corp., La Jolla, CA, and labeled with ^{99m}Tc as previously described (5). Taurocholate sodium and taurodehydrocholate sodium were obtained from Calbiochem and were > 99% pure as determined by thin-layer chromatography (12).

Procedures. Fed animals were anesthetized with pentobarbital sodium (50 mg/kg i.p.). The common bile duct was cannulated with a 20-cm cannula of PE 10 tubing. A gastrectomy was performed and the hepatic artery was prepared as outlined in Fig. 1. The portal vein was then prepared for cannulation as previously described (5). The animal was heparinized with 5,000 IU i.v. The celiac axis was cannulated using a 23 gauge needle connected to Silastic tubing. After successful cannulation, the liver was flushed with 3 ml of physiologic saline containing 1 IU/ml of heparin to ensure uniform perfusion of the whole liver. Then, the portal vein and thoracic inferior vena cava were cannulated as previously described (5). Perfusion of the artery was started at a low pressure that was increased to 60–100 mmHg over 10–20 min. Portal perfusion was begun immediately after cannulation of the portal vein at a physiologic pressure.

The perfusion system is shown in Fig. 2. Arterial and portal pressure were monitored using a strain gauge (P23b; Statham Instruments Inc., Oxnard, CA) (*6* in Fig. 2) and a hydrostatic manometer (*5* in Fig. 2), respectively. Portal resistance was self-adjusting after setting the pressure using the pressure head (*8* in Fig. 2) previously described (13).

The perfusate consisted of bovine erythrocytes (20% vol/vol), BSA (2% wt/vol), and dextrose (0.1% wt/vol) in KRB. It was oxygenated with oxygen-carbon dioxide 95:5% using the membrane oxygenator (*2* in Fig. 2) described by Hamilton et al. (14); 2-mm-diam silicone tubing with a wall thickness of 0.2 mm was obtained from Detakta AG, Alsterdorfenstr. 266, Hamburg, FRG. Perfusate pH was monitored continuously and adjusted with $NaHCO_3$ as necessary throughout the perfusion. Viability of the preparation was judged based on macroscopic aspect of the liver, portal and arterial resistance, bile flow, transaminase and potassium release into the perfusate, and oxygen consumption as previously described (15). All livers reported here met these viability criteria.

Vascular spaces were determined as described by Goresky (16) and adapted by us for the perfused rat liver (5). Briefly, a mixture of indi-

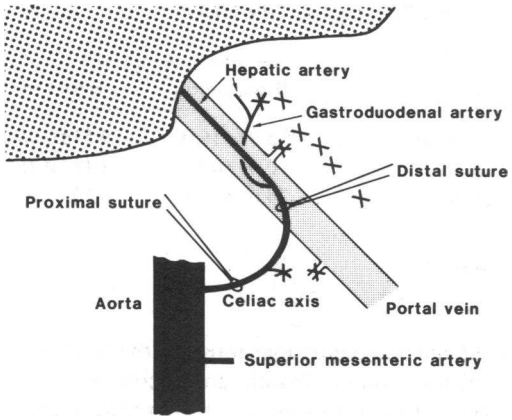

Figure 1. Preparation of the hepatic artery for perfusion. The portal vein overlies the hepatic artery. Since the left hepatic artery derives from the gastroduodenal artery in ~ 30% of animals, the gastroduodenal is ligated distally. To prevent perfusion of intestine, several sutures are posed along the portal vein in the mesentery (*crosses*). Two holding sutures are prepared around the celiac axis which is then cannulated as described in the text.

cators was rapidly injected into the hepatic artery or portal vein, respectively. Immediately thereafter, total hepatic venous outflow was collected at 1- or 2-s intervals for up to 2 min to avoid recirculation of the indicators. Three types of experiments were performed. In the first set (n = 5), ^{51}Cr-erythrocytes (1 μCi) were injected together with [^{14}C]-taurocholate (1 μCi) and $^{3}H_2O$ (50 μCi). These experiments were also used to obtain biliary indicator dilution curves (see below). In the second set of experiments (n = 7), ^{51}Cr-erythrocytes (1 μCi), ^{99m}Tc-albumin (10 μCi) and [^{14}C]sucrose (1 μCi) were coinjected. In these experiments, biliary indicator dilution curves were obtained independently of the blood indicator dilution curves. In the last type of experiments, ^{51}Cr-erythrocytes were coinjected with ^{99m}Tc-albumin and [^{14}C]sucrose into either the portal vein or the hepatic artery while the other vessel was also being perfused (dual perfusion). Then, the perfusion of the other vessel was stopped and the injection was repeated (single perfusion). No biliary indicator dilution curves were obtained in these experiments (n = 5).

