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OSMOREGULATION BY THE PRENATAL SPINY DOGFISH, *SQUALUS ACANTHIAS*

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SUMMARY

1. Late in gestation of the ovoviviparous dogfish, *Squalus acanthias*, the uterine fluids are essentially sea water, while the plasma of the 'pup' is similar to that of the female, i.e. isotonic to sea water/uterine fluids, with significantly less Na and Cl, and substantial concentrations of urea.

2. Early 'candle' embryos are bathed in 'candle' fluid and uterine fluid which contains Na and Cl concentrations intermediate between maternal plasma and sea water levels, K concentrations above sea water levels, and urea concentrations slightly below those found in the maternal plasma. Both fluids are isotonic to sea water and maternal plasma.

3. Incubation of 'candles' with associated embryos in sea water for 4–6 days resulted in significant increases in 'candle' fluid Na and Cl concentrations, and a decline in 'candle' fluid K and urea levels. However, under these conditions, the 'candle' embryo is still able to regulate plasma Na, Cl, K and urea concentrations.

4. The efflux of Cl is approximately 5 times the efflux of Na from the prenatal 'pup'; however, both effluxes are equivalent to those described for adult elasmobranchs.

5. The transepithelial electrical potential (TEP) across the 'pup' is −4·4 mV in sea water, which indicates that both Na and Cl are maintained out of electrochemical equilibrium.

6. Cloacal fluid flows vary diurnally with Na and Cl concentrations significantly above those of the plasma. Rectal gland efflux can account for 50–100% of the Na efflux, but less than 25% of the Cl efflux.

7. Removal of the rectal gland resulted in an increase in plasma Na and Cl concentrations 48 or 72 h after the operation, but in both cases it appears that some extra rectal gland excretory system balances at least some of the net influx of both salts.

8. Our results demonstrate that even very young 'candle' embryos of *S. acanthias* are capable of osmoregulation, and that older embryos ('pups') osmoregulate against sea water *intra-utero* and display the major hallmarks

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of elasmobranch osmoregulation, including a reduced ionic permeability and a functional rectal gland for net extrusion of NaCl. In addition, it appears that other pathways exist for salt extrusion in addition to the rectal gland.

INTRODUCTION

Recent reviews (Pang, Griffith & Atz, 1977; Evans, 1979, 1980*a*, *b*) of elasmobranch osmoregulation indicate the paucity of experimental data defining the mechanisms of regulation by these marine fish. To a considerable extent this is the result of the relative scarcity and large size of the necessary experimental animals. The spiny dogfish *Squalus acanthias* is ovoviviparous with developing young maintained in highly vascularized uteri (actually expanded, posterior regions of the paired oviducts) for periods of approximately two years (Woodhead, 1976). During the latter periods of gestation the yolk-sac-bearing 'pups' are apparently exposed to sea water secondary to material flushing of the uteri with sea water (Burger, 1967). Moreover, it has been reported that 'pups' can be removed from excised uteri and maintained in sea water for periods of at least 40 days (Gilbert, 1958), as long as care is taken to avoid rupture of the yolk-sac membrane. It therefore appears that at least these 'pups' are capable of osmoregulation in sea water. Since 'pups' are plentiful (generally 10–16 per pregnant female) and small (approximately 50–75 g) they represent a source of experimental animals for various studies of shark physiology in general and shark osmoregulation in particular. The present work details an investigation of the ionic and osmotic relationships between early embryos and 'pups', and uterine fluids, as well as the ability of both to osmoregulate *extra-utero*. In addition, various parameters of 'pup' Na and Cl regulation were studied in an effort to compare the pattern of ion regulation in the 'pups' with the few published data on adult elasmobranchs.

MATERIALS AND METHODS

Pregnant female *Squalus acanthias* were caught via hook and line in Frenchman's Bay, Maine and maintained in live cars on the dock of the Mt. Desert Island Biological Laboratory. Females were killed via spinal transection and the uteri removed and placed into shallow, water-filled plastic dishpans. During June, July and August two distinctive classes of embryos (presumably one year apart in gestation age) are found in the uteri: 'pups' which are approximately 18 cm long, weighing in the range of 50–75 g (including a yolk sac which comprises some 30–50 % of the total weight), and 'free-swimming' in the uterus surrounded by a few ml of fluid; and 'candle' embryos, approximately 1–30 mm in length, attached via a large artery to a yolk sac which is approximately 50 mm in diameter. The 'candle' embryos and their individual yolk sacs were surrounded by a fragile 'candle' which is presumably the egg case which is lost before the 'pup' stage (Wourms, 1977). The 'candle' separates a small volume of 'candle' fluid surrounding the developing embryos and another small volume of intrauterine fluid surrounding the 'candle'. 'Candle' embryos used in the present experiments were 25–30 mm in length and possessed large, external gills.

