Because of the suggestion that papverine might exert a local anesthetic effect in some systems we conducted one experiment in which lidocaine (10^{-4}M) was added to the mucosal bath. The drug promptly abolished I_{sc}, but active NaCl absorption was unaffected. One mechanism by which local anesthetics may exert generalized effects on cells is by displacing bound calcium. Accordingly we conducted a series of experiments in which NaCl transport was measured in the presence of the calcium ionophore A23187. The response of I_{sc} to mucosal addition of A23187 (10^{-6}M) was virtually identical to that produced by papaverine and lidocaine, rapid and nearly total inhibition of I_{sc}. Table 4 shows, however, that active NaCl absorption was little, if at all, affected by the ionophore.

Table 4. Effect of A23187 on transmural fluxes and electrical parameters (average of two experiments)

	J^{Na}_{ms}	J^{Cl}_{ms}	J^{Na}_{sm}	J^{Cl}_{sm}	I_{sc}	G_T
control	4.39	5.28	0.82	1.80	6.11	0.54
A23187	3.84	5.47	0.64	2.01	0.97	0.78
Δ	-0.55	0.19	-0.18	0.21	-5.13	0.24

Units are the same as in Table 1.

Discussion

The results presented here show that it is possible to pharmacologically "dissect" I_{sc} and active NaCl absorption in the flounder urinary bladder. Although the data presented fail to establish the ionic basis of I_{sc} it appears unlikely that I_{sc} is a result of intraepithelial salt accumulation due to a "neutral" NaCl absorptive process. The inhibitory effect of ouabain on I_{sc}, however, suggests some link to an Na-K ATPase. The observation by Renfro et al, (Am. J. Physiol. 228:52, 1975) that the flounder bladder acidifies its lumen raises the possibility that I_{sc} could represent active proton secretion perhaps linked to the Na-K ATPase via metabolic CO_2 production.

Little can be said regarding either sites or mechanisms of action of the inhibitors employed in this study. The known inhibitory effects of papaverine on cyclic nucleotide phosphodiesterase suggest the possibility that some of the effects of the drug may result from increased intracellular cyclic nucleotide levels. The rapid inhibition of I_{sc} by papaverine, however, is probably inconsistent with this type of intermediary step. In addition, in a single experiment in which bladders were exposed to 10^{-4}M dibutryl cyclic AMP, I_{sc} declined slowly (50% decline in 3 hrs) and NaCl absorption was, if anything, slightly increased. The similarity of the effects of papaverine, lidocaine and A23187 on I_{sc} strongly suggest that all three of these drugs act, at least in part, by altering the level of intracellular ionized calcium, either by releasing bound ion or facilitating calcium entry.

The flounder bladder is a unique model system in that it combines the properties of an apparently "neutral" NaCl absorptive process with a relatively "tight" epithelium. It is hoped that the drug-induced modifications of NaCl transport and I_{sc} observed in this study will provide useful tools for elucidating the mechanisms of ion translocation by this epithelium. This study was supported by a grant from the NIH-NIAMDD (AM18776).

THE EFFECT OF DECEREBRATION ON EXTRACELLULAR FLUID REGULATION IN CLITELLIO ARENARIUS (OLIGOCHAETA)

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A correlation between the activity of neurosecretory cells in the cerebral ganglia of an intertidal oligochaete (Enchytraeus albidus) and osmotic stress has been demonstrated histologically (Richter and Gersch, 1967). The present

study was designed to determine the effect of decerebration on volume regulation in a related species from the same habitat. Clitellio arenarius was collected from under intertidal rocks at Salsbury Cove, Maine, and maintained in recirculating seawater aquaria (949 mOsm) at 7°C for at least one week prior to use. Worms were cecerebrated (ablated) by transection immediately posterior to the subesophageal ganglia and returned to aquaria for recovery (5 days). Other worms were sham-operated by making an incision in the body wall in the region of the brain followed by a five-day recovery period. Worms were injected by micropuncture with 60 nl of a 50% solution of C^{14} polyethylene glycol (PEG) dissolved in 825 mOsm seawater. This solution matched the osmolality of the body fluids and increased the volume of the worms by less than 2.0%. The C^{14} PEG was injected into both the coelom and blood vessels. After a 24-hour equilibration period in 949 mOsm seawater worms were subjected to 70% seawater (645 mOsm) for 1, 4, 8 or 12 hours before extracellular space determination (as percent of total body water)(Table 1) using the method of Schmidt-Nielsen (1976). Duplicate specimens were weighed, dried at 100°C, reweighed, placed in 50 µl

