liquid junction and a thermal sensitivity ill-suited to experiments conducted in an uninsulated laboratory.

Given a lumen that is negative with respect to interstitial fluid, it is no surprise that when the secreting gland concentrated Cl^-, this ion was consistently actively transported against a substantial electrochemical PD.

The electrochemical PD against which Na^+ was secreted was $+5.7 \pm 1.4$ indicating that Na^+ was actively transported. In only one instance was the electrochemical PD negative and in this instance secretory $[Na^+]$ was only 354 mEq. In the present study as well as that of Palmer, cited above, an appreciable number of perfused glands failed to concentrate Na^+ and/or Cl^-. It is reasonable to suggest that the collected fluid was a mixture of a "true" secretion and fluid leaked by filtration. Although Na^+ was secreted against a small electrochemical PD, the difference at the site of secretion was probably greater. The isolated gland mounted as a flat sheet has been found to have a low conductance of 1.7 mmhos.cm^{-2} (Hogben, C.A.M. & Kalas, J.P., Bulletin Mt. Desert Island Biol. Lab. 5:35, 1965). Had Na^+ simply followed by diffusion the active transport of Cl^-, a greater electrical PD could have been anticipated.

The appearance of K^+ in the secretion in higher concentration than in the perfusate was noted but the degree of concentration was variable and in the later experiments was not much more than the perfusate. This invites speculation that the apparent secretion of K^+ is an injury loss from epithelial cells into the lumen.

SUMMARY

During perfusion, concentration of NaCl by the isolated rectal gland is associated with a small electrical PD with the lumen negative to interstitial fluid. Since the electrochemical PD for chloride is consistently large, it is actively secreted. Sodium is secreted against a small electrochemical PD which when coupled with other observations requires that Na^+ be actively transported. The appearance of potassium in the secretion of this *in vitro* preparation may be due to injury loss from epithelial cells into the lumen.

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1972 #25

ION TRANSPORT BY THE ISOLATED URINARY BLADDER OF A TELEOST, *Hemitripterus americanus*

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The steady-state electrophysiology of the urinary bladder from a stenohaline teleost was investigated by mounting the split bladder in a flux chamber (Hogben, *Gastric Secretion*, Ed. Sachs, Heinz and Ullrich; Academic Press, 1972). Sea ravens caught by commercial fishermen were held in cold running sea water for one to several days. The flux chamber was set at 15C by a thermoelectric

heat-exchanger. Both bladder surfaces, 1 cm^2, were bathed by 12 ml of Forster's flounder saline (J. Cell. Comp. Physiol. 51: 259-72, 1958) gassed by 95 percent O_2, 5 percent CO_2. The chambers had provision for sensing the transmucosal potential difference (PD) through a pair of agar saline bridges whose tips approximated the surfaces. These in turn led to a pair of calomel cells whose signal reached a high impedance millivoltmeter (Corning pH meter, Model 12). The potential difference is expressed as the value at the mucosal surface in reference to the serosal surface, designated as zero. Values have been corrected for a small bridge and calomel cell asymmetry PD. The chambers had current sending electrodes separated at a distance from the mucosa by diffusion barriers. Every 15 minutes a brief, approximately 10 seconds, square pulse of current displaced the mucosal PD by about +35 mv. Mucosal conductance, G, was calculated from the apparent steady-state $\Delta E/I$. Since the transient of E was not ascertained, the reported values may be somewhat less than steady-state G. Except for values given in Table 1, the transmucosal G was corrected for an interbridge resistance of 23±10 (SD) ohms. About 20 minutes elapsed between incision of the fish and mounting the bladder in the flux chamber. There followed an interval of 30-60 minutes until a change of fresh bathing saline. Approximately 30 minutes later tracer amounts of ^{22}Na or ^{22}Na and ^{36}Cl were added to the solution bathing either the mucosal or serosal surface of the isolated bladder. After 30 minutes to attain isotopic steady-state, ionic flux was measured over four hour periods with the mucosa operating at its spontaneous PD. In nine experiments only the flux of ^{22}Na was measured. In 12 others the flux of ^{22}Na and ^{36}Cl were determined simultaneously using both liquid and crystal scintillation detectors. The crystal detector was used to detect the gamma emmission of ^{22}Na. Emmission from ^{22}Na contributed 5-10 percent of the radiation detected by the liquid scintillation detector.