Samples of uterine fluids surrounding 'pups' were removed by syringe at the tim

of killing and stored for later analysis. At the same time, blood samples were taken from the caudal vessels of the female and the resident 'pups' via a heparinized syringe. The plasma was separated by centrifugation. To test the osmoregulatory ability of free-swimming 'pups' in sea water, sixteen siblings (with intact yolk sacs) were placed into running sea water (13–15 °C) and blood was removed from individuals (no more than 2 samples from each fish during the course of the experiment) at 30 min, and 1, 3 or 6 days after transfer. 'Candle' and uterine fluids surrounding earlier embryos were also sampled via syringe, and 'candle' embryo plasma was separated from blood which had been collected in heparinized haematocrit tubes from the cut yolk-sac artery. In some cases whole embryos were removed. In one series of experiments 'candles' (with contained embryos) were maintained in running sea water (13–15 °C) for 4–6 days before sampling. All embryos and uterine, plasma and 'candle' fluids were frozen and shipped to Miami for analysis.

Total body-constituent determination of 'candle' embryos were made after homogenization of a 1·0–1·7 g pool of embryos in 10 ml of distilled water with a Potter–Elvehjelm glass–teflon homogenizer. The homogenate was allowed to sit (with occasional mixing) at room temperature (22–25 °C) for 2·5 h, then centrifuged and the supernatant analysed. Total osmolarity of thawed plasma, uterine and 'candle' fluid samples was determined on a Wescor vapour pressure osmometer, urea by a modified urease reaction (Sigma Kit 640-A), Na and K by flame photometry (IL Model 143), and Cl by amperometric titration (Aminco Cotlove).

To determine the unidirectional rate of Na and Cl efflux either 4 μCi (in 10 μl distilled water) of ^{22}Na or 0·5–1 μCi (in 20–40 μl of distilled water) of ^{36}Cl were injected intraperitoneally into 'pups' which had been free-swimming for at least 48 h. The 'pups' were then placed into 200 ml of sea water in a plastic container maintained at 15–17 °C via external running sea water. After a 1 h period of isotopic equilibration within the body fluids of the 'pup', samples of the bath were removed (for intervals ranging up to 20 h) for analysis on either a Packard Autogamma System (^{22}Na) or a Packard Tricarb Liquid Scintillation System (^{36}Cl, Packard ACS scintillation cocktail). Effluxes were calculated as described previously (Kooistra & Evans, 1976). At the termination of the experiment, blood samples were collected in heparinized microcapillary tubes after tail severance and measured samples of the plasma (separated by centrifugation) were assayed for either ^{22}Na or ^{36}Cl to determine the ionic space of the animal using previously described methods (Kooistra & Evans, 1976).

The transepithelial electrical potential (TEP) across the 'pup' was measured via PE 10 bridges (filled with 3 M-KCl in 2% agar) as previously described (Evans, Carrier & Bogan, 1974) with the exception that the potential was recorded by a Radiometer PHM62 digital pH meter. TEPs were determined when the 'pups' were in sea water as well as after transfer to Na- and K-free artificial sea water (Evans & Cooper, 1976) or Cl-free artificial sea water (Kormanik & Evans, 1979). In all cases 'pups' were transferred to sea water again after the ion-substituted solutions to determine any alterations in relative permeabilities produced by high concentrations of either choline or benzenesulphonate.

Cloacal (presumably urinary and rectal gland) efflux of Na and Cl was determined by cannulating the cloacal opening with approximately 12 cm of surgical tubing

(4 mm OD, 2·5 mm ID) flared at the proximal end and secured with a purse-string suture. The distal end was left open since the trapped air space provided sufficient pressure to prevent back flow of sea water into the cannula. At various times post-cannulation, samples of fluid were removed from the cannula by gentle suction (with a 1 ml disposable syringe and PE 50 tubing) down to the level of the cloacal opening, measured for volume and frozen for subsequent ionic analyses.

