the hexane-extracted radioactivity was identified as phenolic metabolites of benzo(a)pyrene 6.5 weeks after treatment with ^{14}C-BP.

In contrast, benzo(a)pyrene metabolites comprised a greater proportion of the radioactivity stored in tail muscle. At 48 hr after dosing with ^{14}C-BP phenolic metabolites accounted for 38% of the radioactivity in the hexane extracts of muscle homogenate, and 6.5 weeks after treatment approximately 50% of the hexane-extracted radioactivity occurred as phenolic metabolites of benzo(a)pyrene. Moreover, there were slight changes in the amount of radioactivity that remained in the aqueous phase after homogenization and hexane extraction of tail muscle, which were not observed with the hepatopancreas: Two days post-treatment about 91% of the total tail radioactivity was hexane extractable, but only 88% after 6.5 weeks. To date, the metabolites in the aqueous fraction have not been identified but dihydrodiols, polar conjugates of phenols and dihydrodiols, and glutathione conjugates are all likely candidates.

In summary, benzo(a)pyrene-derived radioactivity is very persistent in the lobster ($t_{0.5}$ approximately 2 months), is slowly eliminated via the feces, and may accumulate in egg masses. The radioactivity present in hepatopancreas, the major storage compartment in the lobster, occurs predominantly as benzo(a)pyrene although minor amounts of metabolites are also found.

IONIC CONTRIBUTIONS TO THE SHORT-CIRCUIT CURRENT ACROSS THE OPERCULAR EPITHELIUM

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During previous studies with isolated opercular epithelia, a number of observations on the influence of inorganic ions on the short-circuit current (Isc) were made but not pursued. This year it was decided to return to these observations and investigate further the influences of the major biological ions on the generation of the Isc across this epithelium. This was accomplished by ion substitution experiments by one of the following methods: (1) continual perfusion with ion-substituted Ringer after the establishment of steady-state conditions with normal Ringer, (2) aspiration and rinsing of the chamber several times with ion-substituted Ringer, (3) gradual substitutions by small volume titrations. Epithelia from seawater (SW)-adapted killifish, *Fundulus heteroclitus*, were dissected, mounted in lucite chambers, and short-circuited by methods previously described (Degnan et al., J. Physiol. 271:155-191, 1977).

The Isc across the operculum is a Cl^- current (Karnaky et al., Science 195:203-205, 1977), while the unidirectional Na^+ movements can be described as passive under both short- and open-circuited conditions (Degnan et al., J. Physiol. 271:155-191, 1977; Bull. MDIBL 17:69-71, 1977). Other studies have shown that the transepithelial potential difference (p.d.) across the operculum under open-circuit conditions was primarily Na^+ sensitive and probably a Na^+ diffusion potential on top of an electrogenic Cl^- transport potential (Degnan and Zadunaisky, Amer. J. Physiol., in press).

The effect of Na^+ substitution on the electrical properties across the operculum is summarized in Table 1. Bilateral substitution reduced the Isc 97.9% while unilateral mucosal and serosal substitutions reduced the Isc 85.0% and 17.3%, respectively. The relationship between the bilateral Na^+ concentration and the Isc was a typical Michaelis-Menten curve (Figure 1a). Serosal Na^+ substitution produced an initial increase in the Isc, which peaked near 130% of the control level around 80-100 mM. This was followed by a decline in the Isc to a steady-state level, usually between 50-70% of the control level at zero serosal Na^+ (Figure 1b). Mucosal Na^+ substitution resulted in a linear decrease in the Isc which approached zero as the concentration approached zero (Figure 1c). This response was observed whether there was normal (151 mM) or zero serosal Na^+. The effect of Cl^- substitution on the electrical