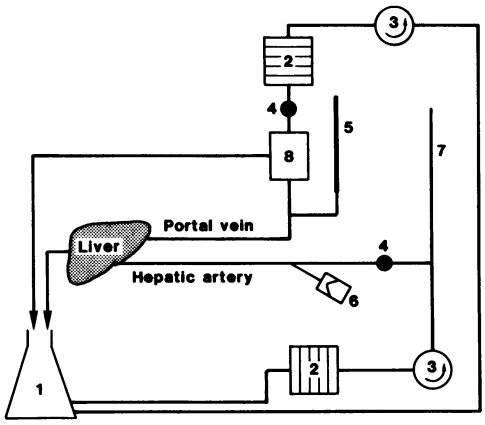

Figure 2. Perfusion system for dual rat liver perfusion. *1* denotes the perfusate reservoir. Each circuit has its own oxygenator (*2*), pump (*3*), and filter (*4*). Pressure is measured by a manometer (*5*) and a strain gauge (Statham) (*6*) in the portal vein and hepatic artery, respectively. Pulsatile flow in the hepatic artery is dampened by a compression chamber (*7*). Pressure in the hepatic artery is regulated by the pump, in the portal vein by a pressure head (*8*).

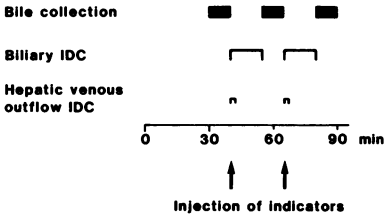

Figure 3. Experimental design for biliary indicator dilution curves (*IDC*) in the absence of exogenous bile salts. The indicator mixture contained ^{51}Cr-labeled erythrocytes, [^{14}C]-taurocholate and $^{3}H_2O$. Biliary IDCs were obtained by obtaining 50 samples of bile at 20-s intervals. The hepatic venous IDC consisted of 30 samples at 2-s intervals.

Before and immediately after obtaining biliary indicator dilution curves, bile flow was determined by collecting bile for 10 min in tared tubes. Bile was collected under water as previously described and validated for biliary indicator dilution curves (11). Two types of biliary indicator dilution curves were obtained. In the first set of experiments, (see Fig. 3) bile flow was determined only after depletion of the bile salt pool; thus, mainly the so-called bile salt-independent fraction (BSIF)[1] was measured. In these experiments, 50 bile samples at 20-s intervals were collected after coinjection of [^{14}C]taurocholate and $^{3}H_2O$ as described above.

In the second set of experiments (see Fig. 4) bile curves were obtained under two experimental conditions, namely after depletion of the bile salt pool and 30 min after beginning an infusion of either 2 μmol/(min · kg) taurocholate sodium, which corresponded to ~ 50 nmol/(min · g liver) (n = 7), or an equimolar amount of taurodehydrocholate sodium (n = 6). Under each of these experimental conditions, termed BSIF and BSDF (for bile salt-dependent fraction), respectively, a set of biliary dilution curves was obtained after coinjection of [^{14}C]sucrose (50 μCi) and $^{3}H_2O$ (25 μCi) into the hepatic artery or the portal vein. Again, 50 samples at 20-s intervals were collected.

Radioactivity of ^{51}Cr was counted in whole perfusate in a gamma-spectrometer (Packard Instrument Co., Inc., United Technologies, Downers Grove, IL). These samples were counted after the decay of the ^{99m}Tc radioactivity (t_2 6 h). ^{99m}Tc radioactivity was counted in the perfusate supernatant by gamma-spectrometry after centrifugating down the red cells. Appropriate corrections for background and isotope spillover were made. The beta isotopes were counted in the perfusate supernatants and in bile on a liquid scintillation counter (Packard Instrument Co.) using Lumagel (Baker Chemical Co., Deventer, The Netherlands) as a scintillator. Quenching was monitored by the external standard method; counts per minute were converted to disintegrations per minute after background subtraction by a built-in microprocessor.

Vascular spaces and t_0, the time spent by indicators in nonexchanging vessels, were calculated as described by Goresky (16).

Vascular spaces were calculated as the product of mean transit time and flow in spite of the fact that one of the main conditions, namely that the system be a closed one (17), was violated. This condition states that a capillary system should have only one common in- and outflow. In our case two inflows (hepatic artery and portal vein) and two outflows (hepatic vein and common bile duct) were present. The latter should not affect our data, since bile flow is only a small fraction of blood flow. Moreover, labels such as erythrocytes have virtually no access to the biliary system. The presence of two inflow systems is more difficult to deal with. Accuracy of the estimation of capillary spaces depends on whether mixing of labels occurs outside or within the capillary bed. In the latter case, true capillary volume will be underestimated. To differentiate between these alternatives, indicator dilution curves were also obtained in single perfused liver, i.e., during perfusion

1. *Abbreviations used in this paper:* BSDF, bile salt-dependent fraction; BSIF, bile salt-independent fraction; IDC, indicator dilution curves; R, water recovery; RSR, relative sucrose recovery; t_0, time spent by indicators in nonexchanging vessels.

