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)

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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

Table 1.--Effect of sea water adaptation on extracellular fluid electrolyte concentration in Carassius auratus (goldfish)

	$[Na^+]$ (mM/L)	$[K^+]$ (mM/L)	Hematocrit &
Fresh water	1.5	0.3	--
1/4 sea water	106	2.4	
1/3 sea water	152	3.3	
SMALL CARASSIUS			
Fresh water	127.6	4.1	
1/4 sea water	149.4	4.1	
1/3 sea water	174.1	4.6	
LARGE CARASSIUS			
Fresh water	139.9	3.6	48.0
1/3 sea water	177.7	3.0	45.8

compared with fresh water controls (Table 2). ^{86}Rb uptake was similarly depressed in cardiac slices from fish adapted to 1/4 sea water but the difference did not attain statistical significance. Total ^{86}Rb uptake in aortic segments averaged 260.7 $\pm$ 69.0 $pM/\mu MK^+$ in fresh water and 213.7 $\pm$ 83.4 $pM/\mu MK^+$ in 1/3 sea water. This difference did not attain statistical significance.

Table 2.--Effect of sea water adaptation on ^{86}Rb uptake by heart and aorta of Carassius auratus (goldfish)

	Fresh Water	1/4 Sea Water	1/3 Sea Water
Cardiac ^{86}Rb Uptake ($pM/\mu M$ Tissue K^+)	161.8	120.1	108.6*
S.D.	$\pm$ 29.9	$\pm$ 35.8	$\pm$ 23.4
n	6	5	6
Aortic ^{86}Rb Uptake ($pM/\mu M$ Tissue K^+)	260.7		213.7
S.D.	69.0		83.4
n	6		8

*Significantly different from control ($p < .05$), Student's unpaired "t" test.

In a preliminary study, $Na^+ - K^+$ ATPase activity measured in a homogenate of ventricular muscle from one large Carassius in fresh water was compared with that from a fish adapted to 1/3 sea water. The measurement of Na^+K^+ ATPase, by an NAD-linked enzymatic assay, kindly performed by J. Epstein and Dr. Victor Sapirstein, revealed a decrease in sodium pump activity in the sea water adapted Carassius (fresh water = 1.74 μM/min/mg protein).

The study was carried out in the stenohaline teleost, Carassius auratus, in order to examine the response to salt loading in a species in which, unlike the eel or eurhaline teleost, sodium homeostasis cannot be maintained by active secretion of sodium via the gills. It has been estimated that Carassius auratus drink approximately 50 ml/kg/day. In 1/3 sea water, this would present a sodium intake of approximately 7.5 mM/kg/day. We have found, in this

preliminary study, that this degree of sodium loading is associated with a significant reduction in sodium pump activity (^{86}Rb uptake) in the cardiac muscle and a less significant reduction in sodium pump activity in the aorta.

Further studies will be required to establish (1) whether this decrease in total ^{86}Rb uptake represents a suppression of a ouabain-sensitive active transport, (2) whether the change in sodium pump activity results from an increase in sodium concentration, an increase in another sea water electrolyte or is a less specific effect of increased extracellular fluid osmolarity, and (3) whether the modulation of vascular sodium pump activity is mediated by a circulating factor i.e., an "endoxin".

INTRACELLULAR CHLORIDE ACTIVITY IN THE ISOLATED RETINAL PIGMENT EPITHELIUM OF THE FROG, RANA CATESBEIANA

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The retinal pigment epithelium (RPE) is a single layer of cells located between the choroidal blood supply and the rod and cone outer segments of the neuroretinal system. The RPE serves as a transport pathway responsible for the exchange of metabolites and ions between the neuroretina and the choroidal blood supply. Active reabsorption of the subretinal fluid by the RPE is very likely a determinant factor in the maintenance of normal retinal adhesion. The transport functions of the RPE have only recently been described. In the toad and the frog preparation a trans-RPE potential difference of 5-30 mV (retinal side positive with respect to the choroidal side) and a net retinal to choroidal transport of chloride, accounting for about two-thirds of the short circuit current, was reported (Lasansky, A. and DeFisch, F.W., J. Gen. Physiol. 49:913-924, 1966; Steinberg, R.H. and Miller, S.S., Exp. Eye Res. 16: 365-372, 1973; DiMattio, J.A., Degnan, K.J., and Zadunaisky, J.A., Exp. Eye Res., in press, 1982).

It has been suggested that along with the apical (retinal) NaK -ATPase pump, there exists an apical Na-Cl coupled transport system which is driven by the Na gradient across the apical barrier. To test this hypothesis in the present study we have analyzed the intracellular Cl activity of the RPE.

After removing the retina and the sclera, hemisections of RPE from the bullfrog (Rana catesbeiana) were horizontally mounted between Sylgaard discs in a modified Ussing type chamber. The transepithelial potential difference was recorded continuously and conventional microelectrodes were employed to measure the electrical potential difference across the apical membrane (Figure 1). Cl-sensitive microelectrodes were prepared following the method

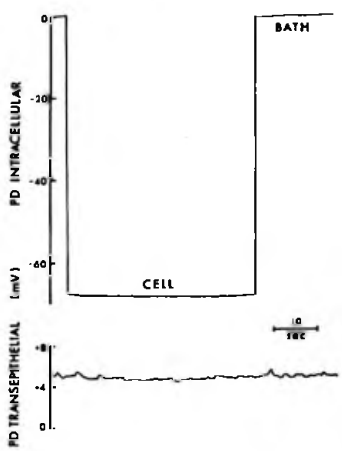

Figure 1.--Original recording of an intracellular membrane potential across the apical membrane of the retinal pigment epithelium. Transepithelial potential was measured simultaneously.

described earlier (Hansen, L.L., Koch, M., Wiederholt, M., Exp. Eye Res. 29:367-378, 1979). Essentially the electrodes were silanized (2% silicone 1107, Dow Corning) and filled with a new chloride liquid ion exchange