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Solute Excretion in *Squalus acanthias* During Adaptation to Dilute Seawater

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When placed in 70% seawater, the spiny dogfish (*Squalus acanthias*) undergoes a diuresis, with increased glomerular filtration rate and increased excretion of sodium and urea (Forster et al, Comp. Biochem. Physiol., 42A: 3, 1972). Serum sodium and urea levels fall, bringing serum osmolality into equilibrium with that of the dilute seawater.

The present investigations were designed to further these observations, particularly with regard to their chronology and the pattern of solute excretion. Female *Squalus acanthias* weighing from 4-6 Kg were caught by trawl line and studied with twenty-four hours. Two 1 to 2-hour control clearance periods were obtained in a seawater pool. The fish were then transferred directly to a pool maintained at a 70% seawater dilution by hoses delivering seawater and freshwater. Efforts were made to avoid sudden water temperature alterations. Immediately on transfer, further clearance periods were obtained; studies were then repeated on the day following (day 2) and two days following (day 3) transfer to the dilute pool. A cloacal catheter for urine collection and caudal vessel catheter for blood specimens were left in situ throughout the study. Eight fish were originally studied, and studies were completed in six.

The results are shown in Table 1. There was a progressive increase in inulin clearance, urine volume, and the excretion of sodium and urea, reaching a maximum at days two and three. Plasma osmolality fell be approximately 15% of which one-third was due to the fall in plasma sodium and attendant anion, one-third to a fall in plasma urea, and one-third to a fall in another unmeasured solute, presumably trimethylamine oxide. On day 2 there was a profound reduction in urinary potassium excretion seen both when this excretion wa expresses in absolute quanitites (UV_K) and as a fract of the filtered potassium. Potassium excretion tended increase towards control levels on the third day. Plas potassium levels were unchanged throughout the stu In a control group of four dogfish studied daily for th days in undiluted seawater, there were no significa changes in any of the measured parameters of so excretion.

While the mechanism of the adaptive changes t take place in plasma and urinary solute levels cannot determined from these studies alone, the transient a profound reduction in urinary potassium excret suggest that the secretion of a mineralocorticoid- substance may be reduced upon introduction to d seawater.

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Table 1

	Day 1 100% Seawater	Day 1 70% Seawater	Day 2 70% Seawater	Day 3 70% Seawater
Urine volume (ml/kg/hr)	0.61±.13	82±.15	2.37±.25**	3.10± 34**
Inulin clearance (ml/kg/hr)	3.1±.65	2.8±0.4	5.5±0.8*	4.9±0.5
Posm (mOsm/kg)	936±5.0	902±4.2	827±4.3**	803±5.5**†
Purea (mM/l)	320±13	338±12	302±5.0	275±8.0*
P_{Na} (mM/l)	261±1.5	254±1.8	235±1.8**	233±2.5**
U_{osm} (mOsm/kg)	661±30	630±20	465±12**	478±18**
UV urea (mM/kg/hr)	30±10	79±16	376±57**	570±121**
U_{Na} (mM/kg/hr)	161±41	201±33	365±40*	453±65**
UV_K (mM/kg/hr)	36.2±6.2	21.8±6.1	6.1±0.9*	23.1±8.4
Excreted/Filtered Na	0.20±.02	0.30±.03*	0.30±.03*	0.41±.04**
Excreted/Filtered K	4.13±5.8	2.37±.70	0.43±.11**	1.39±.42**†

1. Each value represents the mean of two clearance periods for each animal studied.
2. Values are Mean ± SEM.
3. *Significant at $p<.02$ and **$p<.001$ (unpaired) from 100% seawater.
4. †Significant ($p<.02$) difference between days 2 and 3.

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Chloride Cells in *Anguilla* After Partial Adaptation to Fresh Water

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Eels were kept in seawater for four weeks and tran ferred to fresh water for three days. Epstein et al. (tr Bulletin) found a high level of Na-K-ATPase and a hi rate of Na efflux after the three weeks in seawater. Aft 3 days in fresh water the ATP-ase level remained high b the rate of efflux declined. The four-week period in se wate was sufficient for full physiological adaptation.

As previously reported (Doye and Epstein – Cyto ologie *6*, 58-73, 1972), the chloride cells of the fully ada ted seawater eel have a fairly dense cytoplasm pervad by a fully developed maximally close-branched tubular r ticulum with mitochondria evenly distributed from t base almost to the apex of the cell. In eels fully adapt to fresh water the chloride cells tend to be swollen with pale cytoplasm, minimally branched tubular reticulum a fewer mitochondria. Many cells appear degenerated. Se water adapted eels returned to fresh water for seven da

o:ved some signs of mitochondrial damage character-
d by the formation of whorls.

The specimens in the current series were examined the third day after transfer to fresh water. In compari-n with chloride cells of eels fully adapted to seawater, se chloride cells after 3 days in fresh water show a less ll developed tubular reticulum, the cytoplasm is as nse as expected for a seawater specimen but a larger portion of the cells are below the surface epithelium . not exposed to the seawater). In those cells in which tubular reticulum is moderately well developed it is s branched than in animals fully adapted to seawater. at is new in these specimens is the presence of a atly increased vesicular component in the apical third most cells. The following figure is as 12,300 magnifica-n. The mitochondria instead of being evenly distributed m the base almost to the apex of the cell are absent m the apical half to one third of the cell.

