Hepatic versus gallbladder bile composition: in vivo transport physiology of the gallbladder in rainbow trout

M. Grosell, M. J. O'Donnell and C. M. Wood

Am J Physiol Regul Integr Comp Physiol 278:1674-1684, 2000.

Am J Physiol Regulatory Integrative Comp Physiol
278: R1674–R1684, 2000.

Hepatic versus gallbladder bile composition: in vivo transport physiology of the gallbladder in rainbow trout

M. GROSELL, M. J. O'DONNELL, AND C. M. WOOD
Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

Grosell, M., M. J. O'Donnell, and C. M. Wood. Hepatic versus gallbladder bile composition: in vivo transport physiology of the gallbladder in rainbow trout. *Am J Physiol Regulatory Integrative Comp Physiol* 278: R1674–R1684, 2000.—Ion and water transport across the teleost *Oncorhynchus mykiss* gallbladder were studied in vivo by comparing flow and composition of hepatic bile, collected by chronic catheter, to volume and composition of terminally collected gallbladder bile. Differences in composition were comparable with those of other vertebrates, whereas bile flow (75 $\mu l \cdot kg^{-1} \cdot h^{-1}$) was below values reported for endothermic vertebrates. The gallbladder concentrates bile acids five- to sevenfold and exhibits higher net Cl^- than Na^+ transport in vivo, in contrast to the 1:1 transport ratio from gallbladders under saline/saline conditions. Transepithelial potential (TEP) in the presence of bile, at the apical surface, was −13 mV (bile side negative) but +1.5 mV in the presence of saline. Bile acid in the apical saline reversed the TEP, presumably by a Donnan effect. We propose that ion transport across the gallbladder in vivo involves backflux of Na^+ from blood to bile resulting in higher net Cl^- than Na^+ flux. This Na^+ backflux is driven by a bile side negative TEP and low Na^+ activity in bile due to the complexing effects of bile acids.

transepithelial potential; teleost freshwater fish; hepatic bile flow; ion and water reabsorption; bile acid

BILE IS A HEPATIC SECRETION that functions to promote digestion and absorption of lipids from the intestine via the action of bile acids or bile salts. Bile also acts as the medium for excretion of many endogenous and exogenous substances from the blood and liver that are not excreted through the kidneys. Excretory functions in fish have been described in detail (see Ref. 35 for review). The mechanisms of hepatic bile production in fish, however, have received much less attention.

Notable exceptions are certain components of the enterohepatic bile salt circulation. Bile acid composition (7) and mechanisms of hepatic uptake of bile salts (25) in the teleost rainbow trout as well as several elasmobranchs (3, 11, 21, 26, 31, 32) are well characterized. Furthermore, carrier-mediated intestinal bile salt absorption has been reported in teleost fish (17). These studies reveal some differences among elasmobranchs, teleosts, and higher vertebrates in the mechanisms of basolateral membrane bile salt uptake. Reports on hepatic bile flow and composition (i.e., the result of canalicular membrane transport mechanisms) in lower vertebrates are limited to one study of bile flow and composition in elasmobranchs (2) and two studies on hepatic bile flow rates in teleost fish (12, 30). Consequently, one aim of the present study was to refine a technique to continuously collect hepatic bile in vivo for the characterization of bile flow and composition in the teleost rainbow trout (*Oncorhynchus mykiss*).

The role of the gallbladder in storing and concentrating bile in fish is not well described. By contrast, the gallbladder epithelia of various amphibians and mammals have been studied in great detail; this tissue has become a model for epithelia that transport salt and water at high rates and in isosmotic proportions ("leaky epithelia"). The structural simplicity, i.e., a monolayered epithelium with an exclusive or predominant cell type, only a few basic transport processes, and, for some species, large cell size facilitating electrophysiological studies, makes it an appealing model system (for comprehensive reviews, see Refs. 27 and 28). Some of the early, classical work describing the transport processes of this epithelium was conducted on a teleost fish (8–10). Isolated gallbladder epithelia of roach (*Rutilus rutilus*) exhibited a 1:1 Na^+- to Cl^--transport ratio (9). Later, this was also reported for Japanese eel (*Anguilla japonica*) (16) and seems to also apply to isolated gallbladder epithelia of higher vertebrates in general (reviewed by Reuss in Refs. 27 and 28). However, when comparing the composition of hepatic bile with gallbladder bile in several teleosts and one elasmobranch, Diamond (8) observed that the Na^+ concentration of gallbladder bile was higher than the corresponding concentration in the hepatic bile, whereas the opposite was true for Cl^-. This phenomenon also applies to amphibians and mammals (8). Indeed Hunn (18) reported much higher Na^+ than Cl^- concentrations in the gallbladder bile of 25 different teleost fish species. These findings suggest that the gallbladder epithelium in vivo has a higher net Cl^- transport rate than Na^+ transport rate and not a 1:1 Na^+- to Cl^--transport ratio as observed in vitro.

Surprisingly, considering that this discrepancy has been evident for almost four decades, no studies on ion transport of the gallbladder epithelium in vivo have been reported, to our knowledge. In the present study, we refined a technique for continuously collecting hepatic bile for several days in rainbow trout. By comparing the hepatic output of bile acids and major electrolytes, as well as bile volume, with the gallbladder bile

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

volume and composition at the same times, ion and water transport of the gallbladder epithelium was analyzed in rainbow trout during progressive starvation. Starvation was employed as a tool to ensure that bile collected in the gallbladder.

MATERIALS AND METHODS

Rainbow trout, *Oncorhynchus mykiss*, were obtained from Humber Springs Trout Farm, Ontario. The fish were held in 400-l fiberglass tanks (up to 100 fish/tank), each supplied with a flow-through of dechlorinated aerated Hamilton city tap water (in mM: 0.6 Na^+; 0.7 Cl^-; 1.0 Ca^{2+}; and 1.9 HCO_3^-, pH 7.9–8.2) at a rate of at least 5 liters/min. The fish were held at 14.0 ± 1.0°C and were fed a maintenance ratio of dry trout pellets (Martin's Feed Mill, Ontario) at a rate of 1% of their body mass per day.

Experimental design. Bile was collected via chronic cannulation of the hepatic bile duct for 9 successive 12-h intervals ($n \geq 9$ fish in all cases) and via terminal sampling from the gallbladder of noncannulated fish at 24, 48, 72, 96, 144, 168, and 240 h after last feeding ($n = 10$ at each sampling point). Gallbladder surface area was determined in 10 fish. In addition, transepithelial potential (TEP) across the gallbladder (bile-to-blood fluid) was measured in vivo in seven fish, and in vitro in 13 excised preparations. In the latter, the influence of exchanging the mucosal bile for isotonic saline and supplementing the saline with bile acids was also evaluated.

Surgical procedure. Our surgical procedure is the product of extensive testing. The anatomy of the gallbladder and bile duct system, and the common hepatic bile duct cannulation technique are illustrated in Fig. 1. The ideal size of experimental animals for this surgical procedure was ~225 g. Thirty-six hours of starvation were found to provide the ideal conditions for the cannulation of the common hepatic bile duct; the overall success rate of this procedure was just under 50%. A total of 15 experimental animals (average weight 232 g; range 179–278 g), out of 34 originally cannulated, contributed data to this experiment. The remaining fish were not included in the experiment, because no bile was obtained via the catheter in some or all of the 9 successive 12-h intervals.

Fish were anesthetized in 0.1 g/l neutralized MS-222 for surgery. Throughout surgery, the gills were constantly irrigated with aerated MS-222 solution (0.08 g/l). A single midline incision of ~40 mm was made in the anterior direction, starting from just posterior to the pelvic fins. The common bile duct was ligated without damaging the hepatic portal vein (Fig. 1). The gallbladder was held in place for surgery by two sets of silk thread secured by hemostatic forceps. One ligature (not shown in Fig. 1) was made 8 mm from the point at which the hepatic bile duct enters the gallbladder, preventing leakage of the gallbladder contents into the celomic cavity, and a cut was made in the gallbladder wall 4–6 mm from the cystic bile duct. The tip of the catheter (60 cm PE-10 tubing filled with a 140 mM NaCl solution) was inserted through the gallbladder wall, into the cystic bile duct/common hepatic bile duct. Care was taken not to insert the catheter too far into the common hepatic bile duct and thereby interfere with bile flow from the hepatic bile ductules (see Fig. 1). The catheter was held in place by two silk ligatures (Fig. 1); the last ligature was placed as close to the catheter tip as possible, eliminating contact between the gallbladder epithelium and the hepatic bile. Externally, the catheter was anchored by two silk ligatures posterior to the body wall incision. The wound was powdered with oxytetracycline and tightly closed by silk ligatures.