The importance of the rectal gland in 'pup' osmoregulation was studied by extirpating the gland via a ventral incision while the branchial chamber of the 'pup' was irrigated with 0·05 % MS 222 in sea water. A ligature was placed between the proximal end of the gland and its vascular supply before the gland was removed. Sham operations consisted of the same anaesthesia and ventral incision, followed by exteriorization of the gland. In both cases the incision was sutured closed and the 'pup' was returned to running sea water. After 48 or 72 h, blood was collected after tail severance and plasma was separated by centrifugation and frozen for subsequent analyses. Frozen blood and cloacal fluid samples were shipped to Miami and analysed for Na, K and Cl as described above.

All data are expressed as mean ± S.E. (N) and significant differences determined by Student's *t*-test.

RESULTS AND DISCUSSION

Table 1 compares the major constituents of 'pup' plasma, uterine fluid and the maternal plasma with sea water. It is clear that at this late stage of development the uterine fluids are essentially sea water, with decidedly more Na and Cl than either maternal or 'pup' plasma and very little urea. It is interesting to note that 'pup' plasma contains significantly ($P < 0{\cdot}001$) more K than does adult plasma, but in the same range of the K concentrations found in the surrounding uterine fluid/sea water. Our data therefore support the conclusion (Burger, 1967) that late-term embryos of *S. acanthias* are bathed in sea water secondary to uterine flushing. The volume of the uterine fluids is quite small (less than 5 ml per uterus) and therefore (despite the ionic gradients between the fluids and the maternal plasma, and the highly vascularized uterine lining) the uterus probably does not represent a significant site of net flux of ions into the female, especially when compared with the gills. When 'pups' with intact yolk sacs are placed into running sea water, they are able to maintain consistent plasma osmolarity for at least six days (Table 2). However, plasma Na and Cl concentrations increase to levels slightly, but significantly, above initial levels by 3 days, with Cl subsequently falling to a concentration not significantly above the initial levels by 6 days after transfer. Plasma urea levels fall by some 13 % by the third day and are only 83 % of the initial level at the end of 6 days in sea water. Indeed, the decline in urea concentrations is substantially greater (64 mM) than the increase in plasma NaCl concentrations; hence, one must postulate that some other plasma solute has increased in concentration in order to maintain the consistent osmolarity seen in Table 2. The identity of this solute is unknown. It appears that the yolk sac itself may represent a significant leak pathway for at least urea, since 'pups' with ligated yolk sacs only lost some 2–4 % of their plasma urea after 3 days in sea water (A. Oikari & D. H. Evans, unpublished results). Thus it appears that, while osmoregulation b

Table 1. *Major constituents of uterine fluids, 'pup', and maternal plasma compared to MDIBL sea water*

Solute	Sea water	Maternal plasma	Uterine fluids	'Pup' plasma
Total	945	1007*	952±8 (12)	946±2 (17)
Na	450	234±7 (4)	445±4 (12)	249±0·7 (17)
Cl	534	221±9 (4)	520±4 (12)	231±0·4 (17)
K	7·0	3·3±0·2 (4)	7·3±0·5 (12)	7·9±0·7 (16)
Urea	—	357*	8·5±3·3 (12)	342±3 (17)

Total concentration in m-osmol/kg, solute concentrations in mM; $X \pm$ S.E. (N).
* Data from Murdaugh & Robin (1967).

Table 2. *The effect of sea water incubation on plasma constituents of* S. acanthias *'pups'*

Period of incubation	Osm (16)†	Cl (16)	Na (16)	Urea (16)
30 min or less	956± 9·4	231±1·7	241±2·1	384±3·9
24 h	945± 4·0 NS	234±2·5 NS	245±2·8 NS	378±1·9 NS
3 d	957± 8·6 NS	245±4·2**	262±5·3**	331±5·2***
6 d	938±10·0 NS	236±3·4 NS	255±2·7**	317±7·1***

Same concentration units as Table 1.
NS = non-significant.
** $P < 0.01$.
*** $P < 0.001$.
} compared with initial values.
† Number of animals.

