Ophthal. 6: 574, 1967). By comparison, the teleost cornea is also impermeable but lacks the sutural fibers; consequently, this cornea contains more water and does swell when metabolism is inhibited. The potential difference measured in these corneas is probably related to ion transport for deturgescence (Fischer and Zadunaisky, Exp. Eye Res. 25: 149, 1977).

In addition to its protective role, the epithelium of both the elasmobranch and teleost cornea is highly impermeable and functions as a barrier to electrolytes and water. To maintain this function, the epithelium has a high mitotic index which is reflected by this layer's high metabolic activity. Glycolysis, the hexose-monophosphate shunt, and the tricarboxylic acid cycle are well developed in this layer. Glucose oxidation by the HMS and TCA cycle is crucial to the metabolic requirements of this rapidly dividing tissue. Hence, it is not surprising that representative enzyme activities of these pathways are quite similar in both species.

Stromal swelling of the elasmobranch cornea is limited by the passive physical restraint of the stromal sutural fibers. The metabolic requirements of this type of passive mechanism are minimal. It was not possible to measure enzyme levels in the elasmobranch endothelium and stroma. The enzymes are present in extremely low activities which is further manifest by the low metabolic rate of this tissue (Edelhauser and Geroski, Bull. MDIBL, 18: 38, 1978). The endothelium-stroma of the developing dogfish pup cornea, on the other hand, is metabolically quite active. The energy requirements of this developing pup tissue are manifest in the high activity of the TCA enzyme ICDH -- the highest activity measured for any of the corneal tissues studied.

The sculpin cornea, lacking the sutural fibers seen in the elasmobranch, must rely on active transport mechanisms to control stromal hydration. The metabolic requirements of this tissue might be expected to be somewhat greater than that of the corresponding layer (endothelium and stroma) of the adult dogfish, but somewhat less than that of the developing dogfish pup. Enzyme activities of this layer of the sculpin cornea do fall between those of the adult and pup dogfish corneas.

The lower activities of G6PDH measured in muscle suggest that the HMS is less active in this tissue. It is not surprising that the activity of this pathway is greater in actively dividing tissue of the corneal epithelium. Activities of glycolytic and TCA enzymes, by comparison, are much higher in muscle. Of particular importance is the high ICDH to LDH ratio in red muscle compared to white muscle of the shark. The production of ATP would be much greater in red muscle than in white muscle. This data is consistent with the energy requirements anticipated on the basis of the tissue's function.

It thus seems that the measured enzyme activities reflect the particular adaptations made by corneas of marine elasmobranchs and teleosts to extremely different osmotic gradients which enable these tissues to remain transparent.

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DIFFERENTIAL INHIBITION OF NaCl ABSORPTION AND SHORT-CIRCUIT CURRENT IN THE URINARY BLADDER OF THE WINTER FLOUNDER, PSEUDO-PLEURONECTES AMERICANUS

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Ion transport by the isolated urinary bladder of the winter flounder has been previously investigated under conditions of continuous perfusion (Renfro et al, Amer. J. Physiol. 228:52, 1975; Renfro, J. Exp. Zool. 199:383, 1978). These studies showed that the isolated bladder: (1) actively absorbed sodium and chloride at virtually identical rates and (2) was characterized by a small (1-5 mV) mucosa-positive transmural electrical potential difference P.D.). Ouabain abolished both the transmural P.D. and active NaCl absorption.

The purpose of our studies was to investigate the possible interdependence of active ion transport and the electrical properties of the flounder bladder under conditions where the tissue could be mounted as a flat sheet and the electrical properties of the flounder bladder under conditions where the tissue could be mounted as a flat sheet and the ion fluxes as well as short circuit current (I_{sc}) and tissue conductance (G_T) conveniently determined.

Methods

Urinary bladders were removed from flounder which had been maintained for several days to a week in flowing sea water. The bladders were opened longitudinally and pinned out, mucosal side up, on a soft plastic sheet. A portion of the tissue was then gently compressed between two concentric plastic rings which were designed to "snap" together and thus secure the edges of the tissue. Depending on the size of the flounder from one to four, 1.25 cm^2 pieces could be obtained in this way. The tissue, secured by the plastic rings, was mounted in an Ussing Chamber and bathed on both sides by solutions containing (mM/L) Na:147.5, Cl:147:5, K:2.5, Ca:1.5, Mg:1.0, HEPES:15.0, glucose:5.0. Both sides were vigorously stirred with air, the pH being approximately 7.5. All experiments were conducted at room temperature which averaged about 20°C.

The chamber was equipped with four electrodes; two for monitoring the PD and two for passing current across the tissue. An electronic voltage clamp maintained the PD at zero mV and the tissue conductance was determined from the change in transepithelial current produced by a brief (500 msec), 10 mV change in clamping potential. Preliminary experiments revealed that the conductance of the bladder could increase spontaneously, sometimes by as much a factor of 5. It appeared that these changes were the result of smooth muscle contractions. Increases in G_T often followed a pulse of current (conductance determination) and were elicited promptly by the addition of 10^{-6}M carbamyl choline to the serosal bathing solution. Spontaneous and carbamyl choline induced increases in G_T were abolished by the addition of 10^{-5}M Verapamil to the serosal bathing solution. Preliminary experiments suggested that this concentration had no effect on net NaCl absorption of I_{sc}, thus, in all experiments reported below verapamil was present in the serosal bath.

