In order to determine how much mannose was accumulated by the kidney, the kidneys of six fish were extracted at various times after injecting the labelled mannose and PEG (1-8 hrs). Each kidney was sectioned into five pieces of near equal weight, and processed, so that the amount of radioactivity could be measured. It was found that accumulation of the free sugar was low. From 30 kidney pieces, the mean tissue to plasma ratio of D-mannose over that for PEG was 1.6, and the standard error ± 0.10. Since accumulation was low, and scatter considerable, there was no detectable effect of any of the sugars listed in Table 1, or of phlorizin.

There are three possible models which can be proposed to account for the results of these clearance studies. First, glucose, 2-deoxy-glucose and mannose may share a (passive) carrier at the brush border of the renal cell, just as they do at the antiluminal face (Am. J. Physiol. 232:F227, 1977). The difference, however, would be that the former also transports α-methyl-mannoside, while the latter does not. If this were the case, then the effect of phlorizin and α-methyl-glucoside could be explained as an indirect one, since they could conceivably bring about an increase in the effective concentration of glucose, by blocking its uptake via the active carrier. A second explanation is that mannose may be reabsorbed by two separate pathways, one specific for mannose (and α-methyl-mannoside) and the other, the Na^+-dependent glucose carrier. A third explanation, is that there is a carrier for glucose, 2-deoxy-glucose and mannose at the luminal face of the renal cell, identical to the one found on the peritubular side; but, in addition, it may be that the Na-activated glucose carrier is able to accommodate mannose.

Preliminary studies on mannose uptake by vesicles prepared from the brush border of renal cells suggest that either the second or third model may be correct. Evidence was obtained which indicates the existence of a high and low affinity transport site for mannose at the luminal border of the flounder kidney. Uptake of D-mannose by the vesicles showed an overshoot when D-mannose was present at a concentration of 10 uM. This overshoot was reduced by the removal of sodium from the incubation medium. Furthermore, uptake was depressed in the presence of 1 mM glucose, 2-deoxy-glucose and α-methyl-glucoside, as well as mannose. No overshoot in the uptake of mannose was observed when it was present at 100 μM. 10 mM glucose, 2-deoxy glucose, α-methyl-mannoside and α-methyl-glucoside did retard the initial rate of uptake of mannose at the higher concentration. However, more work will need to be done before the pathways for mannose reabsorption are fully defined. This work was supported in part by USPHS grant AM 12619 and the Whitehall Foundation.

AN APPARENT SCN:Cl BI-IONIC POTENTIAL DIFFERENCE OF THE GASTRIC MUCOSA

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Subsequent to the report that the isolated gastric mucosa of the dogfish generates but a meager transepithelial potential difference (PD) (Hogben, Science 129:1224-1225, 1959), SCN, a potent inhibitor of H^+ secretion, has been employed to modulate the PD by applying it only to the serosal aspect (Rehm, Am. J. Physiol. 203:63-72, 1962; Kidder, Am. J. Physiol. 231:1240-1245, 1976). For the gastric mucosa of *Squalus acanthias*, an anion concentration asymmetry results in an apparent bi-ionic PD which has the opposite orientation to that for the isolated mucosa of *Rana catesbiana*.

Experiments were conducted on paired segments of mucosae. A matched pair of Radiometer calomel cells made contact with the two solutions bathing the surfaces of a mucosal segment through 1 M KCl and a pair of iso-osmotic NaCl-3% agar bridges. The signal between the calomel cell pairs was registered with a recording potentiometer, input impedance greater than 1 meg Ω. The observed PD was corrected for the calomel cell-bridge asymmetry PD encountered at the start and end of each experiment, usually negligible and never greater than 1 mV. The composition of elasmobranch saline was Cl 240, HCO_3 30, HPO_4 2, SO_4 4, Na 252, K 10, Ca 10, and Mg 4 mEq/L. Amphibian saline had Cl 92, HCO_3 20,

HPO_4 3, SO_4 1, Na 110, K 2.5, Ca 2.5, and Mg 1 mEq/L. These solutions were modified by equimolar substitution of SCN for Cl and for the bullfrog by substitution of SCN or SCN and NO_3 for Cl such that the sum of monovalent anions mEq/L (Cl, SCN and/or NO_3) remained constant. All solutions were gassed with 5% CO_2, 95% O_2 and contained 5 g glucose/L. The dogfish experiments were conducted at 15°C and those of the bullfrog at 25°C. Fluxes of Cl and SCN through the latter were measured with ^{36}Cl and ^{35}SCN. At the conclusion of an experiment, the label was eluted and counted. This mucosal tracer content was corrected using mean values for the two extracellular spaces and dry weight (Hogben, Fed. Proc. 24:1353-1359, 1965) to yield the intracellular activity (without differentiation with respect to cell type) when the tracer had been added to only one of the two bulk solutions bathing the mucosa. The sum of the relative cell activities when the tracer was migrating from the serosal to mucosal and migrating from the mucosal to serosal solution provided the estimate of cell concentration.