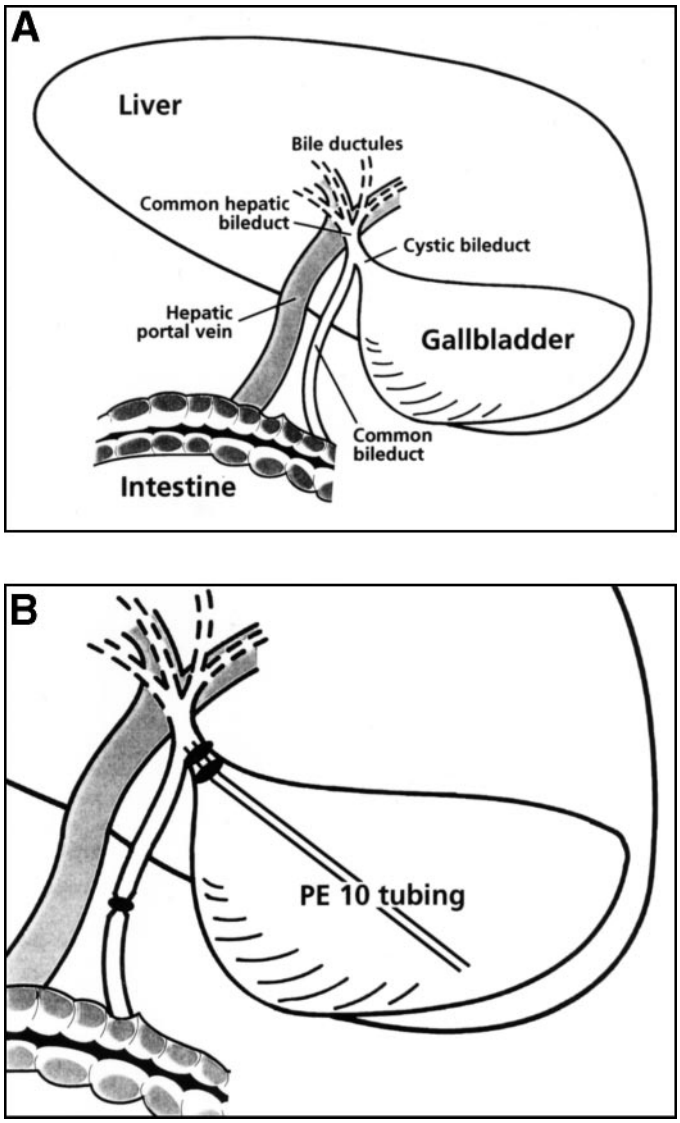

Fig. 1. *A*: diagram of the hepatobiliary anatomy in the freshwater rainbow trout. *B*: cannulation technique used in the present study. A catheter is inserted in the cystic bile duct/common hepatic bile duct. The common bile duct is ligated preventing bile flow to the intestine. PE-10, polyethylene tubing.

After surgery, the fish were placed in individual 4-l fish boxes. Each fish box consisted of black Plexiglass and was supplied with a flow-through of aerated, dechlorinated tap water at a rate of 200 ml/min.

Continuous collection of hepatic bile. The fish were allowed to recover from surgery for 3–12 h in the fish boxes. The catheter end was then placed in preweighed Eppendorf tubes, which were kept on ice in styrofoam containers. At 12-h intervals, the Eppendorf tubes and ice were replaced. The volumes of bile collected were determined gravimetrically for all 9 12-h intervals (i.e., 96 h). The samples were stored at −70°C. For the last eight collection periods, the bile samples were analyzed for the concentration of bile acid, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, total CO_2, and osmolality as described in *Analytical techniques*. Bile from the first collection period was not analyzed for composition, because it was contaminated with the 140 mM saline initially present in the catheter.

Sampling of gallbladder bile. Fish in a single large holding tank (400 liters) were fed to satiation, and then groups of 10 fish were netted out of the holding tank and anesthetized individually at 24, 48, 72, 96, 144, 168, and 240 h after feeding. A blood sample was drawn from the caudal artery with a heparinized syringe, and plasma was obtained immediately by centrifugation. The fish was then killed by a blow to the head, weighed, and the gallbladder exposed by dissection. The gallbladder bile was obtained by a 1-ml syringe fitted with a 26-gauge needle, and volume was determined by weight using preweighed Eppendorf tubes. Samples of plasma and gallbladder bile were frozen in liquid nitrogen as soon as possible after sampling and stored at −70°C for later analysis of bile acid. All bile samples and 10 randomly selected plasma samples were analyzed for Na^+, Cl^-, Ca^{2+}, Mg^{2+}, total CO_2, and osmolality.

Determination of gallbladder surface area. Ten fish were netted out of the holding tank, anesthetized, killed by a blow to the head, and weighed. The entire gallbladder from each fish was obtained by dissection, opened with a longitudinal incision, and the surface area was determined using graph paper.

Determination of transepithelial potential in vivo and in vitro. For the determination of gallbladder TEP, "free flowing bridges" (23) connected via AgCl electrodes to a high-impedance voltmeter (Radiometer pHM84) were employed. The substantial difference in Cl^- concentration between gallbladder bile and plasma creates a significant junction potential and thus inaccurate TEP measurements when AgCl electrodes are used in combination with standard KCl-agar bridges (23). Free-flowing bridges were constructed from barrels of disposable 1-ml plastic syringes that were heated over a gas flame and pulled to form very thin and flexible capillaries (~50 μm ID and 100 μm OD and ~50 cm long). The modified syringe barrels were subsequently filled with 3 M KCl, and a pressure of ~10 cmH_2O was used to force the KCl solution through the capillaries, resulting in a constant and similar Cl^- concentration at the tip of the free-flowing bridges. Because TEP recordings were performed within 30 s, the low flow rate (<1 μl/min) negligibly altered the Cl^- concentration in the bath or the gallbladder (>500 μl). The AgCl electrodes were connected to the KCl solution in the modified syringe for recording of potential difference. Tests demonstrated that the junction potential was reduced to <0.4 mV when the tip of the free-flowing electrodes were bathed in asymmetrical solutions ranging from 1.5 to 150 mM NaCl. As an additional check, when one free-flowing electrode was placed in saline and the other one in gallbladder bile connected to the saline via a KCl-agar bridge, there was no junction potential arising from differences in composition between saline and gallbladder bile.

Trout were prepared for surgery as described in *Surgical procedure*, and the TEP was measured while the fish were lightly anesthetized on the operating table. To gain access to the blood stream, a catheter was inserted into the dorsal aorta as described elsewhere (15). The gallbladder was exposed, and a PE-50 catheter was inserted into the gallbladder. The electrode was inserted into the gallbladder through the PE-50 catheter, and in vivo TEP was recorded with the blood in the dorsal aorta catheter as reference. Recordings were made from seven fish.

For in vitro recordings of gallbladder TEP, fish were killed by a blow to the head, and the gallbladder was obtained by dissection. The isolated gallbladder was cannulated with a length of PE-50 tubing while the bile was still in the gallbladder, and the electrode was inserted into the gallbladder using the surrounding Cortland (34) saline as a reference. The bile was subsequently removed from the gallbladder. After thorough rinsing, the gallbladder was filled with Cortland saline and the TEP under symmetrical conditions was recorded. Recordings were made from eight preparations. In several preparations, the procedure was then reversed so as to replace the saline in the gallbladder with the original bile.

To test the direct effect of bile acid on TEP, the TEP across the gallbladder of an additional five fish was measured, first under saline/saline conditions, and then the saline in the lumen was supplemented with 100 and 200 mM hydrocholic acid (Na salt, Sigma). The procedure was then reversed so as to replace the bile acid-containing saline with bile acid-free saline.