Transmural fluxes of ^{22}Na or ^{36}Cl were measured simultaneously; mucosa to serosa or serosa to mucosa on paired tissues. In all experiments transmural "control fluxes" were measured for at least three, half-hour periods (after at least one hour to achieve steady tracer flow). At this point the agent to be tested was added to the appropriate bathing solution and fluxes were determined for at least 4 additional half-hour periods. The period immediately following the addition was excluded from the data analysis due to the possibility of non-steady tracer flow. Although we did not routinely control for time dependent effects, preliminary experiments indicated that I_{sc} and G_T were stable at room temperature for six hours.

Results

Table 1 shows the combined values for transmural fluxes and electrical parameters for all control flux periods.

Table 1. Transmural fluxes and electrical parameters under control conditions

	J^{Na} ($\mu Eq/cm^2$-hr)	J^{Cl} ($\mu Eq/cm^2$-hr)	I_{sc} ($\mu Amp/cm^2$)	G_T (mS/cm^2)
M → S	3.91 ± 0.60	4.51 ± 0.65	6.76 ± 0.51	0.69 ± 0.22
S → M	0.47 ± 0.08	1.22 ± 0.19	6.05 ± 0.31	0.53 ± 0.09
net or avg	3.44	3.29	6.41	0.61

All values $\bar{x} \pm$ S.E. for 11 pairs of bladders positive I_{sc} = current flow from S to M.

Comparison of the S to M and M to S unidirectional fluxes suggests virtually equimolar active absorption of sodium and chloride in the presence of a small, but non-zero I_{sc} consistent with the net flow of positive charge from serosa to mucosa. Qualitatively, the values in Table 1 are consistent with those of Renfro (see above), but there are some quantitative differences. The average tissue resistance observed in these experiments, 1600 $\Omega\text{-cm}^2$, is approximately 50% greater than reported by Renfro and the S to M sodium flux is similarly about 50% of that reported earlier, suggesting that both differences may be attributable to the differences in the apparent surface area of the perfused and flat-sheet bladder preparations. In addition the <u>ratio</u> of the simultaneously measured S to M Na and Cl fluxes, about 1.3 in Renfro's experiment, was about 2.6 in the present study.

Table 2 shows the effect of ouabain on Na and Cl fluxes and I_{sc}. In this table and those which follow the units of the various parameters are identical to those in Table 1 and the standard errors have been omitted for clarity. In accord with the observations of Renfro serosal ouabain abolishes net NaCl absorption and I_{sc}.

Table 2. Effect of ouabain on transmural fluxes and electrical parameters (average values for two experiments)

	J^{Na}_{ms}	J^{Cl}_{ms}	J^{Na}_{sm}	J^{Cl}_{sm}	I_{sc}	G_T
control	4.49	5.28	0.46	1.74	5.91	0.50
ouabain	0.61	1.18	0.62	2.77	0.37	0.78
Δ	-3.88	-4.10	0.36	1.03	-5.54	0.28

Units are the same as in Table 1.

Early in the course of this study we explored the possibility that papaverine might be employed to relax bladder smooth muscle. We found, however, that papaverine (5 x 10^{-4}M) added to the <u>mucosal</u> bathing solution abolished I_{sc} within seconds, and that this inhibition could be reversed by washing the mucosal surface of the bladder. Table 3 shows the effect of papaverine (5 x 10^{-4}M) on Na and Cl fluxes in two groups of bladders. The first group ($I_{sc} > 0$) was characterized by the typical I_{sc} and net Na and Cl absorption under control conditions. Mucosal addition of papaverine abolished I_{sc} and net NaCl transport. The second group was characterized, under control conditions, by negligible I_{sc} but nevertheless actively absorbed Na and Cl at equal rates. In these tissues mucosal papaverine had no discernable effect on I_{sc} but NaCl absorption was abolished. These results suggested that I_{sc} and NaCl absorption could be dissociated and that papaverine might exert its effects through two different mechanisms.

Table 3. Effect of papaverine on transmural fluxes and electrical parameters

	J^{Na}_{ms}	J^{Cl}_{ms}	J^{Na}_{sm}	J^{Cl}_{sm}	I_{sc}	G_T
control	2.42	2.68	0.41	0.53	8.1	0.68
pap	0.83	1.03	0.46	0.64	0.7	0.51
Δ	1.49	1.65	0.05	0.11	-7.5	-.17
$I_{sc} > 0$ (2 experiments)						
control	4.00	5.04	0.25	0.87	0.4	0.33
pap	0.92	1.82	0.67	0.91	0.4	0.68
Δ	-3.08	-3.22	0.42	0.02	0.0	.35
$I_{sc} \sim 0$ (2 experiments)						

Units are the same as in Table 1.

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_{ms}^{Na}	J_{ms}^{Cl}	J_{sm}^{Na}	J_{sm}^{Cl}	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 dibutryrl 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