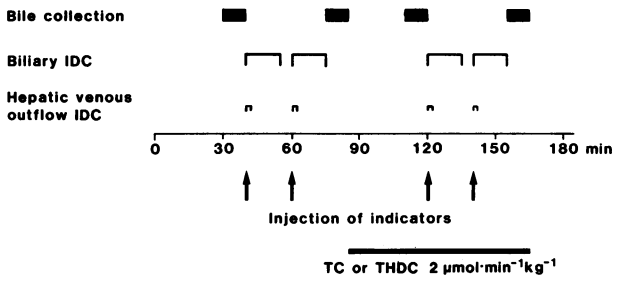

Figure 4. Experimental design for biliary *IDC* in the absence of exogenous bile salts and during stimulation of bile flow with taurocholate or taurodehydrocholate. The indicator mixture contained ^{51}Cr-erythrocytes, ^{99m}Tc-albumin, [^{14}C]sucrose and $^{3}H_2O$. Biliary IDCs were obtained by obtaining 50 samples of bile at 20-s intervals. The hepatic venous IDC consisted of 30 samples at 1-s intervals.

of the hepatic artery or portal vein, only. Since the possibility that this manipulation affects the population of sinusoids being perfused, the vascular spaces will be termed "equivalent" spaces to reflect the fact that in the dually perfused organ the conditions for volume calculation are not strictly met. Biliary water space is calculated as the product of bile flow and biliary mean transit time; the latter was calculated according to standard indicator dilution theory (17). For the calculation of biliary mean transit times, the data were truncated at 16.7 min; at that time the counts of $^{3}H_2O$ were less than twice the background. Extrapolation to infinity, assuming monoexponential decay, leads to, on average, 15% longer transit times. Recovery (R) of indicators was calculated as $R = \sum_{50} h(t)/D$, where $h(t)$ is the frequency function of indicator in each sample and D the amount of radioactivity injected. R was divided by the ratio (Q), where Q = (bile flow)/(blood flow). In this ratio water content of bile (96–97%) and perfusate (95.4%) was disregarded. This correction served to account for labeled water flow in the two phases (perfusate and bile). Relative sucrose recovery (*RSR*), an indirect measure of biliary permeability (11), was calculated as $RSR = R_{sucrose}/R_{H_2O}$.

All results are reported as mean±1 SD. Multiple means were compared by analysis of variance (18) followed by Wilcoxon's rank test for unpaired, or the signed rank test for paired data (19). $P < 0.05$ was considered statistically significant.

Results

With the method described, rat livers could be successfully perfused in about 60% of attempts. The failures (40%) were most often (~ 20%) due to anatomical variations in the arterial supply of the left liver lobe. The remainders were either due to (10% each) unidentifiable perfusate leaks in the arterial inflow or to technical inability to cannulate the celiac axis. The experiments reported had all adequate viability as judged by an alanine aminotransferase release of 14±8 IU/h, a potassium release of 0.4±0.3 meq/h, and a total oxygen consumption of 2.4±0.4 μmol/(min. g liver). The hemodynamic parameters of the preparation are given in Table I. The ratio of portal to hepatic arterial flow was 3.4; as anticipated, vascular resistance was much higher in the arterial than in the portal venous bed.

A typical set of hepatic venous indicator dilution curves after intraportal and intraarterial injection is shown in Fig. 5. The flow-limited pattern initially described by Goresky (16) is clearly seen after intraportal injection: the erythrocyte curve peaks earlier and higher than both the albumin and the sucrose

Table I. Hemodynamic Parameters of the Dually Perfused Rat Liver

	Hepatic artery	Portal vein
Pressure (dyn·cm^{-2} × 10^3)	115.99±16.00	9.51±0.98
Flow (cm^3·min^{-1} g^{-1})	0.33±0.11	1.11±0.25
Resistance (dyn·s·cm^{-5} × 10^3)	24.09±10.72	0.55±0.17

Mean±1 SD are given. n = 12

curve. The delayed and dampened appearance of the two latter curves is due to distribution of albumin and sucrose into the extravascular space. A similar pattern is seen after intraarterial injection. However, the extravascular markers dissociate from the intravascular reference substance later than in the portal venous curve, suggesting that t_0 is prolonged. Another pattern, encountered in three experiments, is shown in Fig. 6. The dilution curve after intraportal injection is similar to the one shown in Fig. 5 *A*, while the intraarterial dilution curve is clearly biphasic. The biphasic nature is presumably due to two populations of portal tracts being perfused at different velocities. In such experiments, prolonged visualization of some areas after intraarterial Patent Blue injection was observed (data not shown). Albumin and sucrose are almost superimposed upon the erythrocyte curve under the first part of the curve, thereby also prolonging t_0. The prolonged t_0 in both patterns of arterial dilution curves is probably due to the passage of labels through the peribiliary plexus, which has a regular capillary endothelium presenting a diffusional barrier to albumin.

