animal treated with hormone showed a decline in BSL following hormone injection, whereas control animals did not. The decline began within 30-60 minutes and BSL was significantly ($p < .05$) depressed by six hours post injections in both of these species. Mean absolute values of blood sugar ranged from 89.46 ± 11.40 (S.D.) mg% at 0 time to 31.24 ± 6.71 mg% at 24 hours for S. acanthias and 42.06 ± 4.95 mg% at 0 time to 13.42 ± 2.85 mg% at 48 hours for R. erinacea. Relaxin, a polypeptide hormone with similar tertiary structure to insulin (Schwabe et al., Ann. N.Y. Acad. Sci. 380:6-12, 1982), had no significant effect on BSL in these species in the same experimental design and conditions. Supported by NSF PCM 8104144 to I.P.C.

THE CANALICULAR PERMEABILITY BARRIER IN THE SMALL SKATE (RAJA ERINACEA)

S.O. Goos, S. Bradley and J.L. Boyer, Mount Desert Island Biological Laboratory, Salsbury Cove, Me ., and Department of Medicine, Yale School of Medicine, New Haven, Ct.

Among vertebrates bile formation appears at the onset to require movement of water and solute from sinusoidal perfusate across the hepatocellular plates to canalicular lumina. The dynamics of this process remains obscure although considerable evidence (Boyer, J., Review in Physiological Reviews 60:303-326, 1980) suggests that an osmotic pressure gradient produced by active secretion of bile salts into the canaliculi provides an energy source for directed fluid movement. Recent work in this laboratory (Reed, J.S., et al., Amer. J. Physiol. 242:G313, 1982) has added evidence that an hydraulic pressure gradient may also be operative in selachians. These gradients impell movement by routes across the hepatocytes and between them. Transcellular flows traverse a complicated succession of membrane and cytoplasmic barriers whereas paracellular flow is impeded only by the junctional complexes and thus presents a simpler system for study. In rats and dogs, simultaneous measurements of the biliary clearances of molecular weight matched molecular species, one anionic, one neutral (^{3}H-sucrose (S) and sodium ^{14}C-ferrocyanide (F) or ^{3}H-methoxy- and ^{14}C-carboxyl-inulin (H,C) yield data (Bradley, S.E. and Herz, R., Amer. J. Physiol., 235:E570, 1978) consistent with a relatively impermeable, negatively charged canalicular junctional barrier that selectively retards passive anionic movement. This approach has been employed in a study of bile formation in excised perfused liver of small skates (Raja erinacea) during changes in bile flow produced spontaneously or by changes in perfusion pressure in order to assess the properties and function of the canalicular membrane.

METHODS--Bile was collected from skate livers, isolated and perfused by 125 ml of Elasmobranch Ringers as previously described by Reed et al (ibid) with the following modifications. Gallbladder drainage was deferred until after isolation of the liver and establishment of adequate portal perfusion with the liver ventral sideup. The gallbladder was incised from the apex to the base. The flap over the common cystic duct on the dorsal side of the gallbladder was identified and a flared 3 cm length of PE205 tubing bent at a right angle was inserted under it. This cannula was secured by 5-0 silk sutured into the gallbladder wall close to the origin of the cystic duct. A preweighed 10 cm length of PE240 tubing was inserted over the PE205 tubing for bile collections. The liver was then inverted to prevent its weight from compressing portal vein, hepatic vein sinuses, and bile ducts.

Bile flow and labelled markers were measured during 3 experimental periods. During the initial period perfusion pressure was established at 10 cm Ringers. Approximately 25 µl of bile was collected in tared PE240 cannulas at intervals that depended on the rate of bile flow (5 to 60 min). After 2-5 collection periods, perfusion pressure was reduced to 5 cm Ringers for 2-5 additional collection periods and then again adjusted to 10 cm Ringers for the final period. In one of 11 experiments this sequence was reversed. The duration of experiments ranged from 3-7 hours.

The 125 ml recirculating fluid contained: sodium ^{14}C-ferrocyanide (25 µCl, NEN, 7135 mCl/m mol) and ^{3}H sucrose (5 µCl, NEN, 11.4 Cl/m mold) in six experiments; and ^{14}C carboxyl inulin (10 µCl, NEN, 2.1 mCl/g) and ^{3}H methoxy inulin (10 µCl 140.5 mCl/g) in five experiments. Perfusate samples (50 µl) were taken in duplicate, 1-4 times during the course of each experimental period and counted in 6 ml of scintillation fluid (Aquasol or

Redisolve). Aliquots of bile (approximately 25 µl) from each collection period were counted similarly.

Table I

Control Biliary Concentration Ratios and Clearances of Methoxy- and Carboxyl-Inulins (C,H) and Ferrocyanide (F)-Sucrose (S) In Isolated Perfused Liver of the Small Skate.

