

TABLE 1

Effect of SLC-76, SLC-76-aliphatic or SLC-76-aromatic fractions on herring gull chick body weight (BW) and organ weights

	BW (g)		(g/kg BW)	Organ weight	
	At dosing	7 d after dosing		Nasal gland (mg/kg BW)	Adrenals (mg/kg BW)
Control (9)	373±30	697±23	41.1±2.5	574±16	62.5±2.4
SLC-76 (6)	365±17	475±33**	49.1±4.0	860±37**	102.8±8.6**
SLC-76 (6) aliphatic	366±27	570±40	43.3±4.9	671±30*	79.1±3.4*
SLC-76 (6) aromatic	353±23	515±37**	46.4±3.0	833±35**	99.0±6.3**

Data given is mean ± SE with the number of birds in each group in parentheses.

* Significantly different from controls, $P < 0.06$.

** Significantly different from controls, $P < 0.01$.

polynuclear aromatics with 3 or more rings whereas many of this class of compound were present in SLC-76 (Hallett, unpublished data). These findings suggest that polynuclear aromatics are the most potent inhibitors of gull growth present in this crude oil. Since we do not yet have residue data, we cannot begin to speculate on the target organs that are involved. However, preliminary experiments with adult Leach's storm petrels (*Oceanodroma leucorhoa*) that were dosed with ^3H -labeled oil and sacrificed 1 day later indicate that about 90% of the oil is retained by the bird and that high levels are found in the gastrointestinal system, blood, muscle and liver. Supported by USPHS Grant ES 00920.

NA AND CL UPTAKE BY DISPERSED RECTAL GLAND TUBULES

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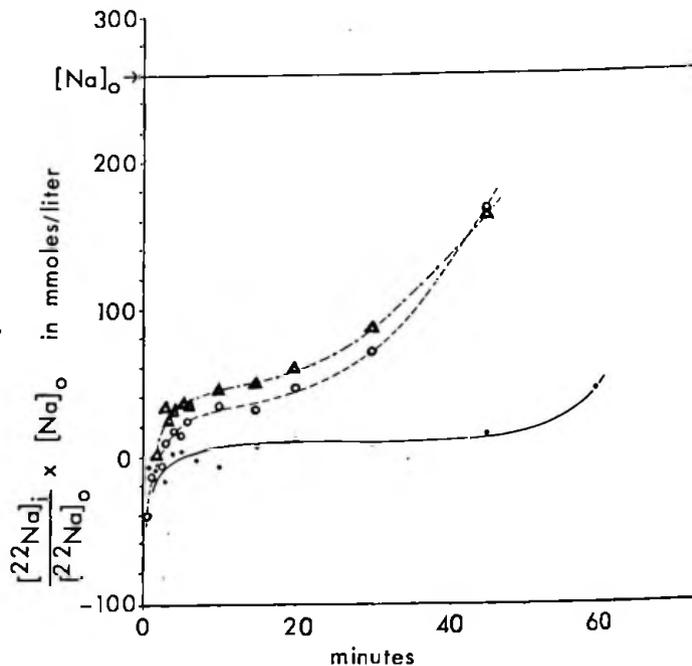
Active Cl secretion by shark rectal gland is Na-dependent and can be inhibited by ouabain and furosemide (Silva et al. Am. J. Physiol. 232:F298). Measurements of intracellular Cl activity and electric potential indicate that Cl accumulates well above electrochemical equilibrium (see Duffey et al., this volume). These observations suggest that the uphill step in Cl transport is an obligatory co-transport with Na across the basolateral membrane. Because of its complex architecture, the intact, perfused gland is not suitable for direct measurements of NaCl co-transport. We therefore attempted to examine cellular uptake of ^{22}Na and ^{36}Cl in isolated rectal gland tubules. If the lumen of the isolated tubule is normally collapsed, initial accumulation should occur almost exclusively from the basolateral side.

After cannulating the vein and artery, the rectal gland was cleared of blood by perfusing with Ca-free dogfish Ringer (see Silva et al Am. J. Physiol. 232:F298, for composition of Ringer). The vein was then clamped and the gland distended with 0.1 mM Ca-dogfish Ringer containing 8 mg/ml of hyaluronidase (Type 5, Sigma) and 5 mg/ml of crude collagenase (Worthington). Thin slices were cut with a Stadie-Riggs microtome and these were incubated for 20 min at ambient temperature in the above enzyme mixture with magnetic stirring and gassing with 1% CO_2 in O_2 . The suspension was then filtered through

gauze, layered over 15% PVP-40 (Sigma) in 0.1 mM Ca-dogfish Ringer, and centrifuged at 50 x g for 10 min in the cold. The pellets, which contained intact tubules (single cells didn't sediment), were resuspended in 2 mM Ca-dogfish Ringer containing 5 mM glucose and 0.1% bovine serum albumin and were incubated as described above, except at 15°C. Phase microscopic examination after 1 hr revealed intact tubules with collapsed lumina and no staining by Trypan Blue. Extracellular and intracellular water were determined with [¹⁴C]PEG-4000 and [³H]H₂O. Tubules were pre-equilibrated with these compounds for 10-20 min before adding ²²Na or ³⁶Cl. At various times tubules were rapidly separated from medium by sedimenting at high speed through silicone oil, essentially as described by Strauss et al. (J. Exp. Med. 144:1009): 200 μl of suspended tubules were layered over 150 μl of oil (1:3 mixture of Dow Corning 510 and Dow Corning 550, both from William F. Nye, Inc., New Bedford, Mass.) in a 400 μl microfuge tube and rapidly centrifuged in an Eppendorf microfuge. The pellets were then extracted for 12 hr in 0.1 N HNO₃. Extracts were dissolved in Bray's solution and analyzed by liquid scintillation spectrometry. K⁺ content was determined by flame photometer and Na⁺ by equilibrium distribution of ²²Na. The extracellular space in the tubule pellets varied from 30 to 50% of total pellet water.