Table 3. *Major constituents of uterine fluids, 'candle' fluids and embryo plasma*

Solute	Uterine fluid (8)	'Candle' fluid (5)	Embryonic plasma (1)
Total	952±9	943±9	Not determined
Na	333±9	317±5	300
Cl	345±6	345±6	353
K	16·4±1	17·6±0·8	10·3
Urea	310±11	308±13	307

Same concentration units as Table 1.
The data from the embryonic plasma is from a single pooled sample of 6 embryos.

free-swimming 'pups' is near-perfect, regulation of NaCl and urea concentrations fails to a varying extent. The fact that near-term 'pups' are surrounded by sea water (Table 1), can survive for prolonged periods of time *extra-utero* in sea water (Gilbert, 1958 and our unpublished results), and display ionic permeabilities and rectal gland effluxes similar to those described for adult elasmobranch (see below), indicates either that the data in Table 1 are relatively abnormal because plasma samples were taken more than once from an individual, or that some sort of secondary regulation takes place after 6 days.

Table 3 compares the major constituents of 'candle' embryo plasma, inter-'candle' fluid and uterine fluid. It is obvious that at this earlier stage of development the uterine fluids are isosmotic to sea water (compare Tables 1 and 2), but contain less Na and Cl, and more K and urea. However, even at this stage the uterine fluids contain greater concentrations of Na, Cl and K, but lower urea concentrations, than does the maternal plasma. The 'candle' fluids and the embryonic plasma are essentially identical to the uterine fluids (with the exception of a reduced K concentration in

Table 4. *Effect of sea water incubation on total body and plasma constituents of* S. acanthias *embryos* (2·5–3 cm)

	Total body concentration (mmol/100 g)		Plasma concentration (mol/l)	
Solute	Control (3)	4–6 days SW (2)	Control (1)	4–6 days SW (2)
Na	14·5 ± 1·5	14·8 ± 0·2	300	271 ± 0
Cl	15·1 ± 1·5	15·9 ± 0·2	353	323 ± 9
K	4·6 ± 0·1	4·8 ± 0·04	10·3	7·8 ± 1·0
Urea	32·9 ± 1·2	32·3 ± 1·0	307	306 ± 62

(N) is number of pooled samples of 6 embryos.

embryonic plasma). The passive role of the 'candle' membrane in this distribution of solutes is indicated by our finding that incubation of candles for 4–6 days in running sea water results in substantial changes in the content of the intra-'candle' fluids. In 4 experiments, the Na and Cl concentrations of the fluids rose to 420 ± 4·2 mM and 496 ± 1·3 mM, respectively, while the K concentrations fell to 8·2 ± 0·2 mM, and the urea levels were reduced to near zero (0·4 ± 0·2 mM). Since the 'candle' membrane appears to be a mechanical, rather than an osmotic or ionic, barrier, it is evident that the early developing embryo is bathed in fluids whose composition is produced and controlled by transport steps at the level of the uterine lining. We have found that the transepithelial electrical potential across early uteri is essentially zero (Kormanik & Evans, 1978) so the Na, Cl and K gradients between the maternal blood stream and the early uterine fluids must be produced by active transport. One could propose further that urea merely leaks into the uterine fluids from the maternal blood stream. Since the 'candle' is permeable to these ions and urea, the solutes merely diffuse into the 'candle' fluids and produce an incubation medium isotonic and iso-ionic to the body fluids of the developing embryo.

The fact that 'candle' embryo body fluids are similar to both the 'candle' fluid and uterine fluid, and contain more Na, Cl and K than 'pups' (compare Tables 2 and 1, plasma values) implies that the 'candle' embryos are incapable of osmotic or ionic regulation. To test this, we examined total body levels and plasma concentrations of Na, Cl, K and urea in embryos taken from 'candles' which had been exposed to sea water for 4–6 days. We found that during this period (when the intra-'candle' fluids were moving toward ionic equilibrium with the sea water, and losing their urea – see above), the embryos were alive and regulating their body ionic and urea content (Table 4). Indeed, it appears that the embryos have actually reduced their blood Na, Cl and K levels. It is therefore clear that ionic and regulatory mechanisms reside in some epithelial membranes of even quite young 'candle' embryos.