Analytical techniques. The Cl^- concentration of bile samples was determined using the colorimetric assay of Zall et al. (37). Cations were analyzed using a Varian 1275 atomic absorption spectrophotometer (AAS) with methods as documented by the manufacturer. The total concentration of bile acids was determined using Sigma kit 450-A modified for microtitre plate use. Osmolality of bile was measured using a Wescor 5100C vapor pressure osmometer. The total CO_2 concentration of the bile was analyzed using a Corning 965 carbon dioxide analyzer.

Sodium ion activity of randomly selected sample fluids was measured using ion-selective microelectrodes based on sodium ionophore II, cocktail A (Fluka), as described previously (24), and compared with total Na^+ concentration measured by AAS in the same samples. Samples of fluids (0.2–0.3 ml) were placed under paraffin oil. Droplets of calibration (150 and 15 mM NaCl, 135 mM LiCl) solutions were placed adjacent to each sample. Na^+ activities in the calibration solutions were determined using tabulated activity coefficients (29); the response of the electrode was close to Nernstian (53–57 mV per 10-fold increase in Na^+ activity). Na^+ activity in bile, saline, and plasma samples was calculated from the measured slope and the change in electrical potential recorded when the reference and Na^+-selective electrodes were moved between the sample and the 150 mM NaCl calibration solution. Na^+ activity was also measured in Cortland saline before and after the addition of 100 mM hydrocholic acid (Na salt) to evaluate the effect of bile acid on Na^+ activity.

Na^+ activity was measured immediately in freshly collected bile and plasma of six fish and then again after freezing the same samples to −70°C to establish that freezing did not influence Na^+ activity.

Calculations, data presentation, and statistical evaluation. Hepatic bile flow (expressed as $ml \cdot kg^{-1} \cdot h^{-1}$) was calculated by relating the volume of bile collected over time to the weight of the individual fish and the time elapsed. The summed bile flow over time ($Hep_{outflow}$) was calculated as follows

$$Hep_{outflow}(T1) = \sum_{T0}^{T1} (V/W)$$

where *T1* is the time elapsed since the start of the period in question (*T0*), V is the volume of bile collected during the time from *T0* to *T1*, and W is the weight of the fish.

Hepatobiliary output of solutes [$Hep_{output}(x)$] summed over time was calculated as follows

$$Hep_{outflow}(x) = \sum_{T0}^{T1} [[V(x)]/W]$$

where [*x*] is the concentration of the biliary solute in question.

Gallbladder bile volume (GBV) is expressed as milliters per kilogram throughout, and the gallbladder [GB(*x*)] pool of solutes at any time (expressed as mol/kg) was calculated as follows

$$GB(x) = V_{GB}/[x]$$

where V_{GB} is the volume of bile present in the gallbladder.

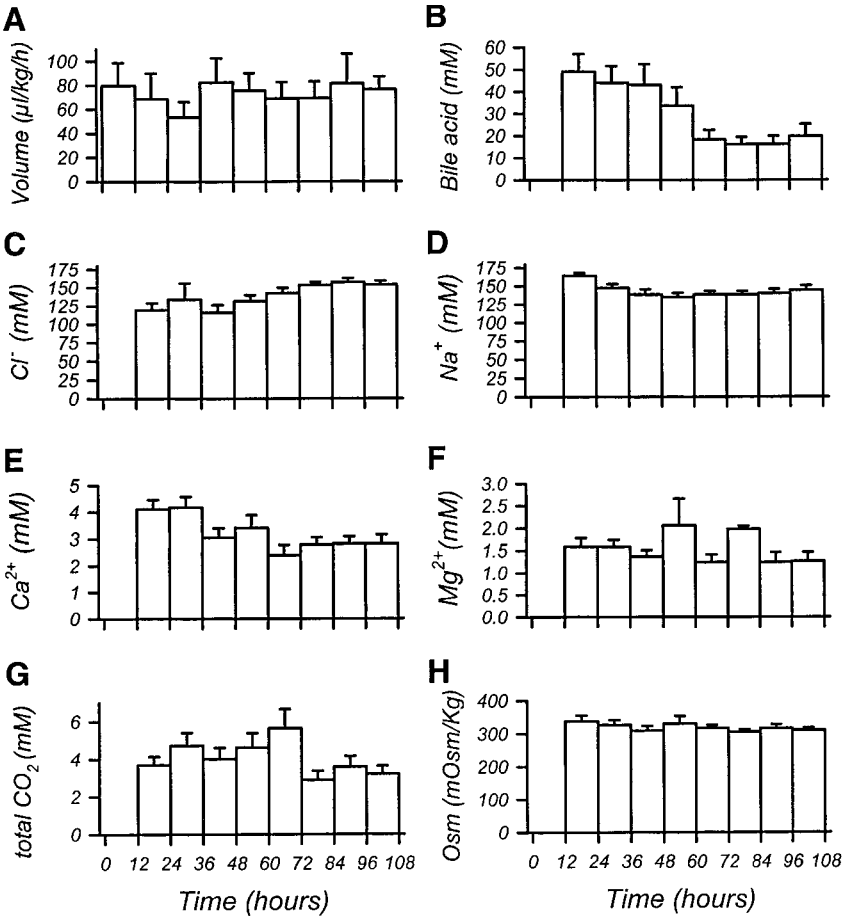

Fig. 2. Hepatic bile flow (*A*; $\mu l \cdot kg^{-1} \cdot h^{-1}$) and concentrations of bile acid (*B*), Cl^- (*C*), Na^+ (*D*), Ca^{2+} (*E*), Mg^{2+} (*F*), total CO_2 (*G*), and osmolality (*H*; mM or mOsm) in hepatic bile of freshwater rainbow trout for up to 108 h after cannulation. Means + SE. n, Number of subjects: 12 h; $n = 9$ (only bile flow), 24 and 36 h; $n = 12$, 48 h; $n = 11$, 60, 72, and 84 h; $n = 13$, 96 h; $n = 11$, 108 h; $n = 9$. Bile acid, Na^+ and Ca^{2+} concentrations were significantly decreased over time ($P < 0.05$).

Reabsorption of solutes by the gallbladder epithelium in vivo was calculated under the assumption that no bile was leaving the gallbladder via the cystic bile duct in these fasted animals (see RESULTS). Thus GB(x) at *time T1* was subtracted from $Hep_{output}(x)$ (over *time T0* to *T1*). Similarly, the reabsorption of water was calculated by subtracting GBV at *time T1* from $Hep_{outflow}$ (over *time T0* to *T1*).

Data are presented as means ± SE with one exception: reabsorption rates are presented just as means, because they were calculated from the mean hepatic output and mean gallbladder pool data.

Simple comparisons between means ($P < 0.05$) were performed by student's paired or unpaired *t*-test, as appropriate. The effect of time on gallbladder bile volume, solute concentration, and osmolality was tested using one-factor ANOVA with time as the main variable. The effect of time on hepatic bile flow, solute concentration, and osmolality was tested using repeated-measures ANOVA with time as the main variable. In cases of statistically significant effect of time ($P < 0.05$), a best-fit regression analysis was applied to test for general trends as a function of time. Cases in which the slope of the best-fit regression was significantly different from zero ($P < 0.05$) are noted in figure legends.

RESULTS

Hepatic bile flow and composition. The mean bile flow rate in the common hepatic bile duct was constant at ~75 $\mu l \cdot kg^{-1} \cdot h^{-1}$ during the entire 108 h of experimentation (Fig. 2*A*). There were significant declines in the concentration of both bile acids (Fig. 2*B*) and Na^+ (Fig. 2*D*), which were complete by 60–72 h. Over the same period, the Cl^- concentration rose (Fig. 2*C*), although for Cl^-, the overall effect of time was not significant. Osmolality of the hepatic bile remained constant throughout (Fig. 2*H*). Concentrations of both Mg^{2+} (Fig. 2*F*) and total CO_2 (Fig. 2*G*) were low and constant, whereas Ca^{2+} levels declined significantly by 60–72 h (Fig. 2*E*). Note that for all biliary constituents except bile acids, concentrations in hepatic bile were generally comparable with those in blood plasma (Table 1). Bile acid levels in plasma were below the detection limit of the assay (20 µM) in contrast to 15–50 mM in hepatic bile. This difference was associated with a small but significant elevation in osmolality in hepatic bile (Fig. 2*H*) relative to plasma (Table 1).