TABLE 1

POTENTIAL DIFFERENCE AND CONDUCTANCE OF ISOLATED SEA RAVEN URINARY BLADDER

Hour	PD mv	Conductance mmhos.cm^{-2}
1	+2.26±.44	2.11±.48
2	+1.22±.35	1.25±.28
3	+0.71±.26	1.05±.21
4	+0.48±.22	0.97±.20
5	+0.23±.20	1.00±.19

$\bar{x}$±SE, n=10

Mean PD corrected for electrode asymmetry. Serosal surface considered the reference. Conductance not corrected for interbridge resistance. Time from mounting bladder in chamber.

By light microscopy, the sea raven urinary bladder appears to be lined by a very simple and orderly single layer of columnar cells (Figure 1a). This epithelium may prove to be the simplest yet studied in the isolated state and simpler than that of the toad bladder (Dibona *et al.*, J. Cell. Biol. 40: 1, 1969). As we were dissecting out the bladder, the epithelium only constituted a thin layer

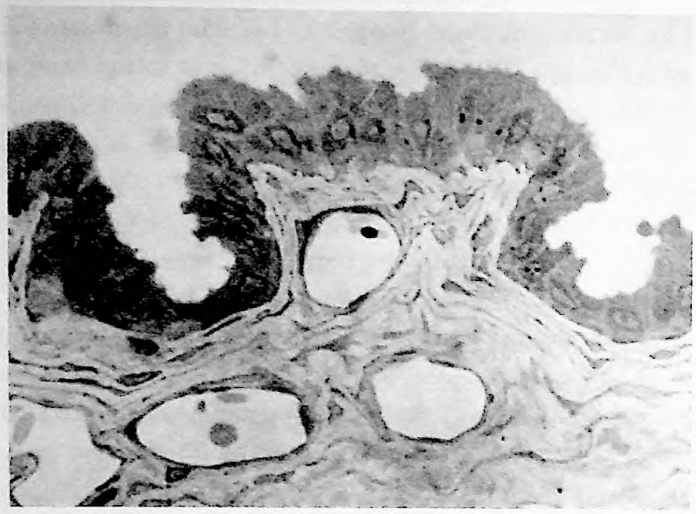

Figure 1a

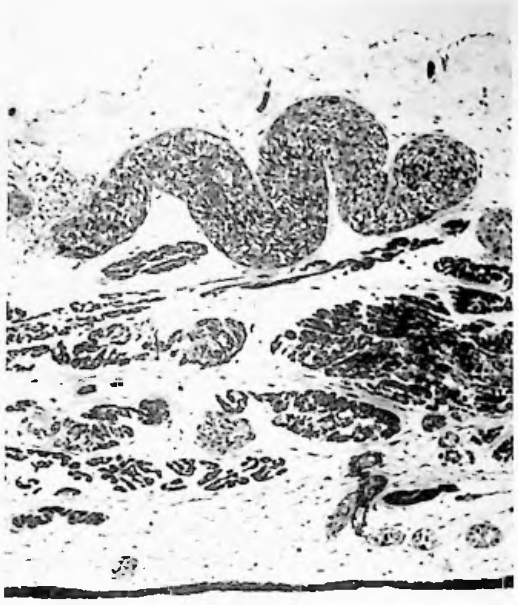

Figure 1b

Figure 1c

FIGURE LEGEND

Figure 1. Appearance by light microscopy of the urinary bladder at the end of an experiment. One micron Epon embedded section fixed with glutaraldyde and stained with methylene blue and azure #2. (a) Orderly single cell layer of the surface epithelium. (b) Full thickness of the mucosa. (c) Peritoneal endothelial surface apparently intact.
(Courtesy of Dr. Karl Karnaky, Jr.)

