

Table 2. SUCCESSIVE HOURLY FLUXES OF UREA AND THIOUREA FOR THE DOGFISH AND BULLFROG GASTRIC MUCOSAE.

Hour	Dogfish	Bullfrog
	$\mu\text{Eq. cm}^{-2}\text{hr}^{-1}$	
1.5 - 2.5	0.56	1.37
2.5 - 3.5	0.70	1.39
3.5 - 4.5	0.63	1.32
4.5 - 5.5	0.65	1.25

mucosal surfaces was 10% greater than the flux in the opposite direction, perhaps due to solvent drag. There was no significant difference between the permeability coefficients when the concentrations were 10 and 100 mM, nor were the fluxes influenced by having urea on one side of the mucosa and thiourea on the other side.

In view of the absence of a striking difference in the permeability of the mucosae of frog and dogfish to urea, it must be concluded that no support was developed for the hypothesis that the transepithelial potential difference in other vertebrates arises from a bicarbonate diffusion potential which had disappeared in the dogfish as a result of an evolutionary change in the plasma membrane required to conserve urea.

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**PURIFICATION OF THE SODIUM-POTASSIUM ACTIVATED ADENOSINETRIPHOSPHATASE FROM THE RECTAL GLAND OF THE DOGFISH, *Squalus acanthias***

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The sodium-potassium activated adenosinetriphosphatase (NaK ATPase) appears to be the molecular machine for the active transport of Na and K by animal cell membranes. A major step in our understanding of the molecular mechanism of Na and K transport would be the complete purification of the NaK ATPase. This has not been accomplished in the 15 years since the discovery of the enzyme. The rectal gland of the dogfish is very rich in NaK ATPase (F.H. Epstein, personal communication; Palmer, R.F., Bull. MDIBL 5(2):31, 1965). Efforts were therefore made to purify the enzyme from this source. Homogenates of the rectal gland were richer by tenfold than homogenates of beef brain cortex, from which our laboratory has achieved purification to about 30% homogeneity (J. Biol. Chem, 246, 531-543, 1971). Table 1 shows results of preliminary purification of the hormone present in dogfish rectal glands.

Calculations indicate that the homogeneous enzyme would have a specific activity of about 2,000  $\mu\text{moles Pi/mg protein/hr}$ , so the data suggest that the partially purified enzyme from the rectal

TABLE 1

PURIFICATION OF THE NaK ATPase FROM THE RECTAL GLANDS OF TEN DOGFISH

Fraction	Total Units ( $\mu$ moles Pi/hr)	Total protein (mg)	Specific activity ( $\mu$ moles Pi/mg protein/hr)
Homogenate	75,500	1,830	41
Crude membranes	34,500	203	170
Purified membranes	24,000	60	400
NaI-treated membranes	19,500	17.3	1,130

gland is about 60% homogeneous. Since further stages of purification (dependent on a zonal centrifuge not available at M.D.I.B.L.) not used here purify the brain enzyme an additional sevenfold there is a reasonable probability that the use of these procedures will achieve complete or almost complete homogeneity. It has been found that the rectal gland NaK ATPase is completely stable on freezing the glands at  $-70^{\circ}$  and storage at that temperature for at least 4 months. Membranes with a specific activity of 200  $\mu$ moles Pi/mg protein/hr can be prepared from stored frozen rectal glands in a yield of about 20-30%. In summary, purification of the NaK ATPase from the rectal gland of the dogfish appears to be highly feasible and offers the real hope that homogeneous enzyme will be obtained in the not too distant future. This work was aided by grants from the National Institute of Neurology and Stroke (NS-01730) and the National Science Foundation (GB-30852).

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#### THE EFFECT OF CO<sub>2</sub> AND CARBONIC ANHYDRASE INHIBITION ON SODIUM ACCESSION TO CSF IN *Squalus acanthias*

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In previous work (Bull Mount Desert Island Biol. Lab 10, 49, 1970 and Am. J. Physiol., Feb. 1972) it has been shown that hypercapnia increases the rate of HCO<sub>3</sub><sup>-</sup> formation in CSF. In this Bulletin (Maren, Welliver, and Istin) hypercapnia (5% CO<sub>2</sub>) increased the Na<sup>+</sup> concentration of CSF. In the following experiment we show the effect of hypercapnia and the carbonic anhydrase inhibitor methazolamide on the rate of transfer of <sup>22</sup>Na from plasma to CSF.