Table 1. *Cl^-, Na^+, Ca^{2+}, Mg^{2+}, CO_2, bile acid concentrations, and osmolality of rainbow trout plasma*

	Plasma
Cl^-	133.9 ± 1.1 mM
Na^+	152.0 ± 1.9 mM
Ca^{2+}	2.06 ± 0.06 mM
Mg^{2+}	1.01 ± 0.03 mM
CO_2	7.89 ± 0.51 mM
Osm	297.1 ± 3.25 mOsm
Bile acids	<20 µM

Values are means ± SE; $n = 10$.

Gallbladder bile volume and composition. Total volume of bile in the gallbladder increased from 0.36 ± 0.14 ml/kg at 24 h after feeding to a maximum of 2.46 ± 0.14 ml/kg 120 h after feeding and stayed more or less constant thereafter at ~2 ml/kg for up to 240 h (Fig. 3*A*).

The composition of gallbladder bile was very different from that of hepatic bile. The mean bile acid concentration of the gallbladder bile ranged from 101 to 359 mM and was thus two to seven times higher than the maximum bile acid concentration in bile collected from the hepatic common bile duct. The bile acid concentration tended to increase with time, and the highest concentrations of bile acid in gallbladder bile were found after 168 and 240 h of starvation (Fig. 3*B*).

As for bile acids, the Na^+ concentration was also much higher in the gallbladder bile than in the bile collected from the hepatic common bile duct. The mean Na^+ concentration, measured by atomic absorption, ranged from 243 to 374 mM in the gallbladder bile, approximately twice as high as the Na^+ concentrations of the hepatic bile, and was apparently little affected by the duration of starvation (Fig. 3*D*).

Similarly, the Cl^- concentration of the gallbladder bile did not change over time. However, in contrast to Na^+, the mean Cl^- concentration in the gallbladder bile was much lower (18–31 mM) than the corresponding concentration in the bile collected from the hepatic common bile duct (~140 mM; Figs. 3*C* and 2*C*, respectively).

The mean Ca^{2+} concentration in the gallbladder bile increased gradually with time after feeding (Fig. 3*E*). Thus by the latter half of the experiment, the Ca^{2+} concentration of the gallbladder bile was substantially higher than the corresponding concentration of the bile collected from the hepatic common bile duct (Fig. 2*E*). The mean Mg^{2+} concentration in the gallbladder rose in a similar manner to values substantially higher than the corresponding values from the hepatic bile (Figs. 2*F* and 3*F*).

Total CO_2 concentration in the gallbladder bile was gradually reduced during 240 h of starvation (Fig. 3*G*) and was much lower than the corresponding concentration in the hepatic bile (Fig. 2*G*). Surprisingly, the osmolality of the gallbladder bile remained much lower than might be expected from the concentrations of the main constituents in the gallbladder bile (Fig. 3*H*). Na^+ and bile acid, the two major constituents in the gallbladder bile together amounted to between 400 and 700 mM total concentration throughout the entire 240 h of starvation. The corresponding osmolality, however, remained constant and much lower at around 305 mOsm (Fig. 3*H*), slightly lower than that in bile collected from the common hepatic bile duct (~320 mOsm; Fig. 2*H*), but slightly higher than in blood plasma (Table 1). These differences in osmolality are consistent with a reduction in Na^+ activity by conjugate formation between Na^+ and bile acids (see DISCUSSION).

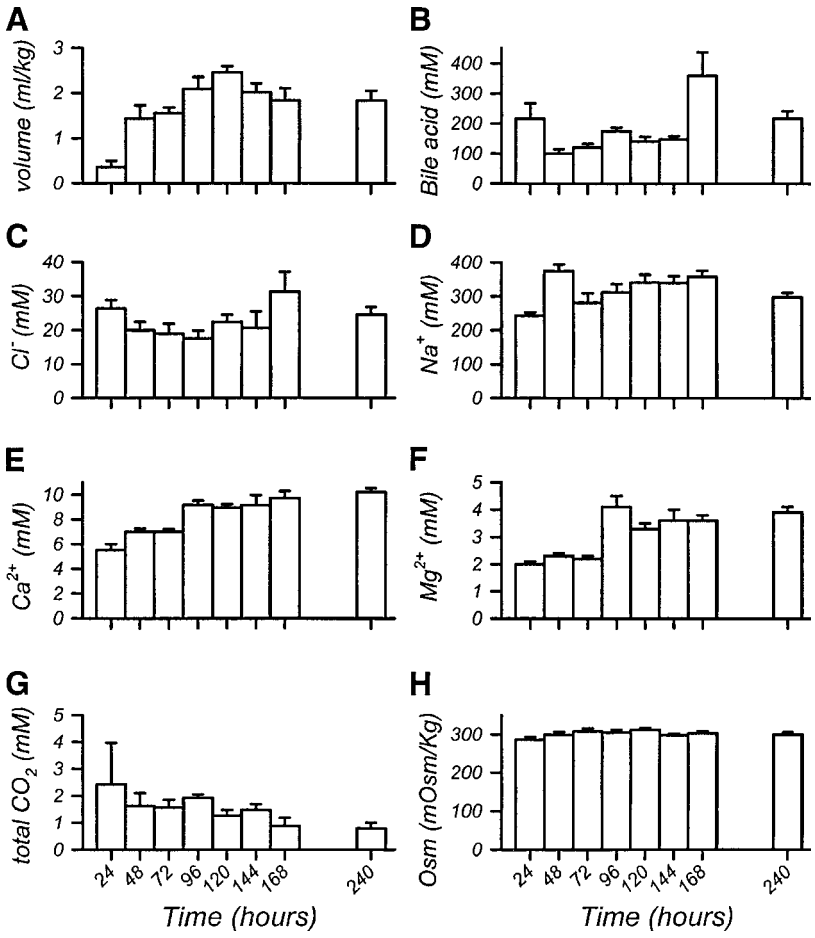

Fig. 3. Gallbladder bile volume (*A*) and concentrations of bile acid (*B*), Cl^- (*C*), Na^+ (*D*), Ca^{2+} (*E*), Mg^{2+} (*F*), total CO_2 (*G*), and osmolality (*H*; mM or mOsm) in gallbladder bile of freshwater rainbow trout for up to 240 h of starvation. Means + SE (n = 10). Volume as well as bile acid, Ca^{2+}, and Mg^{2+} concentrations were significantly increased over time, whereas total CO_2 concentration was decreased ($P < 0.05$).

Fluid and ion reabsorption by the gallbladder epithelium. By relating the accumulated hepatic bile output of fluid ($Hep_{outflow}$) and solutes [$Hep_{output}(x)$] to the corresponding gallbladder pools [GBV and GB(x), respectively] at any given time, the function of the gallbladder epithelium in vivo can be assessed. This is based on the assumption that no bile is leaving the gallbladder via the cystic bile duct during starvation. An almost perfect match between the calculated hepatic bile output of bile acids and the corresponding gallbladder pool during the entire experimental period (Fig. 4*B*) shows that this assumption was justified. For both fluid and all other solutes, it is evident that the gallbladder epithelium of freshwater rainbow trout is indeed an active epithelium involved in reabsorption of solutes and fluid (Fig. 4, *A* and *C-F*). By relating the differences in hepatic output and the corresponding gallbladder pools to the gallbladder epithelium surface area and the time elapsed, the net transport rates of solutes and fluid by the gallbladder epithelium in vivo could be calculated.