TABLE 2

SODIUM FLUX THROUGH *Hemitripterus* URINARY BLADDER

Flux	Period	Ratio of Flux to Membrane Conductance $\mu Eq.hr^{-1}.mmhos.^{-1}$	G $mmhos.cm^{-2}$	PD mv
Mucosa→ Serosa	1	1.38±.66	0.91±.26	+2.0±.7
	2	1.15±.41	0.80±1.8	+1.1±.3
	3	0.95±.25	0.78±.15	+0.5±.3
	4	0.76±.15	0.93±.22	+0.4±.2
	$\bar{x}$	1.06±.37	0.85±.17	+1.0±.2
Serosa→ Mucosa	1	0.59±.06	1.12±.20	+0.9±.4
	2	0.51±.03	1.35±.22	+0.9±.4
	3	0.48±.03	1.62±.27	+0.9±.3
	4	0.46±.03	1.93±.33	+1.0±.3
	$\bar{x}$	0.51±.03	1.50±.23	+0.9±.3

$\bar{x}$±SE, n=11 mucosa to serosa, n=10 serosa to mucosa.
Hourly periods. Wet weights of five mucosae each: mucosa to serosa 134±34; serosa to mucosa 69±24 $mg.cm^{-2}$.

TABLE 3

FLUX OF CHLORIDE AND SODIUM THROUGH *Hemitripterus* URINARY BLADDER

		Ratio of Flux to Membrane Conductance $\mu Eq.hr^{-1}.mmhos^{-1}$				
Flux	Period	Cl^-	Na^+	Σ	G $mmhos.cm^{-2}$	PD mv
Mucosa→ Serosa	1	2.78±1.92	1.78±1.11	4.56±3.20	0.67±.18	+2.8±1.0
	2	2.13±1.22	1.37±0.69	3.50±1.90	0.64±.17	+1.4±0.5
	3	1.46±0.63	1.05±0.42	2.51±1.05	0.72±.21	+0.5±0.5
	4	1.06±0.35	0.79±0.26	1.85±0.60	1.00±.37	+0.3±0.4
	$\bar{x}$	1.85±1.02	1.25±0.62	3.10±1.64	0.76±.22	+1.2±0.4
Serosa→ Mucosa	1	1.32±0.27	0.65±0.08	1.97±0.35	1.00±.17	+1.7±0.5
	2	0.84±0.08	0.50±0.04	1.34±0.11	1.59±.24	+1.6±0.5
	3	0.72±0.05	0.44±0.03	1.16±0.07	1.92±.34	+1.4±0.4
	4	0.66±0.03	0.43±0.03	1.09±0.05	2.37±.47	+1.3±0.3
	$\bar{x}$	0.89±0.09	0.50±0.03	1.39±0.11	1.69±.29	+1.5±0.4

$\bar{x}$±SE; n=6. Values for Na^+ included in Table 2.

covering a relatively thick wall of smooth muscle and connective tissue, Figure 1b. We had hoped that the dissection would have removed the serosal peritoneal endothelium but as illustrated in Figure 1c.the endothelium appears to be intact.

The time course of the spontaneous PD and mucosal conductance over the first five hours after mounting in the chamber is given in Table 1. There was a small PD oriented such that the mucosal surface was positive to the serosal surface; a mean of +2.3 mv, significantly different from zero for the first hour. Among 26 bladders, only one significant negative value, -3.7 mv, was encountered initially, while initial values ranging from +3.9 to +16 mv were recorded in nine other bladders. In no instance was the mean hourly PD of four periods for 21 bladders more negative than -0.5 mv. Characteristically the PD declined towards zero over five to six hours of study as exemplified in Table 1. The initial conductance, G, tended to decline (Table 1) suggesting that the mucosa was recovering from isolation. Later when the mucosa was subjected hourly to a slight hydrostatic pressure head of several cm H_2O with each change of solution, G tended to rise, e.g., Table 3, suggesting that either the so called "tight junctions" are susceptible or that edge damage (Walser, Am. J. Physiol. 219: 252, 1970) might be significant.