In summary, our data indicate that even early embryos of *S. acanthias* are capable of regulating body Na, Cl, K and urea levels, despite the fact that they are incubated in a uterine/intra-'candle' medium which is presumably produced by the uterine lining and contains significantly less Na, Cl, but more K and urea than sea water. Late-term 'pups' are surrounded by small volumes of sea water, but are capable of maintaining plasma Na and Cl levels distinctly below those in sea water, but urea and K levels above those in sea water. Importantly, 'pup' plasma has the same Na, Cl and urea concentrations as maternal plasma, but decidedly greater concentrations of K

Table 5. *Efflux of Na and Cl from 'pups' of* Squalus acanthias

	Rate constant ($\times 10^{-3}.h^{-1}$)	Ionic space ($ml.100\ g^{-1}$)	Plasma concentration* (mM)	Efflux ($\mu mol.100\ g^{-1}.h^{-1}$)
Na	$2{\cdot}9 \pm 0{\cdot}9$ (11)	$63{\cdot}0 \pm 8{\cdot}5$ (11)	$249 \pm 0{\cdot}7$ (17)	45·5
Cl	$21{\cdot}0 \pm 3{\cdot}5$ (8)	$45{\cdot}2 \pm 2{\cdot}4$ (9)	$231 \pm 0{\cdot}4$ (17)	219

The efflux was calculated as the product of the rate constant, ionic space and plasma concentration.
* Data from Table 1.

Table 6. *Transepithelial electrical potentials across* Squalus acanthias *'pups' in sea water and ion-substituted sea water solutions*

SW	−K, −Na ASW*	SW	−Cl, −HCO_3 ASW	SW
$-4{\cdot}4 \pm 0{\cdot}6$** (14)	$-7{\cdot}0 \pm 0{\cdot}6$ (14)	$-4{\cdot}5 \pm 0{\cdot}7$ (13)	$+9{\cdot}1 \pm 0{\cdot}7$ (13)	$-2{\cdot}6 \pm 0{\cdot}5$ (12)

* ASW = Artificial sea water.
** TEP in mV, fish relative to SW.

In order to quantify pathways of Na and Cl balance of 'pups', we measured isotopic fluxes, transepithelial electrical potentials (TEPs), and rectal gland function. The rate of ^{22}Na efflux was the same whether we used pups with intact or ruptured yolk sacs. In addition, analysis of the amount of radioactivity in the yolk sac at the termination of some experiments showed that only $3{\cdot}5 \pm 0{\cdot}7\%$ (6) of the total ^{22}Na radioactivity in the fish was in the yolk sac. It therefore appears that, despite an extensive vascularization, the yolk sac and its contents do not represent a significant Na-leak pathway between the body fluids of the 'pup' and the hypernatric environment. This is to be contrasted with other data (see above) which indicate that the yolk sac may be relatively permeable to urea. The rate constant, ionic space, blood concentration (from Table 1), and calculated efflux of both Na and Cl are presented in Table 5. It is obvious that the efflux of Cl is some 5 times the Na efflux. The magnitude of the Na and Cl effluxes from *Squalus acanthias* 'pups', and the much higher efflux of Cl, are very similar to the few published data from other elasmobranchs (Evans, 1979). It is apparent, therefore, that prenatal 'pups' maintain the extremely low ionic effluxes which is one of the major hallmarks of adult elasmobranch osmoregulation (Evans, 1979).

The measurement of the TEP across a fish can provide us with information about the electrochemical gradients for ions across the permeable epithelium of the fish (presumably gills). Alteration of the TEP by ionic substitutions in the external sea water can assess relative ionic permeabilities of that epithelium. The TEP across *Squalus acanthias* 'pups' (Table 6) is in the same range as those measured across adult sharks *Scyliorhinus canicula* (Bentley, Maetz & Payan, 1976) and two skates, *Raja erinacea* and *Urolophus jamaicensis* (Evans, 1980*a*). Given the previously determined blood Na and Cl concentrations (Table 1) we can calculate (Evans, 1979) the equilibrium potentials for both Na and Cl (+14·8 mV and −20·9 mV, respectively). Thus, it is obvious that neither Na nor Cl is maintained in electrochemical equilibrium, i.e. their low plasma concentration is secondary to active extrusion mechanisms. Table 6 also demonstrates once again the rather low ionic permeability of the 'pup' branchial

