suited for renal function studies in flounder. By comparison ^{14}C-PEG showed no evidence of irreversible penetration into kidney tissue.

We attempted to establish the nature of the association between these labelled compounds and flounder kidney tissue in a single saturation study, using three widely different concentrations of ^{3}H- and ^{14}C-labelled inulin and ^{3}H- and ^{14}C-labelled PEG. Although not conclusive, the results suggest that of these four compounds, only ^{3}H-PEG exhibited saturation kinetics and, hence, binding behavior. Neither ^{3}H- nor ^{14}C-labelled inulin or ^{14}C-PEG showed evidence of saturation kinetics. Thus inulin, either as such or as fructose residues, may be penetrating into cells or other non-exchangeable space, a behavior that is consistent with the absence of saturation kinetics.

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1972 #24

ELECTROPHYSIOLOGY OF THE PERFUSED RECTAL GLAND OF *Squalus acanthias*, IN VITRO

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Palmer first demonstrated that the rectal gland of *Squalus acanthias* perfused *in vitro* secreted a fluid whose chloride concentration was greater than that of the perfusate (Bulletin Mt. Desert Island Biol. Lab. 5:32, 1965). Hayslett and co-workers (Bulletin Mt. Desert Island Biol. Lab. Vol. 12, 1972) have extended that observation. In the present study the isolated rectal gland was perfused via its artery by the same solution used by the latter scientists: Na^{+} 281, Cl^{-} 297 and K^{+} 6 mEq. The artery was catheterized with polyethylene tubing #50. Perfusion was carried out at a hydrostatic pressure head of 85 cm H_2O resulting in a flow of about 2 ml/min. The gland was partially submerged in a petri dish held at 15 C by a thermoelectric heat-exchanger. Luminal fluid was allowed to drain by gravity through #90 polyethylene tubing either into a beaker containing perfusion-saline or collected under oil.

The electrical potential difference, PD, between the perfusion reservoir and the lumen was measured using a pair of salt bridges, polyethylene tubing #240 filled with 275 mM NaCl and 3 percent agar. One salt bridge was placed between the reservoir of perfusion saline and a reference calomel cell, "Radiometer" K401, in a beaker with 1 M KCl. The second bridge was used to establish contact with the outflow and another calomel half-cell. One end of the bridge was in a beaker containing some perfusion-saline into which solution flowed from either the arterial or luminal cannula while the other end was in 1 M KCl bathing the second calomel cell. The convention employed for the PD is such that the reference electrode is in electrical continuity with the interstitial compartment and the reported PD is the value for the lumen with respect to interstitial fluid. At the start and end of each experiment, the asymmetry PD was measured between the pair of bridges and calomel cells by allowing perfusion fluid to flow through the arterial cannula into the beaker of perfusion-saline. The mean asymmetry PD was: initial -1.4 ± 1.2; terminal -0.9 ± 1.4; and mean difference

0.6±0.6 (SE, n=7). Before and after each collection of secretion, 10-20 minutes, the PD was measured between the lumen and perfusion fluid. The mean change across the gland between 'before' and 'after' was -0.1±0.6 (SE, n=9). Electrical continuity was not maintained during the collection of secretion under oil. Reported values for the PD across the gland epithelium are the net PD, correcting the mean gland PD by the mean asymmetry PD for that gland.

Eight experiments were conducted. We have chosen to exclude from further consideration three experiments where the $[Na^+]$ or $[Cl^-]$ of the "secretion" were less than that of the perfusion fluid: 252, 287, and 274 mEq. The $[Cl^-]$ of the first two were 272 and 284 mEq. The third gland would also have been excluded on the basis that there was an unacceptable difference of 11.5 mv between the initial and terminal asymmetry PD. The PD developed by these glands was respectively: -6.6, +2.0 and -6.5 mv. For these three glands, we had not established that "active" secretion was elicited and the dominant process could have been filtration driven by the hydrostatic pressure of perfusion.

TABLE 1

ELECTROCHEMICAL GRADIENTS DEVELOPED BY THE RECTAL GLAND

Expt	PD mv	Secretion $[Na^+]$ mEq	E^{Na}_{ec} mv	Secretion $[Cl^-]$ mEq	E^{Cl}_{ec} mv	Secretion $[K^+]$ mEq	$\dot{V}$ $\mu L.g^{-1}hr^{-1}$
1	-1.7	368	+4.2	-	-	17.4	1.0
2a	-3.9	434	+6.1	453	-14.4	12.4	2.5
b	-2.6	429	+7.3	-	-	8.9	1.5
3a	+0.2	418	+9.3	436	- 9.4	11.6	3.5
b	-1.2	439	+9.0	457	-12.0	7.3	1.6
c	-2.0	432	+7.9	460	-12.9	7.9	0.6
4a	-7.2	414	+1.6	455	-17.8	7.8	2.9
b	-5.4	403	+2.7	442	-15.3	7.9	3.6
5	-9.2	354	-4.2	-	-	8.4	-
$\bar{x}$+SE	-3.7±1.0	410±10	+4.9±1.4	450±4	-13.6±1.2	10.0±1.1	2.2±.4

Values for electrical potential difference, PD, and electrochemical potential difference E^i_{ec}, expressed as the luminal solution with respect to the perfusion fluid.
E^i_{ec} = PD + 2.303 RT/F log c_1/c_2 where 2.303 RT/F at 15C = 57.2 (in mv).
Perfusion fluid: $[Na^+]$ 290, $[Cl^-]$ 297 and $[K^+]$ 6 mEq.

Observations on successful perfusion of five glands are presented in Table 1. The mean PD and SE developed between the gland fluid and perfusate was -3.6±1.0 mv with the lumen negative with respect to interstitial fluid. Among nine observations only one positive value was recorded +0.2 mv. This is well within the limits of resolution employing commercial calomel cells having a restricted

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