TABLE 1. ELECTRICAL VALUES AND H ION SECRETION FOR DOGFISH GASTRIC MUCOSA FOLLOWING CATION SUBSTITUTION.

	E mv	G mmhos.cm^{-2}	Isc μEq.cm^{-2}hr^{-1}	H$^+$ μEq.cm^{-2}hr^{-1}	Δ μEq.cm^{-2}hr^{-1}
HOUR					
Control					
1	+ 2.2 ±.7	1.9 ±.1	+0.16 ±.05	+2.64 ±.14	-2.49 ±.18
2	+ 3.3 ±.7	2.3 ±.1	+0.28 ±.06	+3.07 ±.19	-2.79 ±.18
Both surfaces bathed by 175 mEq K$^+$					
3	+14.9 ±.7	2.9 ±.2	+1.60 ±.10	+2.01 ±.13	-0.41 ±.16
4	+11.9 ±.8	2.7 ±.1	+1.21 ±.11	+1.76 ±.14	-0.54 ±.15
Control					
1	+ 1.7 ±.8	1.8 ±.1	+0.14 ±.06	+2.89 ±.16	-2.75 ±.19
2	+ 2.8 ±.3	2.2 ±.1	+0.24 ±.04	+3.19 ±.17	-2.94 ±.17
Both surfaces bathed by 252 mEq choline$^+$					
3	+ 3.7 ±.3	2.2 ±.1	+0.30 ±.03	+2.08 ±.15	-1.77 ±.17
4	+ 4.0 ±.3	2.5 ±.1	+0.37 ±.03	+2.01 ±.12	-1.64 ±.12

$\bar{x}$ ± SE, n = 10. Δ = algebraic sum of all unidentified net ionic currents in short-circuit state. Isc obtained from the product E·G. High potassium solution obtained by replacing 165 mEq of Na$^+$ by 165 mEq of K$^+$. Extracellular solution having no sodium obtained by replacing 252 mEq of Na$^+$ by 252 mEq of choline$^+$.

1971 #18

PERMEABILITY OF THE DOGFISH AND BULLFROG GASTRIC MUCOSAE TO UREA AND THIOUREA

C. Adrian M. Hogben, Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa

Among the functional attributes which set the elasmobranchs apart from other vertebrates is the fact that although they copiously secrete hydrochloric acid, their isolated gastric mucosae, unlike the mucosae from other vertebrates, do not develop the typical transepithelial potential differences (*Sharks, Skates and Rays*, pp. 299-315, ed. Gilbert, Mathewson and Rall, Johns Hopkins Press, 1964). One explanation advanced for the origin of the usual electrical potential difference is that it is a bicarbonate diffusion potential (Hogben, C.A.M.: Gastric anion exchange: Its relation to the immediate mechanism of hydrochloric acid secretion. Proc. Natl. Acad. Sci. 38:13-18, 1952). Reasoning that the elasmobranchs may have acquired plasma membranes that are uniquely impermeable to urea in order to conserve urea, it was thought that this transformation might also modify the selective permeability to other small solutes such as the common ions.

Gastric mucosae of *Squalus acanthius* and *Rana catesbiana* were separated from their serosal muscle coats. Each, when mounted in a flux chamber, was bathed by a saline solution having a composition approximating the plasma of the appropriate species. The solutions were gassed with 95% O_2, 5% CO_2 and glucose was provided as a substrate. Experiments on the dogfish were conducted at ca. 15 C and on the bullfrog at ca. 25 C. In most experiments the solution on either side of the mucosa contained 100 mM urea or thiourea. To further reduce the likelihood of urea loss by bacterial urease, antibiotics were included in the physiological salt solutions; penicillin G 80 mg/1 and streptomycin SP_4 160 mg/1. Fluxes were traced with ^{14}C labeled urea and ^{35}C labeled thiourea. After addition of the isotope to one half of the flux chamber, 1.5 hours were allowed to attain an isotopic steady state and the appearance of label was then sampled hourly for 4 hours.

Table 1. PERMEABILITY OF THE DOGFISH AND BULLFROG GASTRIC MUCOSAE TO UREA AND THIOUREA.

	Permeability Coefficient $cm.sec^{-1} \times 10^6$		Wet Weight $mg.cm^{-2}$		Conductance $mmhos.cm^{-2}$	
	Urea	Thiourea	Urea	Thiourea	Urea	Thiourea
Dogfish	2.1±0.1	3.6±0.1	197	176	3.5	4.1
Frog	3.9±0.2	6.7±0.2	338	296	3.2	3.4

$\bar{x}$±SE, n = 12

The results are given in Table 1. The apparent difference in the urea permeability coefficients for the two mucosae should be related to their wet weight. The greater wet weight/cm^2 of the bullfrog mucosa indicates that for the present study a cm^2 of "gross" surface area includes more epithelium. If the coefficients are normalized for wet weight, the permeability coefficients of mucosae from the two species are indistinguishable. What is remarkable is not that the dogfish gastric mucosa is relatively impermeable to urea but that the bullfrog gastric mucosa is equally so. Though a more sophisticated analysis is required, one can relate urea permeation to mucosal conductance. Thus for the bullfrog with a conductance of 3.2 $mmhos.cm^{-2}$ and bathed by essentially 100 mM NaCl, the predominant ions Na^+ and $C1^-$ would have permeability coefficents of 4.5×10^{-6} $cm.sec^{-1}$ if current were conducted equally by both ions.

The low permeability to urea exhibited by both species relative to "ionic permeability" invites scrutiny of whether an isotopic steady state was attained permitting calculation of permeability based on the observed flux. The fact that neither species showed a progressive increase in flux over the 4 successive hour periods suggests that isotopic steady state had been attained during the first 1.5 hours, Table 2. However, this may lead us to mistaken confidence because epithelia in general "tighten-up" progressively after isolation. We do not know whether the principal path for urea is between cells or through the epithelial cells. In the latter and worst instance, I calculate that the halftime isotopic steady state would be 2 hours and permeability may have been underestimated by some 20%. If urea passes primarily between the cells yet exchanges laterally with the cell urea pool, the estimate of permeability would be less in error though ultimate attainment of an isotopic steady state would be delayed.

The greater permeability to thiourea encountered in both species presumably reflects the slight but finite lipid solubility of this solute. In the case of urea, the flux of urea from serosal to

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

Hour	Dogfish	Bullfrog
	$\mu Eq.cm^{-2}hr^{-1}$	
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.

1971 #19

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

Lowell E. Hokin, Department of Pharmacology, The University of Wisconsin, Madison, Wisconsin

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 μmoles Pi/mg protein/hr, so the data suggest that the partially purified enzyme from the rectal