toward decreased rate of weight gain (due possibly to parental neglect or cross-contamination from dosed parents). This pilot study with petrels indicates that this species is well suited for pollutant toxicity research, since petrels are abundant, easily accessible in their burrows, and available for long periods during the incubation and chick phases of their breeding cycle.

The results of this successful field study confirm and extend previous laboratory work and emphasize the significant impact that oil pollution could have on young seabirds in the wild. Future research plans involve the effects of oil on the reproductive behavior and physiology of adult seabirds, the behavioral ontogeny of chicks, and mechanisms of contamination of chicks by adults. The impact of other pollutants such as oil dispersants and organochlorines will also be investigated.
This study was supported by United States Public Health Service Grant ES 00920.

## EXPERIMENTS ON THE CELL VOLUME REGULATION IN SLICES OF THE RECTAL GLAND OF THE DOGFISH (*Squalus acanthias*)

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The mechanism(s) of volume regulation in the cells of the dogfish rectal gland was investigated in the light of a pilot experiment carried out jointly with Dr. J. Hayslett in 1972 showing, that as compared with controls, an inhibition of the $Na^+$-pump by 0.5 mM ouabain produced only a slight swelling of the tissue, suggesting the presence of an ouabain-insensitive component of the regulating system.

Slices cut perpendicular to the long axis of the gland (mean thickness 0.3 - 0.4 mm) were employed. The slices were prepared free-hand by the method of Deutsch (J. Physiol. 87:568, 1936), and the fibrous capsule was trimmed off. Incubation media: Dogfish Ringer salines were employed; modifications thereof will be mentioned below. In a number of experiments the salines contained $^3H$-polyethylene glycol (PEG) (1 mg/ml; 1 μCi/ml) as a marker of the extracellular fluid compartment. Samples of tissue were taken: (a) fresh; (b) after loading the cells with $Na^+$ by incubation of the tissue in an isotonic $Ca^{2+}$-free saline at 0°C in the absence of any metabolizable substrate; (c) after incubation of the loaded tissue slices in salines containing glucose as metabolizable substrate. Usually, 10-12 slices (each weighing approximately 10 mg wet wt) were incubated in 2.5 ml saline under appropriate conditions. All tissue samples were gently blotted, and two slices were combined for one analytical procedure. The blotted slices were weighed, and then dried at 85°C overnight. Controls showed that all tissue water was lost under these conditions. The differences between wet and dry wt (W.W. and D.W.) of the tissue were taken to represent tissue water. The dry tissue samples were extracted for not less than 48 h with 5 ml 0.2 N $HNO_3$. In this extract, tissue cations ($Na^+$ and $K^+$) were determined by flame photometry, $Cl^-$ by potentiometric titration, and the activity corresponding to tissue PEG was assayed by scintillation spectrometry. The means and S.E. for each experimental group were determined. The data are expressed in kg $H_2O$ or mequiv. electrolytes per kg tissue D.W. The extracellular space E is given in kg/kg tissue W.W. From the obtained values, the apparent intracellular ionic concentrations may be computed using the usual simplifying assumptions (e.g., availability of all cell water as solvent, uniform distribution of electrolytes in cell $H_2O$, no binding or other interaction of electrolytes with cell constituents, etc.).

The determined parameters for fresh tissue, tissue after loading at 0°C, after aerobic incubation ($O_2$), and the effect of 0.5 mM ouabain thereon, are given in Table 1. It will be seen that in the course of loading the tissue swells slightly by 0.44 kg $H_2O$/kg D.W. ± 0.08 (S.E.), and this process is associated with a marked uptake of $Na^+$ (and $Cl^-$) and some loss of $K^+$. Subsequent aerobic incubation of the loaded tissue did not produce a net loss of water, although regularly a reaccumulation of $K^+$