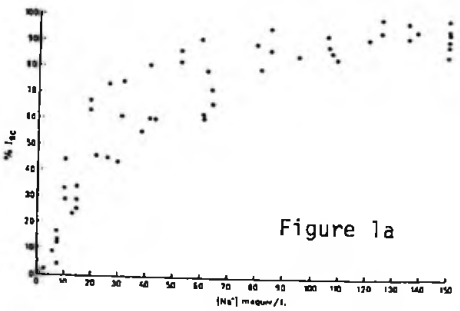

Figure 1a

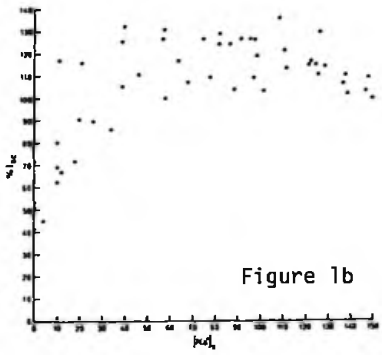

Figure 1b

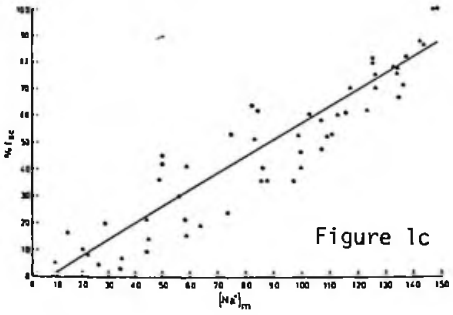

Figure 1c

Figure 1. The dots represent steady-state I_{sc} measurements subsequent to each titration step. The I_{sc}'s were normalized by expressing them as percentages of the controls. a) 6 experiments; b 5 experiments; c) combined data from 4 experiments with normal serosal Na^+ (Δ) and 3 with zero serosal Na^+ (●). Regression line has a slope (S) of 0.62% change in I_{sc}/mM change, an intercept (I) of -5.3%, and a correlation coefficient (r) of 0.94.

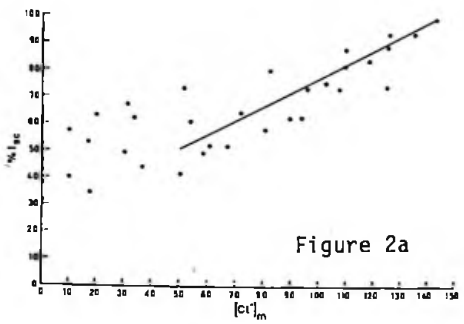

Figure 2a

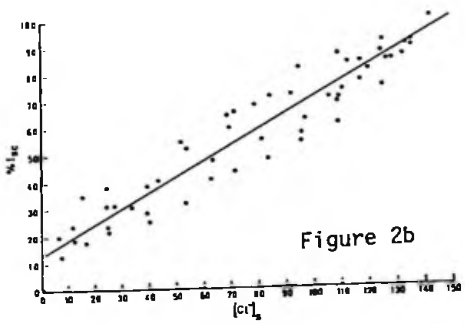

Figure 2b

Figure 2. a) 5 experiments, s = 0.51, I = 24.9, r = 0.90 for the points above 50 mM. b) 5 experiments, s = 0.58, I = 12.9, r = 0.96. Explanations as in Figure 1.

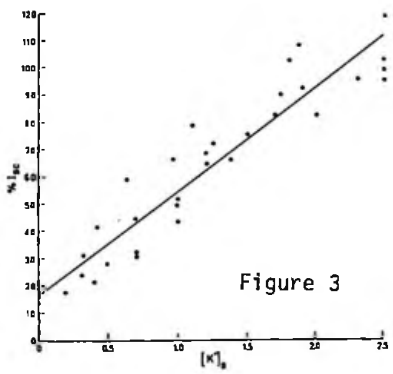

Figure 3

Figure 3. 6 experiments, s = 38.6, I = 16.4, r = 0.95. Explanations as in Figure 1.

TABLE 1.