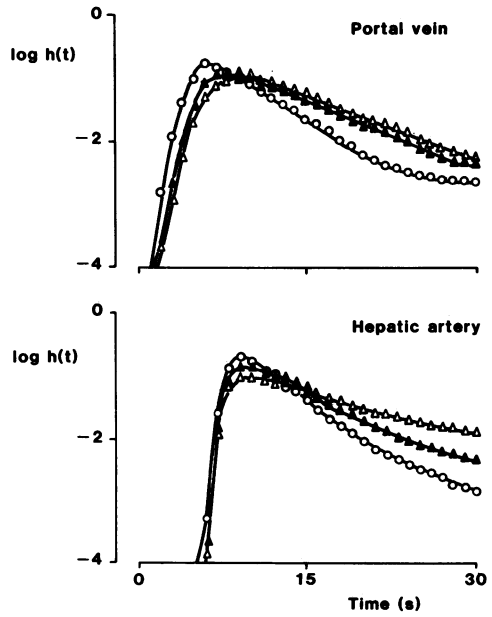

Figure 5. Hepatic venous dilution curves after injection into the portal vein (*top*) and hepatic artery (*bottom*). Decadic logarithms of the frequency function $h(t)$ is given for erythrocytes (○), albumin (▲), and sucrose (△). The three dilution curves separate later in the hepatic artery than in the portal vein, indicating that more time is spent in nonexchanging vessels. Accordingly, t_0 was 3.8 s in the hepatic artery, while it was only 1.3 s in the portal vein.

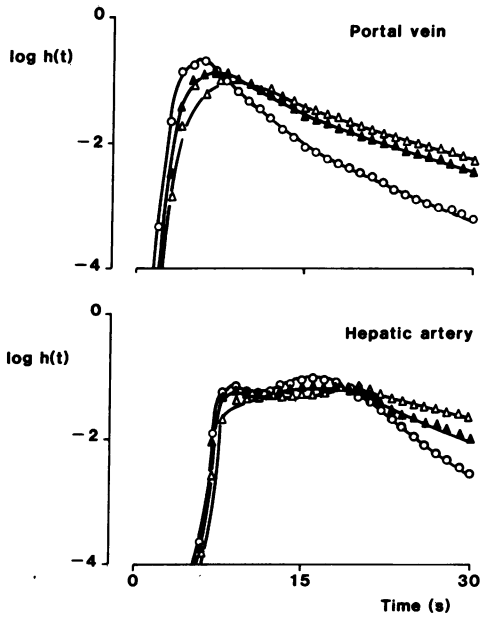

Figure 6. Hepatic venous dilution curves in another dually perfused liver. Legends are as those given in Fig. 3. The portal venous curve (*top*) is comparable to the one shown in Fig. 3 while the hepatic arterial curve is clearly biphasic. There is virtually no separation of the intravascular marker, the erythrocytes (○), from the extravascular markers albumin (▲) and sucrose (△), which suggests that this is due to passage of a diffusion-limited vessel, presumably the peribiliary plexus. t_0 was 1.1 and 6.2 s in the portal vein and hepatic artery, respectively.

The calculated intra- and extravascular equivalent spaces are given in Table II. t_0 averaged 2.37±1.27 and 7.40±2.61 s after intraportal and intraarterial injection, respectively ($P < 0.02$). The erythrocyte space was significantly larger when measured after intraarterial injection. The same held true for extravascular sucrose space and total water space, while extravascular albumin space did not differ significantly between the two vessels (Table II). The results derived from comparing indicator dilution curves that were obtained in dual with those from single perfusion experiments are given in Table III. The intravascular (erythrocyte) space was virtually identical in both vascular beds regardless of the mode (single or dual) of perfusion.

Single pass uptake of taurocholate after injection into the hepatic artery (97.8±10.7%) did not differ significantly from extraction after portal injection (98.3±1.0%; $P > 0.07$). The

Table II. Equivalent Vascular and Extravascular Spaces (ml/g liver) in the Dually Perfused Rat Liver

	n	Hepatic artery	Portal vein	*P*
Erythrocyte space	12	0.217±0.040	0.186±0.017	0.005
Extravascular albumin	7	0.085±0.039	0.063±0.030	0.063
Extravascular sucrose	7	0.149±0.063	0.085±0.024	0.002
Water	5	0.725±0.037	0.677±0.041	0.043

Mean±1 SD are given. The difference between the two vascular beds was evaluated by Wilcoxon's sign rank test.