Table 1

Tissue Water and Electrolytes in Slices of Dogfish Rectal Gland

| TREATMENT | $H_2O$ kg/kg DW | $\varepsilon$ kg/kg WW | Na meq/kg DW | K meq/kg DW | Cl meq/kg DW |
|---|---|---|---|---|---|
| Fresh | 2.85±.05(16) | – | 286±33(16) | 324±30(30) | 347±27(10) |
| Loading (0 C) | 3.21±.07(12) | 0.17±.01(6) | 465±18(11) | 241± 8(12) | 432±16(12) |
| Incubation, control | 3.18±.09(30) | 0.15±.02(20) | 376±31(32) | 321±16(32) | 462±34(16) |
| Incubation, 0.5 mM ouabain | 3.59±.03(14) | 0.14±.01(9) | 683±23(11) | 136± 8(11) | 551±16(11) |

For experimental and analytical conditions, see text.
The incubation of the slices was carried out after loading at 0 C. Values are expressed as the mean ± S.E. , for (n) number of determinations.

was observed, as compared with controls. Ouabain caused a sharp rise in tissue $Na^+$ (and $Cl^-$) and a loss of tissue K well below the level after loading at 0°C, with only a slight swelling. (Mean of 2 experiments 0.35 kg $H_2O$/kg D.W. ± 0.04 S.E.) Such swelling was also observed by electron microscopy of tissue samples (W. L. Doyle, personal communication). 0.5 mM vanadate, an inhibitor of the erythrocyte $Na^+$-$K^+$-ATPase, had no effect on tissue water and electrolytes. Anaerobic incubation ($N_2$) of the loaded tissue had similar, though less pronounced effects as ouabain on tissue electrolytes and water (details not given here). This effect of anaerobiosis was observed only in the absence of glucose as substrate, indicating that the rectal gland is capable of utilizing metabolic energy derived from glycolysis to maintain to some extent the tissue levels of electrolytes.

Experiments not reported here in detail failed to demonstrate an effect of theophylline (10 mM) on the net fluxes of electrolytes and water under conditions where *in vivo* a major secretion of an electrolyte solution takes place (Bull. MDIBL 15:69, 1975). As compared with controls in the presence and absence of external $Ca^{2+}$, external ATP (1 mM) produced a significant uptake of tissue $Na^+$ without affecting tissue $K^+$; similar effects of ATP on tissue electrolytes have been found previously (Rorive and Kleinzeller, Biochem. Biophys. Acta 274:226, 1972).

Consistent with the view that $Na^+$ and $Cl^-$ entry into the cells of the rectal gland is tightly coupled (Kinne et al., Bull. MDIBL 17:98, 1977), the absence of either $Na^+$ or $Cl^-$ in the medium (isotonic $Li^+$ and isethionate media, respectively) produced an actual shrinking of the tissue by 0.39, and 0.29 kg/kg D.W., respectively. Such shrinking would be expected if the $Na^+$ pump extruded $Na^+$ (and $Cl^-$) from the cells while the cellular cation could not be replenished by passive influx. Accordingly, the tissue $Na^+$ was significantly lowered on incubation in the absence of external $Cl^-$.

It is noteworthy that under all experimental conditions the determined tissue $Cl^-$ represents on the average only 64% of the sum of tissue bulk cations, i.e., $Na^+ + K^+$. It may be assumed that under the simplified conditions *in vitro* the difference between the sum of both tissue bulk cations and chloride approaches the value for the tissue nondiffusible anions. The above observation would lead one to expect a considerable Donnan swelling under conditions when the metabolism of the cells, and hence the active extrusion of electrolytes, is interfered with, e.g., by cold, dinitrophenol, anaerobiosis, ouabain, etc. The discrepancy between observation and theory suggests that the cells of the rectal gland are capable of an efficient control of cell volume.

This conclusion is also borne out by experiments showing that halving external $Na^+$, thus reducing the saline osmolarity from 853 mosM to 601 mosM, had only a slight swelling effect on tissue water while significantly decreasing tissue $Na^+$ and $K^+$ (Figure 1).