Table 1. Mean extracellular space as percent total body water ± S.E. (n) in Clitellio arenarius during acclimation to 70% seawater

Hours in 70% Seawater	Control	Ablated	Sham
0	66.63 ± 1.763 (5)	67.85 ± 0.939 (6)	66.44 ± 1.730 (3)
1	55.34 ± 2.670 (4)	61.98 ± 2.171 (4)	58.23 ± 1.480 (3)
4	68.60 ± 1.132 (4)	70.96 ± 2.124 (4)	69.82 ± 0.808 (4)
8	68.50 ± 1.850 (4)	67.89 ± 0.440 (4)	67.94 ± 1.509 (4)
12	68.01 ± 0.518 (4)	67.43 ± 0.709 (4)	68.04 ± 0.683 (4)

distilled water and left for diffusion at 40°C for 24 hours. Osmolality was determined on the supernatant and used in conjunction with the wet weight/dry weight analysis for calculation of grams H_2O/gram solute free dry weight ($G.H_2O/G.S.F.D.W.$)(Table 2) using the method of Schmidt-Nielsen (1976). All data were compared for significant differences using a one-way analysis of variance (Freund, 1962) followed by Student-Newman-Keuls' test (Steele and Torrie, 1960) for separation of significant means.

No significant difference in extracellular space as percent total body water (Table 1) was determined among control, ablated and sham animals at any time period during acclimation to 70% seawater. However, after one hour the percent extracellular space was lower in all three groups than at any other time. During the first four hours of acclimation, control, ablated and sham animals increased in the $G.H_2O/G.S.F.D.W.$ with no significant difference among the three groups (Table 2). However, after 8 hours in 70% seawater ablated worms contained more water than either control or sham animals (Table 2). Control and sham animals showed volume regulation within 8 hours, whereas ablated worms did not appear to demonstrate volume regulatory ability until 12 hours had elapsed. In order to determine in which compartment the water was distributed, intracellular and extracellular water as $G.H_2O/G.S.F.D.W.$ was calculated (Figs. 1 and 2). In all three groups, cellular hydration occurred within one hour followed by a

Table 2. Grams H_2O/gram solute free dry weight ± S.E. (n) in Clitellio arenarius during acclimation to 70% seawater a. $P < 0.05$ b. $P < 0.005$

Hours in 70% Seawater	Control	Ablated	Sham
0	3.653 ± 0.0685 (12)	3.777 ± 0.0546 (12)	3.664 ± 0.0799 (12)
1	4.996 ± 0.0854 (8)	5.190 ± 0.0772 (8)	5.100 ± 0.0916 (8)
4	5.429 ± 0.1254 (8)	5.574 ± 0.0614 (8)	5.445 ± 0.116 (8)
8	5.289 ± 0.1081 (8)	5.733 ± 0.0936[a] (8)	5.352 ± 0.1173 (8)
12	5.217 ± 0.0680 (8)	5.539 ± 0.0837[b] (8)	5.139 ± 0.0870 (8)

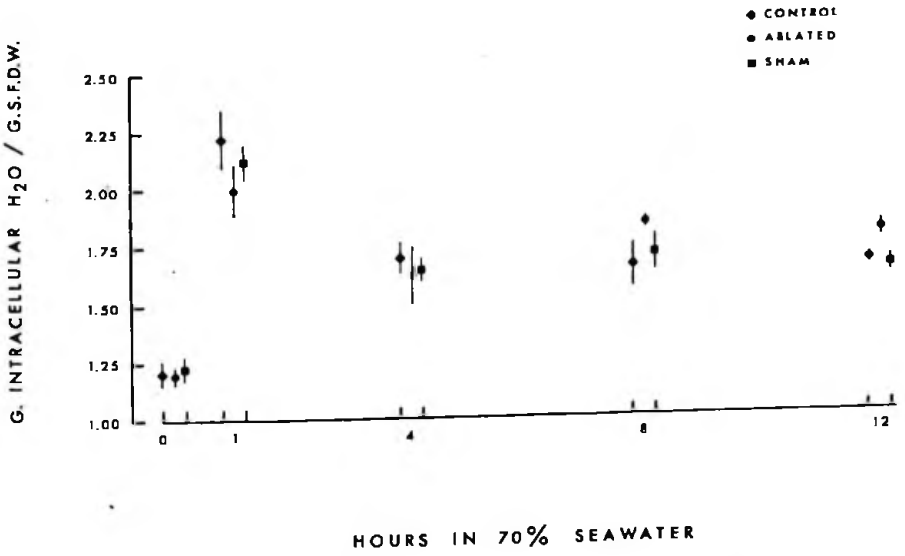

Figure 1. Mean intracellular water (grams/gram solute free dry weight) ± S.E. during acclimation to 70% seawater. n as in Table 1.