In this experiment the numbers of mitochondria per and the numbers of chloride cells in the filament ap-ared unchanged from that in seawater conditions. Des-e a change in configuration of the tubular the total area th s membranous component did not differ significantly m that in specimens adapted to seawater. These mor-ological observations are consistent with the findings Epstein et al. (this Bulletin) in seawater adapted ani-s. However we found 1) many chloride cells located neath the surface epithelium rather than exposed to the ater and 2) alterations in the tubular reticulum from the normal branched configuration in close apposition to mitochondria to a population of vesicles no longer in such close apposition to mitochondria. These results are consistent with the observed decrease in salt efflux.

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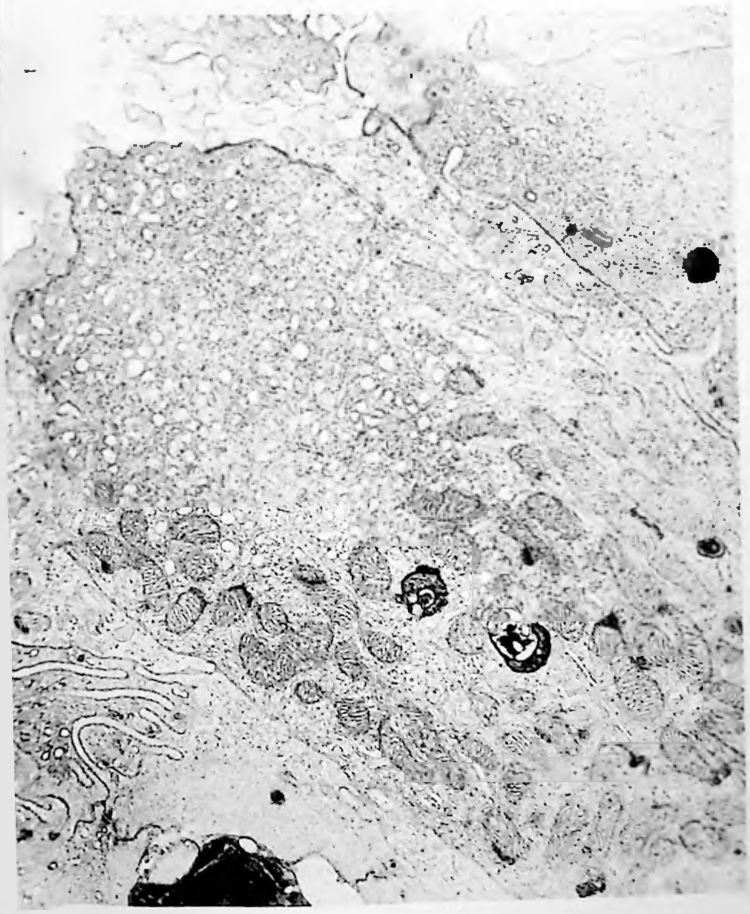

Figure 1
Anguilla gill (12,300 X)

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The Chloride-Cell in Squalus Gill

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The rate of sodium transport in the elasombranch gill has been reported to be about one-tenth of that in teleosts. Boylan (Bulletin MDIBL 5, 1962) found the gill almost impermeable to water and urea. Burger and Horowicz (Bulletin MDIBL 6, 1966) found sodium efflux of the head region to be 2.2 m moles/sq.m/hour, efflux being about one-fifth of influx. There have been only occasional and fragmentary reports of mitochondrial-rich cells in elasmobranch gills and no detailed comparison with chloride cells of teleosts.

The dogfish, *Squalus acanthias*, was studied with histological sections used to orient samples used for electron microscopy. In cross sections of gill filaments there is an artery and vein at the free edge, a central blood sinus and collecting vessels at the attached side of the filament. The respiratory (secondary) lamellae are found at each side of the primary filament. The epithelium of the respiratory lamellae consists of a single cell type characterized by surface ridges, a dense apical cytoplasm with a layer of small ellipsoidal mucous vesicles in a microfilamentous web. The epithelium of the primary filament consists of similar cells, but in two regions one finds much larger cells intersperced among them. At the free edge the large cells are present with some regularity, but it is at the attched edge of the filament that they predominate, being almost as numerous as the ordinary epithelial cells. Failure to observe these large cells in previous electron microscopic studies can be ascribed to their absence from the respiratory lemallae and to their location at the attached edge of the filament. In the electron microscope the large cells show considerable superficial resemblance to the chloride cells of teleost gills in that the mitochondria are very numerous and the cytoplasm is filled with spherical and elongate vesicles and tubules, the lateral and basal borders of the cell have elaborate interdigitating processes and a conspicuous intercellular space. The apical portion of the cell is free of filaments and many of the vesicles are close to the apical membrane. At higher magnifications (Figure 1, pg. 28) it can be seen that there is no regular branching of the tubular reticulum so characteristic of the teleost chloride cell, nor can we find instances of tubular connections to the lateral cell membrane. In the teleost (eel), however, transformation of the branching tubular reticulum to a vescicular form has been observed under some conditions of adaptation (Doyle — #8