The flux of Na^+ through the mucosa is presented in Table 2. Scrutiny of the mean G and PD for the two sets of bladders used to measure the two unidirectional fluxes establishes that they are not well matched. We have chosen to normalize the fluxes by expressing them as the ratios of flux/G. This has merit because passive flux and partial conductance are interconvertible (Ussing, Acta Physiol. Scand. 23:110, 1951). Respectively at 15 C, 1.08 μEq.hr^{-1} = 1.00 mmhos. From G mmhos = $F^2/RT\ (10^{-3}).(96{,}500)^2/(8.315).(288.1).(3{,}600)\ \mu$Eq.hr^{-1} (Hodgkin, Biol. Rev. 26:339, p. 364 equation (19), 1951).

Treated in this way, it is clear from Table 2 that there is an active absorption of Na^+ from mucosa to serosa since the ratio of flux/G was consistently greater over four hours for the mucosa to serosa flux than that for the serosa to mucosa flux. The flux/G ratio, initially greater than 1.08 for the mucosa to serosa flux, declined progressively as the preparation deteriorated. The ratio for the flux in the opposite direction was relatively constant and compatible with passive diffusion.

A similar analysis leads to the conclusion that Cl^- is also being actively absorbed (Table 3). The discrepancy between the observed flux/G ratio and that expected for passive diffusion, 1.08, was even more striking for this ion. There was a similar decline in the ratio with time. For the flux from serosa to mucosa, the initial ratio is greater than that compatible with passive movement.

The sum of the flux/G ratio of the two ions, Cl^- and Na^+, Σ was importantly greater than that expected for the total mucosal conductance except for the latter two hours when serosa to mucosa flux was being measured. If the data had been drawn from a more homogeneous population this would constitute presumptive evidence that either Cl^- and/or Na^+ were being subjected to "exchange diffusion." Scrutiny indicates that though for an individual bladder values are internally consistent, there is inter-bladder heterogeneity permitting of the less likely possibility that some of the bladders were secreting rather than absorbing.

However the heterogeneity encountered between bladders suggests rather that we encountered a mixed population, some bladders were continuing to absorb when flux measurement commenced, and others had already become inert. This study was a preliminary exploration to determine whether it would be feasible to study the electrophysiology of the teleostean urinary bladder *in vitro*. It was not ascertained that the bathing saline was the most appropriate for this species. The Na^+ concen-

tration of the saline was 142.5 mEq in contrast to a mean plasma Na^+ of 184.2 (Forster and Danforth, this issue of the Bulletin). No exogenous substrate was provided.

Perhaps more important factors were the procedures for catching and maintaining the sea raven. This is suggested by the considerable variation in the plasma osmolality of the fish at the time of experiment; for five fish the range was 334 to 444 mOsm (Forster and Danforth, this issue of the Bulletin).

SUMMARY:

The isolated urinary bladder of the sea raven develops a small transmucosal PD with the mucosal surface consistently positive with respect to the serosal surface. In isolation the bladder actively absorbs both Cl^- and Na^-. For the time required for steady-state study of ionic flux, the preparation was not sufficiently stable and further work is required to establish optimal conditions for maintenance *in vitro* and *in vivo*.

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1972 #26

INFLUENCE OF EXTRACELLULAR ACIDOSIS ON ACID SECRETION BY THE ISOLATED GASTRIC MUCOSA OF *Squalus acanthias*

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In the intact mammal it has been recognized that metabolic acidosis favors H^+ secretion by the stomach (Byers et al., Amer. J. Physiol. 202: 429-436, 1962). However metabolic acidosis in the intact animal is accompanied by some degree of respiratory compensation and possibly by less obvious shifts in internal electrolyte balance. The isolated gastric mucosa permits examination of an altered interstitial pH where perturbation is limited to one variable, the extracellular $[HCO_3^-]$.

TABLE 1

H^+ SECRETION

$\mu Eq.cm^{-2}hr^{-1}$

Serosal Solution A	Serosal Solution B	Δ (A-B)
pH 6.5 3 mEq HCO_3^-	pH 7.4 30 mEq HCO_3^-	
3.52±.10	2.97±.17	0.54±.19
	pH 7.0 10 mEq HCO_3^-	
3.40±.08	3.45±.24	-0.06±.20
	pH 6.0 1 mEq HCO_3^-	
3.76±.16	3.01±.33	0.75±.32

X±SE, n=4