Table III. Equivalent Erythrocyte (RBC), Extravascular Albumin (EVA) and Extravascular Sucrose (EVS) Spaces (all in ml/g) in the Perfused Rat Liver

	Hepatic artery		
	RBC	EVA	EVS
Dual perfusion	0.212±0.037	0.091±0.054	0.163±0.073
Hepatic artery perfused alone	0.194±0.043	0.082±0.049	0.115±0.062
	Portal vein		
	RBC	EVA	EVS
Dual perfusion	0.191±0.013	0.063±0.035	0.086±0.023
Portal vein perfused alone	0.189±0.025	0.064±0.044	0.090±0.024

$\bar{x}$±1 SD, $n = 5$.

same held true for biliary excretion over 20 min (94.2±2.0 vs. 94.1±2.1%; $P > 0.30$). The pattern of the biliary dilution curves of taurocholate was similar after injection into either vessel (data not shown), with the mean biliary taurocholate transit time averaging 6.74±0.38 and 5.94±1.11 min after intraarterial and intraportal injection, respectively ($P > 0.30$).

Quite a different pattern emerged for the biliary water dilution curves (Fig. 7, *A* and *C*). After injection into the hepatic artery, water appeared earlier in bile and peaked much higher than after portal vein injection, biliary mean transit time of water averaging 2.14±0.51 and 5.88±0.91 min, respectively ($P < 0.003$). Water recovery (R) corrected for Q, the ratio bile/

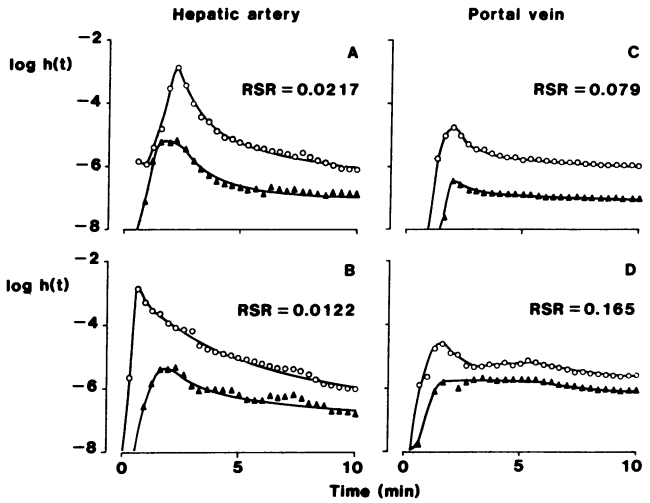

Figure 7. Biliary indicator dilution curves of water (○) and sucrose (▲) after injection into the hepatic artery (*A* and *B*) or portal vein (*C* and *D*) in the absence (*A* and *C*) and presence (*B* and *D*) of an exogenous taurocholate load. Data are shown up to 10 min only although collection was carried out to 16.7 min. Decadic logarithms of the frequency function $h(t)$ are given. The water curve peaked much higher and earlier after injection of the indicators into the hepatic artery under both experimental circumstances. The relative sucrose recovery (*RSR*) decreased in the hepatic artery (*B*) but increased in the portal vein (*D*) during infusion of taurocholate 50 nmol/(min · g liver).

Table IV. Bile Flow and Biliary ^3H_2O Recovery in the Dually Perfused Rat Liver in the Absence (BSIF) and Presence (BSDF) of Exogenous Taurocholate (50 nmol · min^{-1} g liver^{-1})

	Hepatic artery		Portal vein	
	BSIF	BSDF	BSIF	BSDF
Bile flow (μl · min^{-1} g liver^{-1})	1.04±0.08	1.47±0.14*		
Q: (Bile flow)/(Blood flow) (×10^{-3})	3.61±1.30	5.06±1.59*	0.96±0.25§	1.37±0.38*§
R: Recovery of ^3H_2O (×10^{-3})	2.65±0.89	4.43±1.58*	0.16±0.07§	0.38±0.16‡§
R/Q	0.76±0.16	0.90±0.30‡	0.18±0.08§	0.29±0.16‡‖

x̄±1 SD (n = 7). * BSIF vs. BSDF $P < 0.02$, ‡ BSIF vs. BSDF $P < 0.03$, § hepatic artery vs. portal vein $P < 0.002$, ‖ hepatic artery vs. portal vein $P < 0.003$.

blood flow (to correct for water flow in the two phases, perfusate and bile), averaged 0.762±0.188 and 0.163±0.078 after injection into the hepatic artery and portal vein ($P < 0.003$), respectively. Since we have shown previously that water equilibrates completely with bile, (11) the corrected water recovery of 0.762 can be interpreted as 76.2% of the labeled water having reached the biliary system after intraarterial injection stayed therein, or that 24.8% of the labeled water underwent exchange. This figure was much higher after intraportal injection, 83.7% of the labeled water being exchanged.