Table 7. *Cloacal fluid flow, ion concentrations and ionic loss from* S. acanthias *'pups'*

Time period	Flow (μl.100 g.h^{-1})	Na (mM)	Cl (mM)	Na flux* (μmol.100 g.h^{-1})	Cl flux* (μmol.100 g^{-1} h^{-1})
10.00–16.00 (Max. flow)	117±17 (11)	397±40 (10)	468±17 (9)	42·5±6·4 (10)	57·6±11·0 (9)
22.00–8.00 (Min. flow)	52·9±9·0 (12)	387±28 (11)	484±34 (8)	20·5±4·4 (11)	22·6±4·0 (8)
Significance between Max. and Min. flow periods	$P < 0.01$	n.s.	n.s.	$P < 0.01$	$P < 0.01$

* Calculated for individual fish.

epithelium. When a 'typical' teleost is transferred to Na, K-free artificial sea water, the TEP falls by some 65 mV (Potts & Eddy, 1973), far more than the 3·6 mV seen with the 'pup'. In addition, the fact that removal of an approximately equivalent amount of Cl depolarizes the TEP by some 13 mV corroborates our flux data indicating a higher Cl than Na permeability.

We found that the cloacal flow rates displayed a distinct diurnal rhythm with maximal flows evident during the day. To distinguish this rhythm we have separated our data into two collection periods (Table 7). It is evident that, despite a substantial increase in cloacal flow between 10.00 and 16.00 hours, neither the Na nor Cl concentrations of the fluids changed during this time period. Nevertheless, both Na and Cl efflux were twice that calculated for the night period. We found no evidence for diurnal variation in total Na or Cl efflux measured radioisotopically (unpublished results). This is not especially surprising in the case of Cl efflux, since the variable cloacal efflux is only 11–25% of the total efflux (compare Tables 5 and 7), and a diurnal variation in this component might be obscured by variability in the measurements of total efflux. However, the variable cloacal Na loss represents 50 to nearly 100% of the total Na efflux, so we should have seen a diurnal variation in total Na efflux. One can only suppose that, since the cloacal flow and total efflux studies were done on different individuals, individual variability and experimental error obscured any rhythm of total Na efflux that may be present. The alternative, that extra-cloacal (presumably branchial) Na efflux varied reciprocally with the cloacal Na efflux, thereby resulting in no net change in total Na efflux, seems unlikely. Studies of the partitioning of Na efflux from cannulated 'pups' are certainly warranted. The important point to note, however, is that cloacal loss (presumably predominantly rectal gland secretion) represents the major site for Na efflux (compare Tables 5 and 7), while the vast majority of Cl efflux is extra-cloacal. In contrast, Haywood (1975) found that the rectal gland was more important in controlling blood Cl concentrations than blood Na concentrations in the striped dogfish *Poroderma africanum*. It is evident that we need more information on the comparison between Na and Cl control mechanisms in elasmobranchs. The cloacal flow rates and fluid concentrations in Table 7 are in the same range as the few published studies of rectal gland secretion by other, adult elasmobranchs (Evans, 1979), indicating that the rectal gland is fully functional in prenatal *Squalus acanthias* 'pups' and capable of producing a secretion which is

Table 8. *The effect of removal of the rectal gland on plasma Na and Cl concentrations of* S. acanthias *'pups'*

	Sham operated	Rectal gland removed
	48 h experiment	
Na	249 ± 5 (8)	271 ± 7 (7)*
Cl	232 ± 5 (8)	253 ± 6 (7)*
	72 h experiment	
Na	259 ± 5·6 (6)	278 ± 3·0 (6)**
Cl	241 ± 4·5 (6)	256 ± 4·7 (6)**

* $P < 0.02$ when compared to sham-operated fish.
** $P < 0.01$ when compared to sham-operated fish.

distinctly hypernatric and hyperchloric to the plasma (compare Table 7 with Table 1). In fact, the Cl concentration of the cloacal fluids is distinctly above the Na concentration. This discrepancy between the Na and Cl concentration has not been described for the few studies on rectal gland secretion by adult *Squalus acanthias* either *in vivo* (Burger, 1962) or *in vitro* (Silva, *et al.* 1977).