These calculations demonstrated that Cl^- was the electrolyte reabsorbed at the highest rate (0.87 $\mu mol \cdot cm^{-2} \cdot h^{-1}$) by the gallbladder epithelium and that this rate was constant regardless of time elapsed since feeding (Fig. 4*C*). Na^+ was reabsorbed by the gallbladder epithelium as well, however, at a lower and more variable rate than Cl^-. Na^+ reabsorption was 0.32 $\mu mol \cdot cm^{-2} \cdot h^{-1}$ averaged over the entire 108 h of starvation (Fig. 4*D*). Fluid reabsorption by the gallbladder epithelium covaried with Na^+ reabsorption, although the relative fluctuations were smaller. The mean fluid reabsorption rate over the entire 108 h was 5.07 $\mu l \cdot cm^{-2} \cdot h^{-1}$. A substantial fraction of the Ca^{2+}, Mg^{2+}, and total CO_2 secreted by the liver into the bile were reabsorbed by the gallbladder epithelium (Fig. 4, *E-G*), but due to the low concentration, the absolute reabsorption rates (expressed as $\mu mol \cdot cm^{-2} \cdot h^{-1}$) were very low. As expected, the gallbladder epithelium did not reabsorb bile acid (Fig. 4*B*). Reabsorption rates for osmolality were not calculated because Na^+-conjugate formation in the bile (see *Ion transport by the gallbladder epithelium*) would result in large overestimation of this reabsorption rate. The average concentrations of Cl^-, Na^+, Ca^{2+}, Mg^{2+}, and CO_2 in the fluid reabsorbed by the gallbladder epithelium over the entire 108 h were 171 mM, 63.1 mM, 0.2 mM, 1.3 mM, and 3.6 mM, respectively.

Na^+ activity versus concentration. Ion-selective electrode measurements revealed a much lower mean activity of Na^+ ions (130 mM) in randomly selected gallbladder bile samples in which total Na^+ concentration averaged 356 mM (Fig. 5). Na^+ activity was not affected by freezing. The composition of rainbow trout plasma is given in Table 1. As for bile, the Na^+ activity was lower than the Na^+ concentration (Fig. 5). How-

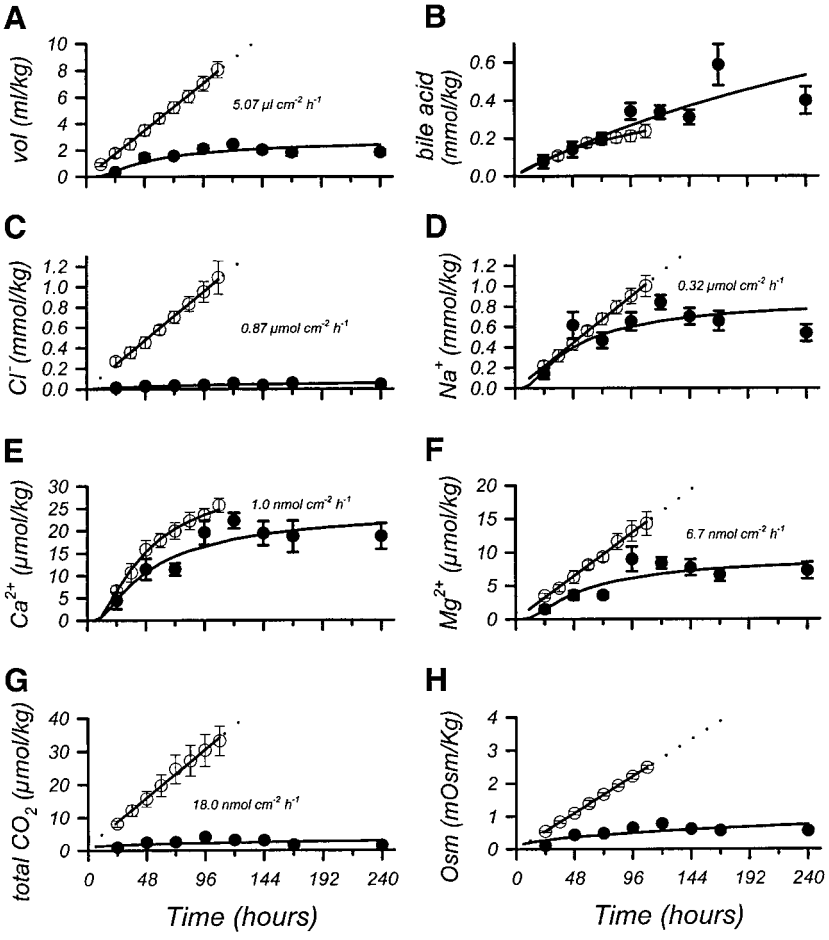

Fig. 4. Comparison of hepatic bile output summed over time (open symbols) and total gallbladder bile (dark symbols) in freshwater rainbow trout for up to 108 and 240 h, respectively. *A*: hepatic bile flow (ml/kg) and gallbladder bile volume (ml/kg). Hepatic output of bile acid (*B*), Cl^- (*C*), Na^+ (*D*; all in mmol/kg), osmolality (*E*; mOsm/kg), Ca^{2+} (*F*), Mg^{2+} (*G*), and total CO_2 (*H*; all in µmol/kg) for up to 108 h together with total amount of bile acid, Cl^-, Na^+, total CO_2, osmolality (mOsm), Ca^{2+} and Mg^{2+} present in the gallbladder for up to 240 h of starvation. Mean reabsorption rates over 108 h of fluid ($\mu l \cdot cm^{-2} \cdot h^{-1}$), Cl^-, Na^+ ($\mu mol \cdot cm^{-2} \cdot h^{-1}$), Ca^{2+}, Mg^{2+}, and CO_2 ($nmol \cdot cm^{-2} \cdot h^{-1}$) are reported in *A-G*, respectively. Bile acid was not reabsorbed by the gallbladder epithelium. Reabsorption of osmolality equivalents are not reported because Na-conjugate formation would result in large overestimates of this reabsorption rate.

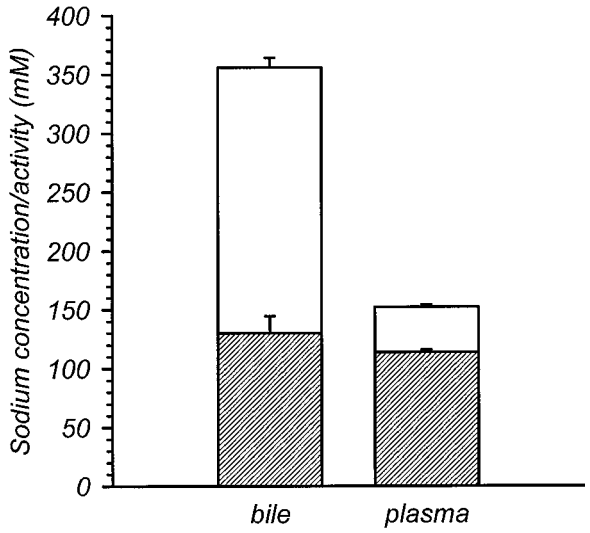

Fig. 5. Total Na^+ concentration as assayed by atomic absorption (open bars) and Na^+ activity as assayed by Na ion-selective electrode (hatched bars; both in mM) in randomly selected gallbladder bile samples ($n = 7$) and plasma samples ($n = 10$).

ever, the relative difference between the total Na^+ concentration and the Na^+ activity in plasma was much lower than in gallbladder bile (Fig. 5).

To test the specific effect of bile acid on Na^+ activity, the effect of hydrocholic acid (100 and 200 mM) addition to the apical saline was evaluated. Addition of 100 mM hydrocholic acid (Na salt) to Cortland saline increased the Na^+ activity by only 29 mM in contrast to an expected increase of ~100 mM.

TEP in vivo and in vitro. The potential across the gallbladder epithelium in vivo was approximately −13 mV (bile side negative relative to blood; Fig. 6). The same potential was recorded in isolated gallbladders containing bile, with saline as a reference. In contrast,

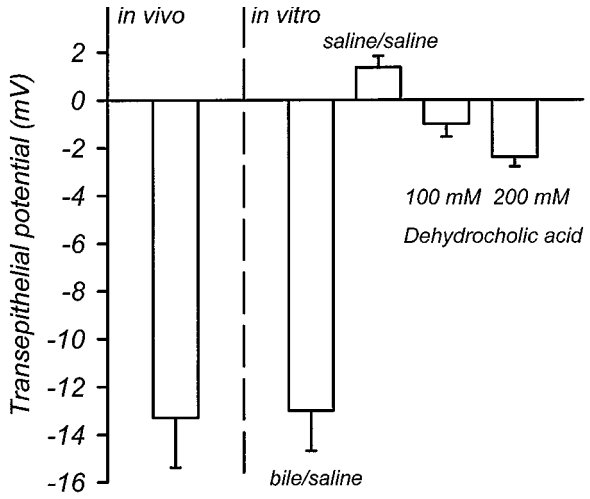

Fig. 6. Transepithelial potential of the rainbow trout gallbladder in vivo ($n = 7$) and in vitro, under symmetrical saline/saline conditions ($n = 8$), under (saline + 100 mM hydrocholic acid)/saline conditions ($n = 5$), (saline + 200 mM hydrocholic acid)/saline conditions ($n = 5$), and under bile/saline conditions ($n = 8$). In all cases, manipulations were performed on the bile side (mucosal surface); saline was retained on the blood side (serosal surface).

the potential was approximately +1.5 mV (i.e., bile positive) in isolated gallbladders when the bile was replaced with saline, i.e., under symmetrical conditions (Fig. 6). In several isolated gallbladders, the original bile side negative potential (approximately −13 mV) was completely restored when the saline was replaced with the original bile.