Expt.No.	Vw	B/P_C	B/P_H	C_C	C_H	C_C/C_H
	ul/min			ul/min	ul/min	
21	4.33	0.05	0.09	0.21	0.39	0.53
23	0.60	0.42	0.45	0.25	0.27	0.93
24	1.20	0.61	0.61	0.74	0.74	1.00
25	0.76	0.58	0.57	0.44	0.43	1.01
26	1.32	0.62	0.65	0.81	0.86	0.94
$\bar{X}$	1.65	0.46	0.48	0.49	0.54	0.88
±SD	.53	0.24	0.23	0.27	0.25	0.20
		$B/_F$	B/P_S	C_F	C_S	C_F/C_S
15	7.24	0.29	0.32	2.13	2.30	0.93
17	1.39	0.44	0.72	0.61	1.00	0.61
18	0.38	0.46	0.49	0.18	0.19	0.94
19	0.69	0.68	0.58	0.47	0.40	1.18
20	0.67	0.67	0.62	0.45	0.42	1.07
27	0.44	0.65	0.59	0.28	0.26	1.10
$\bar{X}$	1.80	0.53	0.55	0.69	0.76	0.97
±SD	2.69	0.59	0.14	0.72	0.81	0.20

Abbreviations: Vw; weighted mean bile flow (total flow during delay period divided by delay time). B/P; biliary - perfusate radioactivity ratio; C-clearance.

RESULTS AND DISCUSSION--In all experiments, perfusate radioactivities changed relatively very little throughout although a regular tendency to fall was observed. In contrast, biliary radioactivities rose slowly to a more-or-less persistent equilibrium level over a period of 67 to 284 minutes (averaging 155 ± SD 68 minutes, n=11). To correct for this prolonged delay, bile-perfusate concentration ratios (B/P) were computed using mean values for biliary radioactivities during a period of equilibrium ranging from 50 to 209 minutes (138 ± 56.2 minutes). On the assumption that intrahepatic biliary transit times are normally distributed in the skate and that transit for all contributing biliary units had been completed at the end of the observed delay period, the mean biliary radioactivity for each marker was divided by its mean perfusate radioactivity during the delay period to obtain its B/P ratio (Table 1). To assess the relationship between bile flow and these values, weighted mean values for bile flow (Vw) during the delay period were computed from total bile flow and duration of that period.

Analysis of the values thus compiled and presented in Table 1 demonstrated no significant difference between B/P ratios for C,H,F or S, which averaged 0.46, 0.48, 0.53 and 0.55 respectively. These findings suggest that paracellular flow may comprise approximately one-half the total bile flow assuming that these solutes enter bile via paracellular rather than transcellular routes. It was further noted that both neutral and anionic molecular B/P ratios were significantly and inversely correlated with Vw ($p < 0.01$ for C and H, $p < 0.001$ for F and S). These findings are consistent with a diffusion dependent component for entry of solutes into bile as well as solvent drag, at these very low bile flow rates obtained in the skate (Reed, JS et al,: Amer. J. Physiol.). Although both Vw and B/P varied markedly from experiment to experiment so that clearances varied widely in both series, the anionic-neutral clearance ratios were nevertheless close to unity in all but two studies (Exp. 21 for C_C/C_H, Expt. 17 for C_F/C_S), averaging 0.88 ± 0.20 for all values of C_C/C_H and 0.97 + 0.20 for C_F/C_S, indicating little if any bioelectric interference with relative anionic movement into the bile.

Owing to the variability within and between livers it was difficult to assess the effect of changes in perfusion pressure upon C,H,F, and S excretion. The data in Table 2 obtained in 10 studies in which the pressure effect could

Table II

Bile Flow During Changes In Perfusion Pressure In Isolated Perfused Liver of the Small Skate

(All values are $\bar{X}$ ± SD, (n) = number of successive clearance periods averaged)

	Perfusion Pressure - cm. Ringer					
Expt. No.	10 cm		5 cm		10 cm	
15	7.24 ± 1.25	(6)	3.74 ± 1.42	(7)	3.73 ± 1.04	(4)
17	2.01 ± 0.95	(4)	1.06 ± 0.58	(4)	3.24 ± 3.49	(3)
18	0.40 ± 0.14	(4)	0.33	(1)	-	
19	0.86 ± 0.40	(4)	0.87 ± 0.28	(4)	0.40 ± 0.45	(2)
20	0.67 ± 0.03	(3)	0.42 ± 0.23	(3)	0.67 ± 0.09	(2)
21	4.33 ± 1.30	(6)	3.75 ± 1.28	(5)	1.92	(1)
24	1.92 ± 0.54	(6)	0.61 ± 0.07	(4)	0.71 ± 0.08	(2)
25	1.06 ± 0.27	(4)	0.58 ± 0.06	(3)	0.94 ± 0.20	(3)
26	1.32 ± 0.13	(4)	0.93 ± 0.06	(3)	1.02 ± 0.11	(3)
27	-		0.44 ± 0.06	(4)	0.56± 0.16	(2)
$\bar{X}$ ± S.D.	2.20 ± 2.22	(9)	1.27 ± 1.32	(10)	1.52 ± 1.19	(9)