The data in Table 8 demonstrate that removal of the rectal gland does result in a significant increase in both plasma Na and Cl, when measured either 48 or 72 h after extirpation of the gland. The increase in plasma Na concentration observed 48 h after gland removal in the present experiments is only slightly greater than described for adult *Squalus acanthias* under similar circumstances (Forrest *et al.* 1973). It is interesting to note that the difference between the sham-operated controls and the experimentals is actually less after 72 h than (in another experiment) after 48 h, indicating that some type of extra-rectal gland regulation may have become more important after 48 h. If we assume that the difference between the plasma Na levels in the sham and in the operated fish represents the true net influx of Na secondary to the removal of the rectal gland, and since we know that the Na space is 63 ml. 100 g^{-1} (see Table 5), we can calculate the net Na influx during the 48 h experimental period to be 29 μmol. 100 $g^{-1}.h^{-1}$. This is remarkably close to the mean of the diurnal rate of Na efflux via cloacal secretion (32 μmol. 100 $g^{-1}.h^{-1}$, Table 7). It therefore appears likely that removal of the rectal gland was followed by a net Na influx equivalent to that normally balanced by cloacal secretion, i.e. there was no compensatory alteration of extra-rectal gland (renal or branchial) Na secretion in the first 48 h. However, similar calculations using the data from the 72 h experiment indicate that the average net Na influx for 72 h was only 17 μmol. 100 $g^{-1}.h^{-1}$, far below the net influx expected if the 'pup' was unable to compensate for the loss of the rectal gland. One must therefore propose that, at least after 48 h, the 'pup' either decreases its already low Na permeability to decrease the net passive influx of Na, or else initiates some other excretory mechanism, presumably at the level of the kidney or gills. The data for Cl demonstrate extra-rectal gland control even more clearly. From Tables 5 and 8 we can calculate that the net Cl influx was 20 μmol. 100 $g^{-1}.h^{-1}$ for the 48 h experiment and only 9·4 μmol. 100 $g^{-1}.h^{-1}$ for the 72 h experiment, both decidedly below the mean measured cloacal (predominantly rectal gland) secretory rate of 40 μmol. 100 $g^{-1}.h^{-1}$. Thus, it is clear that, while removal of the rectal gland does impair regulation of both

Na and Cl, other compensatory mechanisms exist and reduce the increase in plasma Na and Cl below that expected. In fact, Burger (1965) found that adult *Squalus acanthias* are able to survive for prolonged periods of time in sea water after removal of the rectal gland.

Our data therefore indicate that, despite a fully functional rectal gland, prenatal *Squalus acanthias* 'pups' apparently have extra-rectal gland mechanisms for both Na and Cl regulation. Chan, Phillips & Chester Jones (1967) have previously found that neither the rate of Na efflux nor the plasma Na concentration of the lip shark, *Hemiscyllium plagiosum*, changes after extirpation of the rectal gland. In addition, Haywood (1975) found that after ligation of the rectal gland in the striped dogfish, *Poroderma africanum*, blood Na and Cl levels increased (Cl much more than Na) for 2–3 days and then either levelled off (in the case of Na) or declined sharply (Cl). One must conclude therefore that, at least in three species of elasmobranchs, excretory pathways for both Na and Cl exist in addition to the rectal gland. We have recently succeeded in perfusing the isolated head of *Squalus acanthias* 'pups' (Evans & Claiborne, 1982); it is hoped that this system will allow us to investigate branchial transport pathways more directly.

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REFERENCES

BENTLEY, P. J., MAETZ, J. & PAYAN, P. (1976). A study of the unidirectional fluxes of Na and Cl across the gills of the dogfish *Scyliorhinus canicula* (Chondrichthyes). *J. exp. Biol.* **64**, 629–637.

BURGER, J. W. (1962). Further studies on the function of the rectal gland in the spiny dogfish. *Physiol. Zool.* **35**, 205–217.

BURGER, W. H. (1965). Roles of the rectal gland and the kidneys in salt and water excretion in the spiny dogfish. *Physiol. Zool.* **38**, 191–196.

BURGER, W. (1967). Problems in the electrolyte economy of the spiny dogfish, *Squalus acanthias*. In *Sharks, Skates and Rays* (ed. P. W. Gilbert, R. F. Matheson and D. Rall), pp. 177–185. Baltimore: Johns Hopkins University Press.