The addition of 100 and 200 mM hydrocholic acid (Na salt) to the luminal saline under saline/saline conditions reversed the blood side negative TEP. The effect of hydrocholic acid on TEP exhibited concentration dependence, reversing the TEP to −1.0 and −2.4 mV, bile side negative at 100 and 200 mM, respectively (Fig. 6).

DISCUSSION

Hepatic bile flow and composition. The technique employed for collecting hepatic bile from the common hepatic bile duct revealed a constant hepatic bile flow in resting starved restrained rainbow trout for up to 108 h after cannulation. Thus progressive starvation from 36 h after last feeding (starvation time before surgery) throughout this study did not influence hepatic bile flow. The mean bile flow rate of ~75 $\mu l \cdot kg^{-1} \cdot h^{-1}$ observed in the present study is consistent with values of 50–75 $\mu l \cdot kg^{-1} \cdot h^{-1}$ previously reported for rainbow trout (12, 30). The latter flow rates were obtained from both spinally transected and free-swimming rainbow trout during experimental periods of a few hours, and different surgical procedures were employed. The constant bile flow rate over 108 h observed in the present study thus seems realistic. Because it is at the high end of the previously reported values, we were apparently successful in preventing contact between the hepatic bile and the gallbladder epithelium and thereby preventing reabsorption of fluid from the hepatic bile. The observed bile flow rate in freshwater rainbow trout is comparable with bile flow rates of two marine elasmobranchs, the spiny dogfish, *Squalus acanthias*, with a flow rate of 33–74 $\mu l \cdot kg^{-1} \cdot h^{-1}$ and the skate, *Raja erinacea*, with a rate of 75–111 $\mu l \cdot kg^{-1} \cdot h^{-1}$ (2). The only bile flow rates reported from elasmobranchs and teleosts are considerably lower than the bile flow rates of higher vertebrates: the rat with a bile flow rate of 3,900; rabbit, 4,920; dog, 336; cat, 780; guinea pig, 9,600; and finally humans with a bile flow rate of 216 $\mu l \cdot kg^{-1} \cdot h^{-1}$ (20, and references therein). At least in part, this difference could be related to temperature, because bile flow is generated by the active transport of bile acids and other organic anions and cations. The studies of bile flow in elasmobranchs and teleost fish were conducted at temperatures at least 20°C lower than the mammalian studies. Indeed, increased temperature has been reported to increase hepatobiliary excretion of the bile acid, taurocholate, in rainbow trout (6, 19).

The concentration of Mg^{2+} and total CO_2 remained relatively constant in hepatic bile throughout the 108 h of experimentation. In contrast however, the bile acid concentration was markedly reduced, and the Na^+ and Ca^{2+} concentrations somewhat decreased as time progressed. Interestingly, the fall in bile acid concentration

was balanced by an approximately equimolar increase in Cl^- concentration. With the exception of the bile acid concentration, the concentrations of all measured ions were similar to the corresponding ion concentrations in the plasma. This is in close agreement with observations reported for the above mentioned species of elasmobranchs and mammals. Despite the similarity in ionic concentrations, the osmolality of the hepatic bile was higher than plasma osmolality. This difference can be accounted for by the high concentration of bile acid in the hepatic bile compared with the very low concentration in plasma (<20 μM), which provides part of the driving force for paracellular diffusion of water and electrolytes, thereby contributing to the bile flow (1, 5, 20, 22, 33).

In higher vertebrates, two components of bile formation have been documented extensively: *1*) bile acid-dependent bile flow and *2*) bile acid-independent bile flow (for reviews, see Refs. 1, 5, and 22). In brief, transcellular, carrier-mediated transport of bile acids (bile acid-dependent bile flow) and of other organic anions as well as HCO_3^- and organic cations (bile acid-independent flow) creates a hyperosmotic environment in the bile canaliculi. This hyperosmotic environment drives a passive paracellular movement of water and electrolytes from the blood plasma into the bile. Consequently, the electrolyte composition of hepatic bile has been shown to parallel that of the blood plasma (in vivo studies) or perfusate (in vitro studies).

The bile acid concentration in the hepatic bile of rainbow trout in the present study (15–50 mM) is within the range reported for mammals (20, 33), and a bile acid-dependent component to bile flow in rainbow trout seems likely. The reduced bile acid concentration and thereby reduced hepatic bile acid output observed as time progressed was, however, not parallelled by a reduced bile flow. This could indicate that even the lowest bile acid concentration observed (15 mM) was sufficient for maintaining a constant bile flow, or that the bile acid-independent component to bile flow could account for most, if not all, of the bile formation. It is interesting that the osmolality of the hepatic bile remained constant throughout the experimental period despite the marked reduction in bile acid concentration. This could indicate that the concentration of other components, such as Cl^- (which was in fact increased), glutathione, bilirubin, and/or organic cations (all involved in the bile acid-independent bile flow) were increased in compensation for the reduced bile acid concentration.

Gallbladder bile volume and composition. In the present study, progressive starvation of the experimental animals was used as a tool to collect bile in the gallbladder. The conservation of bile acid accumulation in the gallbladder (Fig. 4*B*) demonstrate that the approach was successful.

The maximal gallbladder bile volume in the rainbow trout is comparable with volumes reported for European eel, *Anguilla anguilla* (14), and a number of other marine and freshwater teleost fish species after 5–10 days of starvation (M. Grosell, C. M. Wood, and F. B. Jensen, unpublished data). The ionic composition of the gallbladder bile is very similar to compositions reported for other teleost fish species, reptiles, amphibians, and mammals (8, 18). For these organisms, gallbladder bile is characterized by Na^+ concentrations exceeding those in hepatic bile and plasma and Cl^- concentrations much lower than than those in hepatic bile and plasma. The absolute concentrations of Na^+ and Cl^- in plasma, and thus in hepatic bile of marine elasmobranchs, are quite different from those of higher vertebrates, including teleost fish, due to their unique osmoregulatory stategy. The relative relationships, however, are the same as in the higher vertebrates, i.e., higher Na^+ and lower Cl^- concentrations of the gallbladder bile.

Ion transport by the gallbladder epithelium. All these findings indicate that the gallbladder epithelium in vivo has a higher net transport of Cl^- than of Na^+. Furthermore, these observations suggest that the mechanism of this asymmetrical anion-to-cation transepithelial transport ratio could be similar throughout a wide phylogenetic range of vertebrates. This analysis is in contrast to all reported findings of ion-transport properties of isolated gallbladder epithelia determined under "symmetrical" conditions in vitro in the absence of bile acid on the mucosal side (see introduction) and cannot be explained by the current proposed transport models. Consequently, we propose an alternative model for transepithelial ion transport based on the present analysis of in vivo ion transport by the gallbladder epithelium of freshwater rainbow trout (Fig. 7).