be followed, clearly show a tendency for average bile flow (V) to fall when perfusion pressure was reduced and to rise when it was elevated. The figures for V were compiled for successive clearance periods in order to define the extent of variation in V during each pressure interval. Average V fell in all but one experiment when perfusion pressure was reduced from 10 to 5 cm Ringers but did so significantly (> 2 SD) in only two. These changes appeared

to exert no obvious effect upon B/P ratios; however further analysis and appropriate collection for delay will be necessary for more precise evaluation.

The findings in this study support the hypothesis that a hydraulic pressure gradient is one component of bile formation in the skate. The canalicular membrane also does not demonstrate a significant bio-electric barrier to solute entry into bile and may be more permeabile than in mammals.

VASCULAR SODIUM PUMP ACTIVITY IN SEA WATER ADAPTED CARASSIUS AURATUS (GOLDFISH)

J. Lowenstein, J. Zadunaisky and A. Evans, Department of Medicine and the Department of Physiology and Biophysics, New York University Medical Center, New York, N.Y.

It has been reported that salt loading in rats subjected to renal artery clipping (1 kidney 1-clip hypertension) or DOC administration demonstrates decreased vascular $Na^+ - K^+$ pump activity as measured by the uptake of ^{86}Rb by tail artery or aortic segments (Pamnani, M. et al., Hypertension 3:II-96-101, 1981). Incubation of tail artery segments from control rats in plasma supernatant from salt loaded rats similarly demonstrates decreased ouabain-sensitive ^{86}Rb uptake, suggesting the presence of a circulating inhibitor of vascular $Na^+ - K^+$ ATPase. A circulating ouabain-like substance, termed "endoxin" has also been extracted from the plasma of salt loaded dogs (Gruber, K.A. et al., Nature 287:743-6, 1980) and from bovine hypothalamus (Haupert, G.T. and Sancho, J.M., Proc. Nat'l. Acad. Sci. USA 76:4658-4660, 1979) and guinea pig brain (Fishman, M.C., Proc. Nat'l Acad. Sci. USA 76:4661-4663, 1979).

The present study was undertaken to examine the effects of salt loading in the fresh water teleost, Carassius auratus (goldfish) on cardiac and vascular ^{86}Rb sodium pump activity. Dilute sea water adaptation of the "conformist" Carassius auratus could lead to a less traumatic model for these studies.

Carassius auratus were adapted to 1/4 sea water for 6-7 days and then to 1/3 sea water for an additional 4-5 days (Zadunaisky, Exp. Eye Res. 14:91-110, 1972). Goldfish weighing 2.3 to 10.9 grams were subjected to percutaneous cardiac puncture for measurement of plasma electrolyte by flame photometry after adaptation to 1/4 or 1/3 sea water. One or two days later the fish were pithed, the heart removed, the ventricle isolated and sectioned with a sharp scalpel and fragments weighing .5 to 3 mg were incubated in Ringer's solution (NaCl 135 mM/L, KCl 2.5 mM/L, CaCl 1.5 mM/L, $MgCl_2$, 1.0 mM/L, $NaHCO_3$ 16 mM/L, glucose 5.5 mM/L) containing approximately 5 nM carrier free ^{86} Rubidum chloride (^{86}Rb). Incubations were carried out at room temperature in a bath gassed with 95% oxygen and 5% CO_2. At 15 minutes, the tissue was removed, blotted, weighed and digested overnight in 100 μl concentrated nitric acid. The volume of the digest was adjusted to 1.0 ml with distilled water, one half was removed for scintillation counting (Aquasol) and the remainder diluted 1.4 with lithium chloride (20 mM/l) and the potassium concentration measured by flame photometry.

Larger Carassius auratus, weighing 60 to 164 grams were adapted to sea water for 10 days. Fish kept in fresh in fresh water or adapted to 1/3 sea water were pithed, a blood sample taken by direct cardiac puncture and the proximal aorta dissected free of connective tissue and blood clots. Segments of aorta, opened lengthwise, weighing 1-2 mg were incubated in ^{86}Rb containing Ringer's solution and treated as described above.

In order to obviate errors inherent in weighing small fragments of wet tissue, Rb uptake was expressed as picomoles Rb taken up per tissue K^+ content. All data were normalized to a bath ^{86}Rb content of 1×10^6 cpm (5 nM).

The adaptation to sea water was associated with a significant increase in plasma sodium concentration in both the small and larger Carassius auratus (Table 1). The hematocrit, measured only in the larger fish, did not change significantly (Table 1).

Total ^{86}Rb uptake in cardiac slices was significantly lower in Carassius auratus adapted to 1/3 sea water as