CHAN, D. K. O., PHILLIPS, J. G. & CHESTER JONES, I. (1967). Studies on electrolyte changes in the lipshark *Hemiscyllium plagiosum* with special reference to hormonal influence on the rectal gland. *Comp. Biochem. Physiol.* **23**, 185–198.

EVANS, D. H. (1979). Fish. In *Comparative Physiology of Osmoregulation in Animals*, vol. 1 (ed. G. M. O. Maloiy), pp. 305–390. London: Academic Press.

EVANS, D. H. (1980*a*). Kinetic studies of ion transport by fish gill epithelium. *Am. J. Physiol.* **238**, R224–R230.

EVANS, D. H. (1980*b*). Osmotic and ionic regulation by freshwater and marine fishes. In *Environmental Physiology of Fishes* (ed. M. A. Ali), pp. 93–122. New York: Plenum Press.

EVANS, D. H., CARRIER, J. C. & BOGAN, M. B. (1974). The effect of external potassium ions on the electrical potential measured across the gills of the teleost, *Dormitator maculatus*. *J. exp. Biol.* **61**, 277–283.

EVANS, D. H. & CLAIBORNE, J. B. (1982). Hemodynamic effects of epinephrine and carbachol on the isolated, perfused head of the dogfish 'pup' (*Squalus acanthias*). *Bull. Mt Desert Isl. biol. Lab.* (In the Press).

EVANS, D. H. & COOPER, K. (1976). The presence of Na–Na and Na–K exchange in sodium extrusion by three species of fish. *Nature, Lond.* **259**, 241–242.

FORREST, J. N., SILVA, P., EPSTEIN, A. & EPSTEIN, F. H. (1973). Effect of rectal gland extirpation on plasma sodium in the spiny dogfish. *Bull. Mt Desert Isl. biol. Lab.* **13**, 41–42.

GILBERT, P. W. (1958). The ability of yolk-sac dogfish pups to survive outside the uterus. *Bull. Mt Desert Isl. biol. Lab.* **68**.

HAYWOOD, G. P. (1975). A preliminary investigation into the roles played by the rectal gland and kidneys in the osmoregulation of the striped dogfish *Poroderma africanum*. *J. exp. Zool.* **193**, 167–176.

KOOISTRA, T. A. & EVANS, D. H. (1976). Sodium balance in the green turtle, *Chelonia mydas*, in seawater and freshwater. *J. comp. Physiol.* **107**, 229–240.

KORMANIK, G. A. & EVANS, D. H. (1978). Preliminary studies of osmoregulation by the premature 'pup' of the dogfish, *Squalus acanthias*, and the uterine lining of the female. *Bull. Mt Desert Isl. biol. Lab.* **18**, 65–69.

KORMANIK, G. A. & EVANS, D. H. (1979). HCO_3-stimulated Cl efflux in the gulf toadfish acclimated to sea water. *J. exp. Zool.* **208**, 13–16.

MURDAUGH, H. V. Jr. & ROBIN, E. D. (1967). Acid base metabolism in the dogfish shark. In *Sharks, Skates and Rays* (ed. P. W. Gilbert, R. F. Matheson and D. P. Rall), pp. 249–264. Baltimore: Johns Hopkins University Press.

PANG, P. K. P., GRIFFITH, R. W. & ATZ, J. W. (1977). Osmoregulation in elasmobranchs. *Am. Zool.* **17**, 365–377.

POTTS, W. T. W. & EDDY, F. B. (1973). Gill potentials and sodium fluxes in the flounder *Platichthys flesus*. *J. comp. Physiol.* **87**, 29–48.

SILVA, P., STOFF, J., FIELD, M., FINE, L., FORREST, J. N. & EPSTEIN, F. H. (1977). Mechanism of active chloride secretion by shark rectal gland: role of Na–K–ATPase in chloride transport. *Am. J. Physiol.* **233**, F298–F306.

WOODHEAD, A. D. (1976). Reproductive ecology of spiny dogfish, *Squalus acanthias*. *Bull. Mt Desert Isl. biol. Lab.* **16**, 103–106.

WOURMS, J. P. (1977). Reproduction and development in chondrichthyan fishes. *Am. Zool.* **17**, 379–410.