The classic work by Diamond (8–10) demonstrated that the gallbladder of teleost fish, when isolated, absorbs an isotonic NaCl solution. This has since been shown also to apply to mammalian gallbladders (see

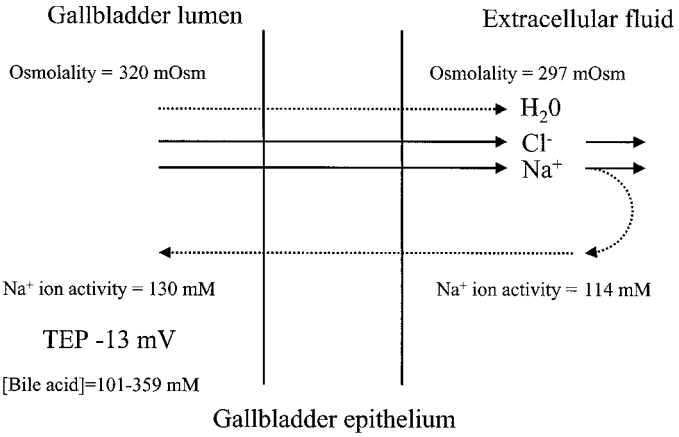

Fig. 7. Proposed transepithelial ion-transport model for vertebrate gallbladder epithelium in vivo. Directions of fluxes observed in steady state are depicted. Na^+ and Cl^- are extruded from the cell across the basolateral membrane. Na^+ diffuses back across the gallbladder epithelium into the bile because of the relatively low (compared with plasma) Na^+ activity of the gallbladder bile and the negative transepithelial potential (TEP) resulting from the Donnan effect of bile acids that complex Na^+. Thus there is transepithelial Na^+ recycling. It should be noted that this model can account for a transepithelial Cl^- transport higher than the corresponding net Na^+ transport as observed in vivo.

Refs. 27 and 28 for comprehensive reviews). Reuss (27, 28) proposed a model of ion transport by the gallbladder epithelium based primarily on data obtained from *Necturus* and the guinea pig. In the Reuss model, Cl^- is transported in exchange for HCO_3^- and Na^+ in exchange for H^+ into the epithelial cell across the apical membrane. The catalytic action of carbonic anhydrase in the gallbladder reforms CO_2, which diffuses back into the cell where it is hydrated and refuels the cycle of Cl^-/HCO_3^- and Na^+/H^+ exchange. Na^+ is extruded across the basolateral membrane by the Na^+/K^+-ATPase in a 3:2 exchange for K^+, and K^+ subsequently acts together with Cl^- as a substrate for a basolateral K^+/Cl^- cotransporter mediating Cl^- transport across the basolateral membrane. To account for a 1:1 transepithelial Na^+/Cl^--transport ratio in this model, in which two K ions are available for K^+/Cl^- cotransport while three Na ions are extruded across the basolateral membrane, Reuss suggests diffusion of Na^+ back across the basolateral membrane to refuel the Na^+/K^+-ATPase. This model proposed by Reuss (27, 28) elegantly accounts for the transepithelial net Na^+/Cl^--transport ratio observed in vitro under symmetrical conditions. It comes short, however, in explaining the evidently higher net Cl^- transport than Na^+ transport in most (if not all) vertebrates in vivo in which bile rather than saline is present at the apical surface of the gallbladder epithelium.

We propose a transepithelial rather than a basolateral membrane recycling of Na^+ to account for the low Na^+ net flux compared with Cl^- net flux. In this model, Na^+ and Cl^- are extruded across the basolateral membrane and Na^+, in part, diffuses back across the epithelium. This diffusion of Na^+ back across the epithelium reduces the net Na^+ flux across the epithelium and can thus account for a lower Na^+ than Cl^- transepithelial net flux. At first glance, the diffusion of Na^+ from the lower plasma Na^+ concentration (152 mM) to the apparently higher Na^+ concentration in the gallbladder bile (350 mM) seems unlikely. This diffusion, however, is possible because of the low free Na^+ activity in the gallbladder bile (130 mM in bile vs. 114 mM in plasma; Fig. 5) and the TEP of −13 mV (bile side negative). The equilibrium potential required for maintaining this observed Na^+ activity gradient is approximately −7 mV (bile side negative). Thus there are thermodynamically favorable conditions for the proposed Na^+ backflux. The low Na^+ activity and reduction in osmolality in gallbladder bile observed in the present study is in agreement with observations reported by Diamond (8) and is due to the formation of bile acid-Na^+ conjugates. Our observation that the addition of 100 mM hydrocholic acid (as Na salt) to saline only increased the Na^+ activity by 29 mM demonstrates this Na-conjugate formation in the presence of bile acid acid.

The TEP of −13 mV (bile side negative) in the presence of bile at the apical surface is unusually high for a leaky epithelium and cannot be explained by the higher net Cl^- than Na^+ transport. The TEP is abolished when bile is replaced with saline, indicating that the presence of one or more components of gallbladder bile causes a diffusion or Donnan potential. Impermeant anions have previously been suggested to be the reason for the lumen negative potential observed in isolated rat hepatocyte couplets (13). If the substantial cation-to-anion gap in gallbladder bile is considered, the presence of an anion other than Cl^- in gallbladder bile seems highly likely, and bile acids are a probable candidate. The concentration of impermeant anions needed to explain the bile negative TEP of −13 mV can be estimated to be ~50 mM from the following equation (1)

$$e^{[(FR/T)(\mathrm{TEP})]} = (1 + \mathrm{A}^-/\mathrm{Cl}^-)^{1/2}$$

where A^- is the impermeant anion, F is Faraday's constant, R the gas constant, T is absolute temperature, and the Cl^- concentration is set to 25 mM, the mean value at 240 h (see Fig. 3). Bile acids could account for this 50 mM concentration of impermeant anions. The average concentration of bile acid in the gallbladder bile was variable (101–359 mM) but averaged ~220 mM at 240 h. This value is substantially higher than the concentration of impermeant anions needed to explain the TEP. However, Na^+-bile acid conjugate formation reduced the free Na^+ activity by ~65% (Fig. 5). Similarly, the activity of the bile acid anion must be comparably reduced by Na^+-bile acid conjugate formation, which leaves a predicted bile acid free anion concentration close to the 50 mM of impermeant unknown anion needed to explain the TEP of −13 mV. In isolated gallbladders, the addition of bile acid to the luminal saline under saline/saline conditions revealed that this impermeant anion can indeed reverse the TEP from blood side negative to bile side negative. Even addition of 200 mM hydrocholic acid (as Na salt), however, was not as potent as gallbladder bile in terms of generating bile side negative TEP. This could be due to the difference in ionic composition of the saline and gallbladder bile and/or different characteristics of bile acids, but could also indicate that other components in gallbladder bile could act as impermeant anions.

On the basis of the observed ion fluxes, Cl^- transport clearly exceeds the total recorded cation transport. Not all cations were, however, included in the present study. K^+ could have contributed to the total cation transport, but, given the low K^+ concentration in blood plasma and thus in hepatic bile, it is unlikely that K^+ alone could account for the cation gap. Possibly, net acidic equivalent transport (not measured) could explain the remaining cation gap maintaining macroscopical electroneutrality. The observed cation gap was ~0.4 $\mu mol \cdot cm^{-2} \cdot h^{-1}$, and the gallbladder surface area was 11 cm^2/kg, which would be associated with a net acid flux of only 4.4 $\mu Eq \cdot kg^{-1} \cdot h^{-1}$ from bile to blood, which would be negligible relative to the net acidic equivalent flux of the whole animal during starvation (36).

The water reabsorption of the gallbladder in vivo in the present study was 4.5–6.0 $\mu l \cdot cm^{-2} \cdot h^{-1}$, which is

approximately half of the values reported by Hirano and Bern (16) and Diamond (10) for isolated gallbladders of a number of teleost fish species under symmetrical saline/saline conditions in vitro. The osmolality of the rainbow trout gallbladder bile is similar but slightly higher than the corresponding plasma osmolality (a difference of 7–10 mOsm). If assumed that the interstitial fluid and plasma osmolalities are similar, it thus appears that water is transported against an osmotic gradient. Water transport against an osmotic gradient was also observed by Diamond (10) in isolated gallbladders of teleost fish. Diamond tested the effect of osmolality difference on the water transport rate and found water transport against an osmotic gradient of up to 30 mOsm. At an osmotic difference of 10 mOsm as in the rainbow trout in vivo, Diamond (10) found water transport rates of 7–8 $\mu l \cdot cm^{-2} \cdot h^{-1}$, in close agreement with the in vivo water transport rates determined in this study.