THE EFFECT OF Na^+ SUBSTITUTION ON THE ELECTRICAL PROPERTIES ACROSS THE ISOLATED OPERCULAR EPITHELIUM OF THE SEAWATER-ADAPTED TELEOST, *FUNDULUS HETEROCLITUS*

	I_{sc} ($\mu A/cm^2$)	p.d. (mV)	R ($\Omega \cdot cm^2$)
CONTROL (14)	150.1 ± 12.6	16.8 ± 1.0	122.9 ± 12.8
Na^+-FREE, M & S	3.1 ± 0.6	1.3 ± 0.4	380.8 ± 63.1
PERCENT CHANGE	97.9	92.5	209.8
P	< 0.001	< 0.001	< 0.001
CONTROL (14)	137.9 ± 19.8	20.1 ± 2.9	149.7 ± 20.8
Na^+-FREE, M	20.2 ± 9.1	4.1 ± 1.4	285.1 ± 59.5
PERCENT CHANGE	85.0	79.7	90 5
P	< 0.001	< 0.001	< 0.025
CONTROL (19)	136.6 ± 16.1	16.7 ± 1.6	143.6 ± 18.6
Na^+-FREE, S	113.0 ± 14.9	22.8 ± 2.5	225.0 ± 26.3
PERCENT CHANGE	17.3	36.8	56.7
P	< 0.02	< 0.01	< 0.005

Table 1. Na^+ was substituted with equimolar amounts of choline or Tris. The data are expressed as mean ± s.e.m. and the number of experiments given in parentheses. M = mucosa; s = serosa.

TABLE 2.

THE EFFECT OF Cl^- SUBSTITUTION ON THE ELECTRICAL PROPERTIES ACROSS THE ISOLATED OPERCULAR EPITHELIUM OF THE SEAWATER-ADAPTED TELEOST, *FUNDULUS HETEROCLITUS*

	I_{sc} ($\mu A/cm^2$)	p.d. (mV)	R ($\Omega \cdot cm^2$)
CONTROL (9)	203.9 ± 35.5	24.6 ± 2.8	161.7 ± 25.9
Cl^--FREE, M & S	6.9 ± 2.8	1.6 ± 0.5	250.4 ± 90.4
PERCENT CHANGE	96.6	93.4	54.8
P	< 0.001	< 0.001	< 0.40
CONTROL (9)	131.2 ± 15.3	18.4 ± 1.7	256.0 ± 29.7
Cl^--FREE, M	61.1 ± 15.3	10.4 ± 1.7	257.0 ± 45.8
PERCENT CHANGE	53.4	43.6	0.4
P	< 0.005	< 0.001	> 0.90
CONTROL (6)	156.7 ± 9.7	20.0 ± 1.2	161.7 ± 30.2
Cl^--FREE, S	14.2 ± 6.2	2.6 ± 0.9	205.1 ± 38.3
PERCENT CHANGE	90.0	87.1	26.8
P	< 0.001	< 0.001	< 0.60

Table 2. Cl^- was substituted with equimolar amounts of methylsulphate. Other specifications as in Table 1.

TABLE 3.

THE EFFECT OF K^+ SUBSTITUTION ON THE ELECTRICAL PROPERTIES ACROSS THE ISOLATED OPERCULAR EPITHELIUM OF THE SEAWATER-ADAPTED TELEOST, *FUNDULUS HETEROCLITUS*

	I_{sc} ($\mu A/cm^2$)	p.d. (mV)	R ($\Omega \cdot cm^2$)
CONTROL (7)	101.1 ± 10.3	14.1 ± 2.1	144.3 ± 20.2
K^+-FREE, M & S	31.1 ± 6.9	3.6 ± 1.2	124.7 ± 23.8
PERCENT CHANGE	70.0	74.2	13.6
P	< 0.001	< 0.005	< 0.50
CONTROL (12)	142.0 ± 22.8	18.0 ± 2.4	139.9 ± 15.8
K^+-FREE, M	135.7 ± 21.9	18.6 ± 2.1	152.7 ± 17.3
PERCENT CHANGE	4.4	3.2	9.2
P	< 0.05	< 0.60	< 0.20
CONTROL (18)	134.7 ± 15.7	18.7 ± 2.0	183.7 ± 21.5
K^+-FREE, S	16.0 ± 3.6	2.8 ± 0.5	233.0 ± 34.7
PERCENT CHANGE	81.0	84.9	26.9
P	< 0.001	< 0.001	< 0.10

Table 3. K^+ was substituted with equimolar amounts of Na^+. Other specifications as in Table 1.