Water recoveries, corrected and uncorrected, for the seven experiments where a taurocholate challenge was given, are reported in Table IV. The bile salt infusion appropriately stimulated bile flow by 41% ($P < 0.02$). The corrected water recovery was significantly increased after injection into either hepatic artery or portal vein (Table IV) suggesting that labeled water exchange in the biliary tree was diminished during stimulation of bile salt secretion.

Water and sucrose appeared in bile with a similar pattern both in the absence and presence of an exogenous bile salt load after intraportal injection as previously described (11, Fig. 7, *C* and *D*). The sucrose curve after intraarterial injection peaked later and decayed more slowly than the corresponding water curve (Fig. 7, *A* and *B*). The RSR, i.e., the biliary recovery of sucrose divided by that of water, was much lower in the hepatic arterial bed than in the portal vein bed both in the absence and presence of an exogenous taurocholate load ($P < 0.02$). This suggests that the biliary epithelium is less permeable to sucrose than the portally perfused canaliculi. When bile salt-dependent bile flow was stimulated by taurocholate (Fig. 8), the RSR decreased by 33% ($P < 0.02$) after intraarterial injection whereas it actually increased by 36% ($P < 0.03$) after intraportal injection.

The results of the studies using taurodehydrocholate are reported in Table V. Taurodehydrocholate increased bile flow by 69% ($P < 0.025$), which is significantly more than taurocholate ($P < 0.003$). Its effects on corrected ^3H_2O-recovery (*R/Q*) were similar to those of taurocholate, i.e., a significant increase in both vascular beds. Taurodehydrocholate significantly decreased the RSR after intraarterial injection, an effect similar to that of taurocholate. In contrast, taurodehydrocholate did not significantly affect RSR after intraportal injection of the indicator (Table V).

Biliary water space was calculated as the product of bile flow and of mean biliary transit time truncated at 16.7 min. The data are given in Table VI. It was significantly larger after intraportal than after intraarterial injection during either bile salt-independent– or bile salt-dependent–stimulated bile formation ($P < 0.001$). Neither taurocholate nor taurodehydrocholate infusion affected the calculated biliary water space ($P > 0.05$) when the indicators were given into the hepatic artery (Table VI). In contrast, when the indicators were given into the portal vein, it increased significantly after stimulation of bile flow by either taurocholate or taurodehydrocholate (Table VI).

Discussion

Our study is the first to formally evaluate the influence of hepatic arterial perfusion on biliary physiology; in the absence of exogenous bile salts, there is considerable tracer water exchange, ~ 80%, downstream of the canaliculus. Tracer exchange is diminished in both vascular beds when bile flow is stimulated by bile salts. We confirm our earlier data in the portally perfused rat liver demonstrating that taurocholate, but not taurodehydrocholate, increases permeation of sucrose into bile. In contrast to these events in the portal bed, biliary permeation of sucrose is less in the hepatic arterial bed and stimulation of bile flow by either taurocholate or taurodehydrocholate further decreases sucrose permeation.

Viability of the dually perfused organ is comparable to that of the portally perfused liver (15) when judged by oxygen consumption, transaminase, and potassium release. Bile flow in the absence of exogenous bile salts is somewhat less than we have reported with the portally perfused liver (6, 11) but the

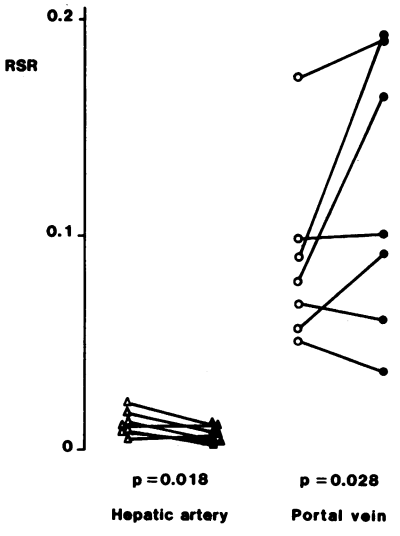

Figure 8. Relative sucrose recovery (*RSR*, biliary sucrose recovery divided by biliary water recovery) after injection of indicators into the hepatic artery (*triangles*) and portal vein (*circles*). RSR was lower in the hepatic artery in the absence (△) and presence (▲) of an exogenous taurocholate load. Taurocholate significantly decreased RSR in the hepatic artery but increased it in the portal vein.