Perspectives

The present investigation suggests that the currently accepted model for ion transport by the gallbladder may not apply to in vivo conditions. Further studies on the ion-transport properties of the gallbladder epithelium are needed to verify our proposed model. These studies would have to employ isolated gallbladder epithelia but should now include the potential role of bile acids and other gallbladder bile components in modulating transepithelial net ion fluxes and TEP. The influence of temperature and bile acid concentration on hepatic bile flow in lower vertebrates remains unclear. Studies employing intravenous infusion of bile acids combined with continuous hepatic bile collection at different temperatures are needed to evaluate the presence of a bile acid-dependent bile flow and temperature-dependent bile flow in lower vertebrates.

We greatly appreciate the excellent technical assistance of Erin Fitzgerald.

This work was supported by a Danish National Research Council grant (9700849) to M. Grosell and National Sciences and Engineering Research Council research grants to C. M. Wood and M. J. O'Donnell.

Address for reprint requests and other correspondence: M. Grosell, McMaster Univ., Dept. of Biology, 1280 Main St. West, Hamilton, ON, Canada L8S 4K1 (E-mail: grosellm@mcmaster.ca).

Received 2 June 1999; accepted in final form 11 January 2000.

REFERENCES

1. **Boyer JL, Graf J, and Meier PJ.** Hepatic transport systems regulating pH_i, cell volume and bile secretion. *Annu Rev Physiol* 54: 415–438, 1992.
2. **Boyer JL, Schwarz J, and Smith N.** Biliary secretion in elasmobranchs. I. Bile collection and composition. *Am J Physiol* 230: 970–973, 1976.
3. **Boyer JL, Schwarz J, and Smith N.** Biliary secretion in elasmobranchs. II. Hepatic uptake and biliary excretion of organic anions. *Am J Physiol* 230: 974–981, 1976.
4. **Brown AC.** Biophysics of transport across membranes. In: *Physiology and Biophysics* (19th ed.), edited by Ruch TC and Patton HD. Philadelphia and London: W. B. Saunders, 1965, 820–842.
5. **Curtis BJ and Wood CM.** Renal and urinary bladder responses of trout to isosmotic NaCl and $NaHCO_3$ loading. *J Exp Biol* 173: 181–203, 1992.
6. **Curtis LR, Kemp CJ, and Svec AV.** Biliary excretion of [^{14}C]taurocholate by rainbow trout (*Salmo gairdneri*) is stimulated at warmer acclimation temperature. *Comp Biochem Physiol* 84C: 87–90, 1986.
7. **Denton JE, Yousef MK, Yousef IM, and Kuksis A.** Bile acid composition of rainbow trout, *Salmo gairdneri*. *Lipids* 9: 945–951, 1974.
8. **Diamond JM.** The reabsorptive function of the gallbladder. *J Physiol* (*Lond*) 161: 442–473, 1962.
9. **Diamond JM.** The mechanism of solute transport by the gallbladder. *J Physiol* (*Lond*) 161: 474–502, 1962b.
10. **Diamond JM.** The mechanism of water transport by the gallbladder. *J Physiol* (*Lond*) 161: 503–527, 1962c.
11. **Fricker G, Hugentobler G, Meier PJ, Kurz G, and Boyer JL.** Identification of a single sinusoidal bile salt uptake system in skate liver. *Am J Physiol Gastrointest Liver Physiol* 253: G816–G822, 1987.
12. **Gingerich WH, Weber LJ, and Larson RE.** Hepatic accumulation, metabolism and biliary excretion of sulfobromophthalein by rainbow trout (*Salmo gairdneri*). *Comp Biochem Physiol* 58C: 113–120, 1977.
13. **Graf J, Henderson RM, Krumpholz B, and Boyer JL.** Cell membrane and transepithelial. Voltages and resistance in isolated rat hepatocyte couplets. *Membrane Biol* 95: 241–254, 1987.
14. **Grosell MH, Hansen HJM, and Rosenkilde P.** Cu uptake, metabolism and elimination in fed and starved European eels (*Anguilla anguilla*) during adaptation to water-borne Cu exposure. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 120: 295–305, 1998.
15. **Grosell MH, Hogstrand C, and Wood CM.** Cu uptake and turnover in both Cu acclimated and non-acclimated rainbow trout *(Oncorhynchus mykiss*). *Aquat Toxicol (Amst)* 38: 257–276, 1997.
16. **Hirano T and Bern HA.** The teleost gall bladder as an osmoregulatory organ. *Endocrinol Jpn* 19: 41–46, 1972.
17. **Hokanen RE and Patton JS.** Bile salt absorption in killifish intestine. *Am J Physiol Gastrointest Liver Physiol* 253: G730–G736, 1987.
18. **Hunn JB.** Inorganic composition of gallbladder bile from freshwater fishes. *Copeia* 3: 602–605, 1975.
19. **Kemp CJ and Curtis LR.** Thermally modulated biliary excretion of [^{14}C]taurocholate in rainbow trout (*Salmo gairdneri*) and the Na^+,K^+-ATPase. *Can J Fish Aquat Sci* 44: 846–851, 1987.
20. **Klaassen CD and Watkins JB.** Mechanisms of bile formation, hepatic uptake, and biliary excretion. *Pharmacol Rev* 36: 1–67, 1984.
21. **Miller DS, Fricker G, Schramm U, Henson JH, Hager DN, Nundy S, Ballartori N, and Boyer JL.** Active microtubule-dependent secretion of a fluorescent bile salt derivative in skate hepatocyte clusters. *Am J Physiol Gastrointest Liver Physiol* 270: G887–G896, 1996.
22. **Nathanson MH and Boyer JL.** Mechanisms and regulation of bile secretion. *Hepatology* 14: 551–566, 1991.
23. **Neher E.** Correction for liquid junction potentials in patch-clamp experiments. *Methods Enzymol* 207: 123–131, 1992.
24. **O'Donnell MJ, Dow JAT, Huesmann GR, Tublitz NJ, and Maddrell SHP.** Separate control of anion and cation transport in Malpighian tubules of *Drosophila melanogaster. J Exp Biol* 199: 1163–1175, 1996.
25. **Råberg CMI, Ziegler K, Isomaa B, Lipsky MM, and Eriksson JE.** Uptake of taurocholic acid and cholic acid in isolated hepatocytes from rainbow trout. *Am J Physiol Gastrointest Liver Physiol* 267: G380–G386, 1994.
26. **Reed JS, Smith ND, and Boyer JL.** Determinants of biliary secretion in isolated perfused skate liver. *Am J Physiol Gastrointest Liver Physiol* 242: G319–G325, 1982.
27. **Reuss L.** Ion transport across gallbladder epithelium. *Physiol Rev* 69: 503–545, 1989.
28. **Reuss L.** Salt and water transport by the gallbladder epithelium. In: *Handbook of Physiology. The Gastrointestinal System*. Bethesda, MD: Am. Physiol. Soc., 1991, sect. IV, p. 303–322.

29. **Robinson RA and Strokes RH.** *Electrolyte solutions* (2nd ed.). London: Butterworths, 1970.
30. **Schmidt DC and Weber LJ.** Metabolism and biliary excretion of sulfobromophthaein by rainbow trout (*Salmo gairdneri*). *J Fish Res Board Can* 30: 1301–1308, 1973.
31. **Smith DJ, Grossbard M, Gordon ER, and Boyer JL.** Isolation and characterization of a polarized isolated hepatocyte preparation in the skate, *Raja erinacea*. *J Exp Zool* 241: 291–296, 1987a.
32. **Smith DJ, Grossbard M, Gordon ER, and Boyer JL.** Taurocholate uptake by isolated skate hepatocytes: effect of albumin. *Am J Physiol Gastrointest Liver Physiol* 252: G479–G484, 1987.
33. **Strange RC.** Hepatic bile flow. *Physiol Rev* 64: 1055–1102, 1984.
34. **Wolf K.** Physiological salines for freshwater teleosts. *Prog Fish Cult* 25: 135–140, 1963.
35. **Wood CM.** Excretion. In: *Physiology and Ecology of the Pacific Salmon*, edited by Groot C, Margolis L, and Clarke WC. Vancouver: UBC Press, 1995.
36. **Wood CM.** Acid-base and ionic exchanges at the gills and kidney after exhaustive exercise in the rainbow trout. *J Exp Biol* 136: 461–481, 1988.
37. **Zall DM, Fisher D, Garner MD.** Photometric determination of chlorides in water. *Anal Chem* 28: 1665–1678, 1956.