regulatory volume decrease within 4 hours (Fig. 1). No significant difference in intracellular water among the three groups was demonstrated until after 12 hours when the cells of the ablated animals were more hydrated than those of the controls or shams. Extracellularly, ablated animals contained more water than did other groups at all time periods after onset of stress. Differences were significant at 8 hours ($p < 0.055$) and 12 hours ($p < 0.005$). In summary, decerebration interfered with the time course and extent of extracellular volume regulation possibly by neurosecretory control of nephridial output. This work was supported by NIH grant 5 RO1 AM15973-09.

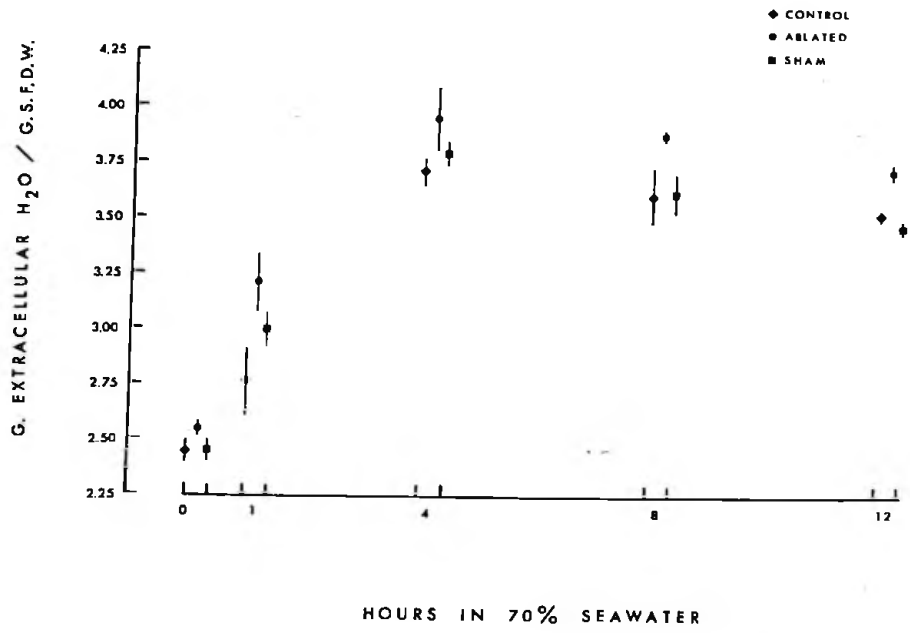

Figure 2. Mean extracellular water (grams/gram solute free dry weight) ± S.E. during acclimation to 70% seawater. n as in Table 1.

ACIDIFICATION OF THE URINE BY THE URINARY BLADDER OF THE FEMALE LITTLE SKATE, RAJA ERINACEA

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We report here the results of preliminary experiments to determine the function of the urinary bladder of the mature female skate. Our results show that the bladder participates in acidification of the urine.

Although the morphology of the female skate urinary bladder is well described in both the older (Borcea, Arch. Sool. Exp. et Gen. 4: 199-484, 1906) and recent (Lacy et al., Bull. Mt. Des. Is. Biol. Lab. 15: 56-58, 1975) literature, no published values for the ionic composition of skate bladder urine exist. Shown in Table 1 are the results of ion analyses performed on serum and bladder urine obtained from mature female skates (800-1300 g)

Table 1. Composition of serum, collecting duct urine and bladder urine in the little skate, Raja erinacea. Mean values ± S.E.; number below mean value is n.
All values, except pH, are in mmoles/l

	pH	Na^+	K^+	Cl^-	Mg^{2+}	Ca^+	Urea
Serum	7.46(0.05) 5	253(4) 18	4.5(0.2) 18	285(15) 15	1.9(0.3) 9	5.6(0.4) 11	347(5) 3
Collecting Duct Urine*	--	180(16) 23	31.6(8.8) 23	209(16) 9	154(29) 9	10.4(1.6) 9	--
Bladder Urine	5.01(0.07) 12	187(20) 19	110(18) 17	286(27) 15	238(53) 3	11.6(1.8) 12	40.0(14.4) 3

*Data from Stolte, et al., (J. Exp. Zool. 199: 403-410, 1977).