Table V. Bile Flow, Corrected Biliary ^3H_2O Recovery (R/Q), and Relative Biliary Sucrose Recovery (RSR) in the Dually Perfused Rat Liver before (CTR) and during Taurodehydrocholate Infusion (TDHC) (50 nmol · min^{-1} g liver^{-1})

	Hepatic artery		Portal vein	
	CTR	TDHC	CTR	TDHC
Bile flow (μl · min^{-1} g^{-1})	1.12±0.05	1.89±0.03*		
R/Q	0.804±0.267‡	1.018±0.159*	0.360±0.342	0.652±0.424*
RSR	0.012±0.003§	0.011±0.003*,§	0.280±0.195	0.132±0.051

$\bar{x}$±1 SD are given, $n = 6$. * Significant effect of TDHC $P < 0.025$, ‡ significant difference between hepatic artery and portal vein $P < 0.05$, § significant difference between hepatic artery and portal vein $P < 0.005$.

increase seen after taurocholate infusion corresponded to the apparent osmotic activity of this bile salt, which has been reported to be between 6 and 10 μl/mol (3, 4, 6, 20, 21), in the rat. This is comparable to the choleretic property of taurocholate in vivo (4, 20), which again attests to the viability of the dually perfused organ.

Two other reports have described techniques for hepatic arterial perfusion (9, 10). Ahmad et al. directly cannulated the hepatic artery with a large needle (9) while Gardemann et al. perfused the hepatic artery via the aorta (10); both publications report viabilities similar to ours but neither group stated their failure rate. With our method, which is more time-consuming than either of the previously published methods, we still had a failure rate of 40%. This was mostly due to anatomical variations which are rather important and can lead to uneven perfusion of the organ. Therefore, we feel it important to assess uniformity of perfusion in each experiment. We could decrease the initial failure rate of 50% to the current one of 40% by not directly ligating the gastroduodenal artery (Fig. 1); this vessel feeds the left hepatic artery in about one out of five livers. The setup of the perfusion apparatus as outlined in Fig. 2 allows selfregulation of portal flow (13) and differential oxygenation of the two vascular beds. This system is similar to the one described by Ahmad et al. (9), however, this feature was not exploited in the present experiments.

The indicator dilution technique can be used to calculate hepatic vascular and extravascular spaces (16) provided that the conditions of stationarity, absence of recirculation, absence of stagnant pool, and closedness are met (17). The first two conditions are met in the isolated perfused organ, the absence of indicator recirculation being one of its main advantages. Stagnant pools, which may have been present in experiments of the type shown in Fig. 6 will lead to an underestimation of the volume. No statistical difference was observed between experiments such as those shown in Figs. 5 and 6 (data not shown), therefore, a major distortion of our data by this phenomenon appears unlikely.

Table VI. Biliary Water Spaces (μl/g liver) in the Dually Perfused Liver in the Absence (BSIF) and Presence (BSDF) of Taurocholate (TC) or Taurodehydrocholate (TDCH), Both Infused at 0.2 μmol · min^{-1} 100 g body wt^{-1}

	n	Hepatic artery	Portal vein
BSIF	13	2.08±0.41	5.41±1.08
TC	7	2.56±0.38*	8.41±1.69‡
TDHC	6	2.36±0.66*	8.99±2.13§

Mean±1 SD are given. Differences were evaluated by Wilcoxon's rank sign test.
* No significant difference between BSIF and BSDF, ‡ significant effect of bile salt $P < 0.01$, § significant effect of bile salt $P < 0.05$.

More serious is the violation of the criterion of closedness, i.e., of the requirement that the system has only one inflow and one outflow (17). The second outflow, namely bile, is trivial and will not affect any of our data since (*a*) the ratio of bile/blood flow is ~ 10^{-3} and (*b*) erythrocytes have no, and albumin very little, access to the biliary system. We attempted to appreciate the importance of dual inflows by perfusing the same liver in single and dual fashion (Table III). An underestimation of spaces can occur when the closedness criterion is violated (17). Our results (Table III) suggest that the sinusoidal erythrocyte and albumin volumes are accurately reflected by our technique while a systematic error is incurred in the estimation of sucrose spaces. The latter is probably true for water spaces as well. We have therefore termed the spaces "equivalent spaces" to draw attention to the fact that some systematic bias is associated with that data. Equivalent erythrocyte, extravascular sucrose, and water space, but not extravascular albumin space, were appreciably larger when measured in the hepatic artery than in the portal vein (Table II). This is at variance with a previous publication (9). From the difference in water space one can estimate that ~ 5% more tissue was perfused by the hepatic artery. This probably represents portal tract structures and the biliary epithelium. The discrepancy between extravascular equivalent sucrose space (larger after intraarterial injection) and extravascular equivalent albumin space (identical after injection into either vessel) is not surprising. Sucrose can diffuse out of tight capillaries, such as those making up the biliary plexus, while albumin cannot. Thus, sucrose labels the extravascular space of portal tracts and the biliary epithelium fed by the hepatic artery. The virtual identity of the extravascular albumin space after intraarterial and intraportal injection can be interpreted as the efferent arterioles from the peribiliary plexus draining into the portal/sinusoidal system close to the portal tract. This is in agreement with an earlier study (9) and with studies of corrosion casts of rat liver (22).