properties across the operculum are summarized in Table 2. Bilateral substitution reduced the Isc 96.6% while unilateral mucosal and serosal substitutions reduced the Isc 53.4% and 90.9%, respectively. The bilateral Cl^- substitution produced a linear decline in the Isc, confirming previous reports (Degnan et al., J. Physiol. 271:155-191, 1977). Mucosal Cl^- substitution produced an initial linear decrease in the Isc with a change to a smaller slope occurring around 50 mM (Figure 2a). Serosal Cl^- substitution produced a linear decline in the Isc, which approached zero as the concentration approached zero (Figure 2b). The effect of K^+ substitution on the electrical properties across the operculum are summarized in Table 3. Bilateral K^+ substitution reduced the Isc 70.0%, which could be completely accounted for by serosal K^+ substitution since there was no effect of mucosal K^+ substitution. The relationship between the serosal K^+ concentration and the Isc was linear (Figure 3).

Under conditions of unilateral ionic substitutions, the Isc across the operculum is no longer an accurate measurement of the net Cl^- secretion rate. Such substitutions establish a diffusion gradient which could generate an ionic current that interferes with the Isc measurement. The observation that this epithelium is largely Na^+ permeable suggests that the largest diffusion artifact in the Isc measurements would result from unilateral Na^+ substitutions. Cl^- flux measurements under these conditions are planned. However, evidence for the ability of this epithelium to transport Cl^- under these conditions was obtained with the use of isoproterenol. This β-adrenergic activator stimulates the Isc across the operculum which can be accounted for by an increase in the Cl^- secretion rate (Degnan and Zadunaisky, Fed. Proc. 37:652, 1978). Its effect is initiated within 1 minute of addition to the chamber with a peak effect occurring within 15-20 minutes.

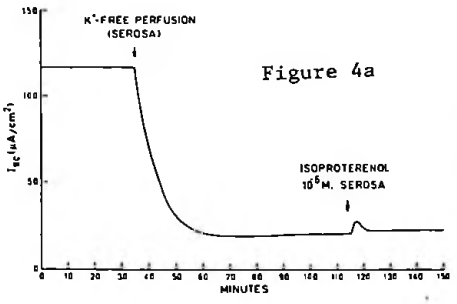

Figure 4a

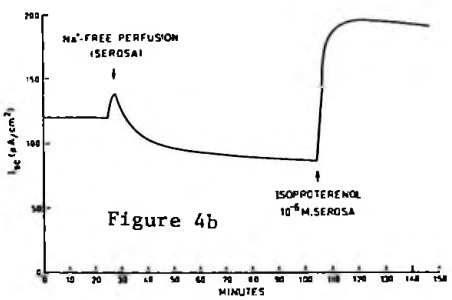

Figure 4b

Figure 4. Lines represent continuous I_{sc} recordings while the serosal side was perfused with the ion-free Ringer and subsequently exposed to isoproterenol. (a) typical response of 4 K^+-free perfusion experiments. (b) typical response of 5 Na^+-free perfusion experiments.

The absence of saturation kinetics in the relationship between the Cl^- concentration and the Isc suggests a flexible system that can vary its transport rate directly with changing plasma Cl^- levels. The depressing effect of mucosal Cl^- substitution on the Isc can explain the depressing effect of SW Cl^- substitution on the transepithelial p.d. across the operculum (Degnan and Zadunaisky, Amer. J. Physiol., in press) and possibly other chloride cell containing epithelia, such as the flounder gill (Potts and Eddy, J. Comp. Physiol. 89:29-48, 1973). If Cl^- were freely permeable to these epithelia, SW Cl^- substitution would be expected to increase the p.d. The observed decreases in the p.d. can be explained by having relatively Cl^- impermeable epithelia with SW Cl^- substitution acting to decrease the electrogenic Cl^- secretion and its associated transport potential. The sensitivity of the Isc across the operculum to serosal K^+ confirms the Na^+,K^+-ATPase dependency of this Cl^- transport (Degnan et al., J. Physiol. 271:155-191, 1977) and the serosal localization of this enzyme in the chloride cell (Karnaky et al., J. Cell Biol. 70:157-177, 1976). The inability of the operculum to respond to isoproterenol (Figure 4a), suggests that the Cl^- transport is effectively inhibited in the absence of serosal K^+. The lack of any effect of mucosal K^+ substitution suggests either there is no apical Na^+,K^+-ATPase, or, if there is, it is not involved in transepithelial Cl^- transport.