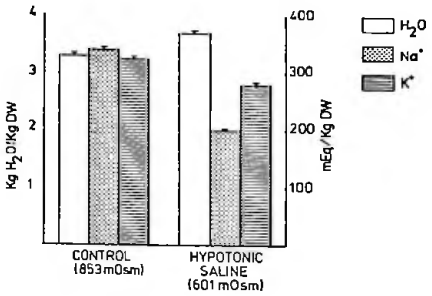


Figure 1. The effect of hypotonicity on tissue water and electrolytes in slices of the dogfish rectal gland. The tissue was incubated aerobically ($O_2$) for 60 min in standard dogfish saline (853 mosM, control) and in a saline in which only the NaCl concentration was halved, all other constituents being the same as in the control (601 mosM). Steady state values, means ± S.E. are given.

The rather small swelling effect of ouabain suggests the presence of an ouabain insensitive component of cell volume regulation in the dogfish rectal gland.

This investigation was supported in part by USPHS grant AM 12619 and the Whitehall Foundation.

RENAL CLEARANCE OF DMO AND METHYLAMINE BY THE WINTER FLOUNDER (*Pseudopleuronectes americanus*)

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Intracellular pH is most often measured from the distribution of an inert weak acid across the cell membrane (Physiol. Rev. 49:285, 1969). One critical postulate is that the marker be passively distributed between the intra- and extracellular fluid in accordance with the hydrogen ion gradient. Failure to monitor successfully changes in the intracellular pH of teased flounder tubules using 5,5-dimethyl-2,4-oxazolidinedione (DMO), made us suspect that the kidney may be able to handle actively this molecule. Therefore, clearance studies were carried out on DMO, as well as on another often used marker for measuring pH, i.e., methylamine, employing the technique described by Pritchard and Kleinzeller (Am. J. Physiol. 231:603, 1976).

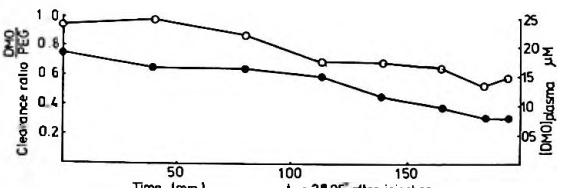


FIGURE 1 RENAL CLEARANCE OF DMO

● Clearance of DMO to PEG
○ Plasma concentration of DMO

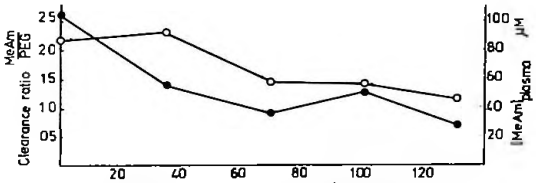


FIGURE 2 RENAL CLEARANCE OF METHYLAMINE

● Clearance of Methylamine to PEG
○ Plasma concentration of Methylamine

As may be seen from Figure 1, the clearance ratio of DMO to PEG (polyethylene glycol) was less than one, and was observed to decline over time. The clearance ratio for two fish ranged from 1.0 to 0.32. The mean rate of urine flow was 0.30 ± 0.04 ml/hr, based on a total of sixteen clearance periods each of which represents 96 µl of urine. (All values are expressed as mean ± the standard error.) The mean clearance of PEG was 0.45 ± 0.09 ml/hr. Although there is notable scatter in these two values, neither parameter was observed to vary in any defined manner.

To determine the extent of DMO tissue accumulation, the kidneys of two fish were extracted, sectioned into five pieces of near equal weight, and treated appropriately. The tissue to plasma ratio of DMO over that for PEG thus obtained was 2.40 ± 0.15.

Further experiments will need to be carried out to show unequivocally active uptake of DMO by the flounder kidney. In any event, these preliminary results raise questions as to the advisability of using DMO to measure the intracellular pH of kidney cells.