In a previous investigation we have shown that tritiated water, injected as a bolus into the portal vein, adequately labels biliary water (11). Thus, our finding of a reduced recovery of biliary tracer water after portal as compared with that after arterial injection has to be interpreted as evidence for the ex-

change of tracer by tissue fed by the hepatic artery: under these experimental conditions, unlabeled water can enter the biliary tract only from the hepatic artery. The recovery of 0.18 of tracer (Table IV) can be interpreted to mean that, in relation to volume flow, only 18% of the labeled water has come from the canaliculus after intraportal injection. It is of interest to note that stimulation of bile flow by bile salts leads to an increase in biliary water recovery after injection into both vessels (Tables IV and V). The finding that both taurocholate and taurodehydrocholate had this effect suggests that the phenomenon is due to a shortening of the transit time of bile which in turn is due to the increased flow rather than to some alterations of the physical characteristics of the system by the infused bile acid.

Smith and Boyer were the first to show that the biliary epithelium is permeable for a variety of inert solutes including sucrose (8). Our data concerning the RSR confirm this finding. The RSR, a measure of permeation of sucrose into bile (11) analogous to the better established steady-state bile to plasma ratio of inert solutes (23), increased in response to taurocholate (Fig. 8) but not to taurodehydrocholate (Table V) in the portal venous bed (Fig. 8), which confirms our earlier studies in the portally perfused rat liver (6). Our data do not permit us to determine whether this reflects permeation across the tight junctions as suggested by lanthanum permeation studies (24) or increased transcellular entry of sucrose as suggested by the demonstration of increased vesicular traffic (7, 25) and the finding that chenodeoxycholate leads to an increased incorporation of inulin into intracellular vesicles (26). In contrast to the events in the portal venous bed, RSR decreased in response to both bile salts after injection of the indicators into the hepatic artery (Fig. 8 and Table V). This is probably due to the decreased transit time of bile through the biliary ductules.

As one would anticipate, the biliary water space was considerably smaller after intraarterial than after intraportal injection (Table VI). This reflects entry of the bulk of labeled water relatively downstream into the biliary system. Collection of bile was carried out to 16.7 min, only; this underestimates water space by $\sim$ 15% when compared with transit time extrapolated to infinity assuming monoexponential decay. We elected not to extrapolate the data to infinity since only very low levels of radioactivity were contained in the last samples which made an estimate of the terminal exponential uncertain.

The size of the biliary space determined after intraarterial injection corresponds quite closely to the biliary dead space reported by Haecki and Paumgartner (27) while the biliary space after intraportal injection probably reflects total biliary plus canalicular volume. Its value is quite close to the morphometrically determined canalicular volume fraction of 0.0056 reported by Weibel et al. (28). Thus, the biliary indicator dilution technique could be used as a nondestructive way to estimate canalicular volume and biliary dead space. Its utility as a physiological tool is strengthened by the finding that canalicular volume increased after stimulation of bile flow by taurocholate (Table VI). We believe this to be the equivalent of the increased canalicular diameter observed on scanning electron micrographs by Layden and Boyer after a choleretic bile acid infusion (29).

Rather large rats were used in the present study, their age ranging from 6 to 8 mon. This was necessitated by the vascular anatomy, the hepatic artery being very difficult to cannulate in the more commonly used 3–4-mo-old rats. This is unlikely to affect our results since biliary function only declines at higher ages (> 1 yr) (30).

In conclusion we have established a novel system of dual rat liver perfusion that permits studies of the hepatic artery as well as the portal vein. The livers perfused in this way have similar viabilities as the portally perfused organ. We have characterized the vascular and extravascular spaces in the two vascular beds. Finally, we have studied the effect of bile salts on exchange of tracer water and biliary permeation of sucrose. Our data demonstrate extensive tracer water exchange in the peribiliary plexus fed by the hepatic artery. Bile acids increase permeation of inert solutes, such as sucrose, at the canalicular but decrease it at the biliary epithelial level. Finally, the biliary indicator dilution technique can be used to estimate the different biliary spaces and thus may be a useful physiological tool.

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