Tubules incubated in regular dogfish Ringer for 30-90 min had intracellular K⁺ and Na⁺ concentrations of 94 ± 3.3 (1 SEM) and 51 ± 16 mM respectively (extracellular K⁺ and Na⁺ concentrations were 5.5 and 260 mM respectively). Intracellular Na⁺ thus proved to be greater and K⁺ less than the values previously reported for fresh intact glands (Silva et al. MDIBL Bull. 14:16) and for incubated 200 μm thin slices (Fine and Hays, MDIBL Bull. 15:37). We did obtain, however, a Na⁺ concentration of only

Figure 1. Uptake of ²²Na by dispersed rectal gland tubules. Extracellular marker and drugs added 20 min before ²²Na. Open circles: 0.5 mM ouabain; triangles: 0.5 mM ouabain and 1 mM furosemide; closed circles: no additions.



10 mM in a shorter incubation in a modified dogfish Ringer which contained no urea (Figure 1). The apparently negative intracellular Na⁺ concentrations observed in the first few minutes (Figure 1) indicate relatively slow equilibration of the extracellular spaces, the PEG having been added 10-20 min before the ²²Na. Initially negative values were also observed with ³⁶Cl (Figure 2).

Cl⁻ uptake kinetics were studied in a low (18 mM) Cl⁻, urea-free dogfish Ringer (0.5 mmoles of SO₄⁼ and mannitol replacing each mmol of Cl⁻). As shown in Figure 2, equilibration is rapid (50% in 1.3 min) and the equilibrium intracellular concentration is 64% of the extracellular concentration. This is probably well above electrochemical equilibrium although the intracellular electric potential

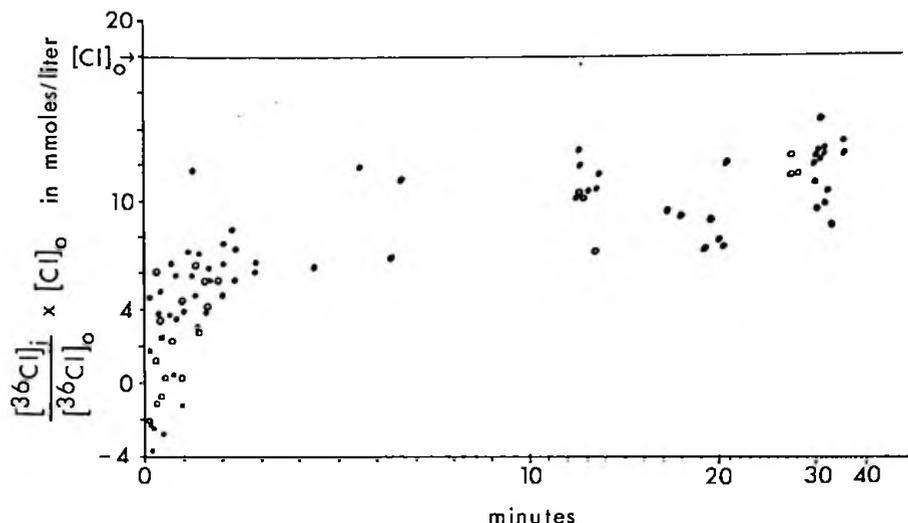


Figure 2. Uptake of ^{36}Cl by dispersed rectal gland tubules. Extracellular marker and furosemide added 20 min before ^{36}Cl . Open circles: furosemide (1 mMi); closed circles: control.

of the isolated tubules is not known (-80 mV in isolated, perfused glands--see Duffey et al., this volume). No inhibition of Cl^- uptake by furosemide could be detected. Since the scatter was large, however, a small effect could easily have been missed.

We conclude from these studies that viable suspensions of isolated rectal gland tubules can be prepared by proteolytic digestion but that such preparations are not especially well suited for determinations of initial rates of uptake of Na^+ and Cl^- . This work was supported by NIH grant AM-21345.

THE POSITIVE INOTROPIC ACTION OF CATECHOLAMINES ON ISOLATED ATRIUM AND VENTRICULAR MYOCARDIUM OF THE ELASMOBRANCH, *Raja erinacea*

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There is no anatomical or physiological evidence to suggest that there is a direct sympathetic innervation of the elasmobranch heart. J. Z. Young in a comprehensive study of the autonomic nervous system of selachians, that involved 10 species including 3 Rajiformes, found none after a systematic search of all possible paths including the ductus Cuvieri and the walls of the esophagus. Also, he found no ganglion cells in the walls of the atrium and ventricle (Q. J. Micr. Sci. 75:571-624, 1933). Inhibitory "parasympathetic" cardiac nerves, however, arise from the last branchial branch and from the visceral branch of the vagus, but B. R. Lutz also was unable to find either anatomical or physiological evidence of cardiac sympathetic adrenergic nerves in the little skate (*Raja erinacea*), 2 other species of *Raja* or in *Squalus acanthias* (Am. J. Physiol. 93:669, 1930).

A close connection between β -adrenergic stimulation and taurine fluxes has been reported in the rat heart (Huxtable, R. and J. Chubb, Science 198:409-411, 1977). We chose to test this hypothesis by characterizing taurine uptake in an *in vitro* skate atrium preparation because of this elasmobranch's complete lack of sympathetic adrenergic innervation to the heart and found that indeed no β -adrenergic stimulation of taurine transport could be demonstrated. The active carrier-mediated, Na- and energy-dependent β -amino acid transport system was not affected by isoproterenol nor dibutyryl cyclic AMP