The observations that the Isc was almost completely dependent on mucosal Na^+ and partially dependent on mucosal Cl^- suggests that these ions may play a direct regulatory role in the euryhaline response to changing salinities. The transfer of fish from SW to freshwater is accompanied by an immediate reduction in the branchial Na^+ and Cl^- secretion rates (Motais et al., J. Gen. Physiol. 50:391-422, 1966; Epstein et al., Amer. J. Physiol. 224:1295-1299, 1973). Rapid mucosal Na^+ substitutions with isolated opercular epithelia reduced the Isc's to near zero levels within minutes suggesting that this response is fast enough to account for the effects observed with intact fish. The relative effects of mucosal and serosal Na^+ substitutions on the Isc suggests that either the coupled NaCl entry at the

serosal border of the chloride cell, proposed by Silva et al. (Amer. J. Physiol. 233:298-306, 1977), is not a step in the Cl^- transport pathway across this epithelium or, if it is, the high Na^+ permeability and the diffusion of Na^+ from the mucosal side is sufficient to maintain this coupled entry and to activate the ATPase. The fact that the Isc could be stimulated by isoproterenol under serosal Na^+ substituted conditions (Figure 4b), suggests that this transport mechanism was not impaired by this substitution. This work was supported by NIH grants GM 25002 and EY 01340.

RECTAL GLAND VASCULATURE

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Continued interest in the mechanisms of salt secretion has fostered numerous physiological and morphological studies of the selachian rectal gland. We have employed methacrylate corrosion casting techniques and scanning electron microscopy to further identify the general organization of the rectal gland vasculature of *Squalus acanthias*

Two rectal glands were obtained from *S. acanthias* following methyl methacrylate infusion via the ventral aorta. The preparative methods were identical to those described for a study of *S. acanthias* gills (Olson and Kent, this bulletin).

The rectal gland receives arterial blood from the posterior mesenteric artery (also called rectal artery; Bulger, Anat. Rec. 147:95, 1963, or digitiform artery; Hoskins, J. Morphol. 28:329, 1917). As it approaches the anterior third of the gland it bifurcates sending rostral and caudal branches into the outer capsule. These arterial branches course the length of the gland and periodically give rise to singular or paired circumferential arteries which encircle the gland. The latter vessels ramify freely within the outer capsule to form a dense arterial web which supplies the sinus vessels of the secretory parenchyma. Paired veins are closely associated with the mesenteric artery and form an extensive venous net around the artery and over the exterior portion of the rostral and caudal branches. Smaller paired veins course lateral to each circumferential artery and through many small branches form a venous network which overlays the arterial vasculature.

The secretory parenchyma is invested with sinus-like vessels that occupy the spaces between the tubules thus giving them a triangular appearance in cross section. The sinus vessels in the peripheral half of the tubular tissue are radially oriented and frequently connected to each other by short cross branches (Figure 1). Midway into the gland the sinus vessels undergo a rather abrupt change and appear to orient around tubules in both radial and longitudinal axes (Figure 2). The sinus vessels drain into one or several central vessels that anastomose into a single vein which leaves the gland at the level of the post-valvular intestine. Rarely, a singular arterial vessel will course centrally through most of the tubular tissue and then abruptly return to the capsular tissue.

Three types of vessels communicate between the rectal gland and the post-valvular intestine. 1) As the posterior branch of the rectal artery approaches the recto-intestinal junction it divides into three vessels, two of which enter directly into the intestinal tissue. Thus there is an anatomical arterial bypass of the tubular sinuses. 2) The large singular central venous vessel which drains the tubular sinuses leaves the gland in this region and reflects cephlad into the intestine. 3) The capsular venous network, which is so intimately associated with the capsular arterial vessels, also follows the latter vessels as they enter the intestinal tissue. In addition, several capsular veins are closely associated with the sinus vein as it leaves the gland but no anastomoses between the two have been found. The capsular venous vessels appear to communicate with the mucosal sinus veins of the intestine.