The sequential response of the transmucosal PD of the isolated dogfish gastric mucosa to a series of solution changes is displayed in Figure 1a. Prior to the first change at 20 minutes the mucosal segment had been dissected in the customary Cl+(HCO_3)-saline and mounted with the identical solution at the two surfaces. After 45-60 minutes, to allow stabilization in the isolated state, the spontaneous PD was recorded for 20 minutes. The solution at the serosal surfaces was then changed to one in which 25 mM of Cl replaced 25 mM of SCN establishing a bi-ionic gradient. The PD fell by about 1 mV, observing the convention of the serosal solution being the reference solution. In other words,

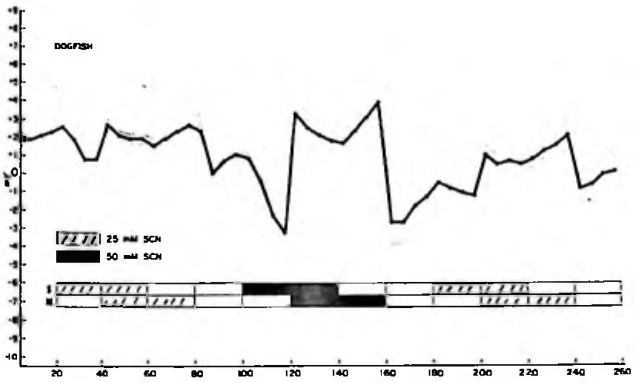

Figure 1a. The mean spontaneous PD of dogfish gastric mucosae has been plotted as a function of time. The convention for the orientation is that the sign of mucosal solution is given with respect to the serosal solution as the reference. About the continuous line the stippled envelope embraces ± one standard error. Below the PD record, the horizontal rows of blocks indicate the solutions bathing the serosal, S, and mucosal, M, surfaces. Until 20 minutes, both surfaces were bathed by the same customary CL-saline. At 20 minutes the solution at the serosal surface was replaced by one in which 25 mM SCN had been substituted for 25 mM Cl. At 40 minutes both surfaces were bathed by the same 25 mM SCN-saline. Progressive solution changes were executed at 20-minute intervals until the conclusion at 260 minutes. (n = 4 mucosal segments from 4 fish)

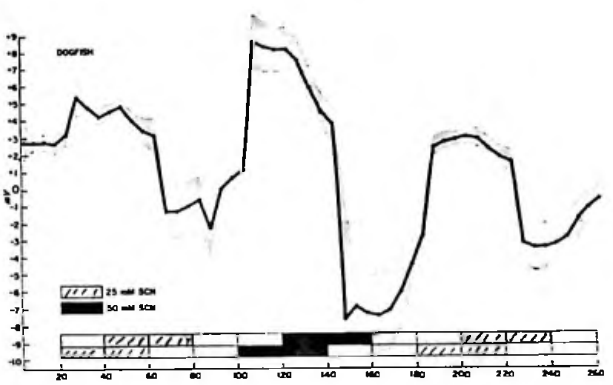

Figure 1b. Similar to Figure 1a. As before the upper row of blocks designates the solution in contact with the serosal surface and the lower row that bathing the mucosal surface. Thus at 20 minutes the mucosal solution was replaced by 25 mM SCN-saline. The 4 mucosal segments used in this series were paired segments adjacent to those taken from the same fish whose mean PD was given in Figure 1a. Indeed the only procedural difference between Figures 1a and 1b was the indicated sequence of solution changes.

the small PD change would be compatible with SCN having a greater mobility than Cl. Though the subsequent shift of the PD associated with the sequential changes of solutions was never very great, at each step the response was consistent with relative mobilities being $U_{SCN} > U_{Cl}$.

Concomitantly, a paired segment of mucosa was subjected to solution changes but the change started with the mucosal solution being replaced by 25 mM SCN-saline, Figure 1b. From the stomach of 4 fish there were 4 such pairs with each pair of segments treated identically but for the sequence of change. This seemingly trivial difference in strategy led to a striking accentuation of the apparent bi-anionic PD but again with the apparent mobilities being $U_{SCN} > U_{Cl}$. For this sequence, taking the shift of the PD when 50 mM SCN was at only one surface, the apparent bi-anionic PD could be as great as 0.16 mV per mEq/L asymmetry.

The data displayed in the two figures have been condensed by obtaining the mean PD 18 minutes after a solution change. For each 20 minutes when the mucosa was exposed to asymmetric solutions, there was an antecedent and subsequent period when the segment was bathed by identical solutions. The asymmetry PD was computed as the difference between the 18' mean of these 2 periods and the 18' asymmetric PD. Adopting a convention that a negative sign would signify a difference compatible with $U_{SCN} > U_{Cl}$, values for each concentration difference from each mucosal segment were reduced to a single datum and summarized in Table 1. Irrespective of the sequence of change the minimum estimate of the SCN:Cl bi-anionic PD is 0.1 mV per mEq/L.

TABLE 1 Apparent SCN:Cl Bi-ionic PD of the Gastric Mucosa

	[SCN]:[Cl] 25 mM	[SCN]:[Cl] 50 mM	n
Squalus acanthias	-2.3±1.0	-5.2±1.3	8
Rana catesbiana	+2.0±1.2	---	9

$\bar{x}$ ±95% Confidence Limits

A negative sign corresponds to $U_{SCN} > U_{Cl}$.

TABLE 2 Rate Constants of SCN Flux, Bullfrog Gastric Mucosa

mM	S→M	M→S	n
0.5	15±2	11±1	5
2.5	15±1	9±1	8
15	11±0.7	6±0.4	8
20	11±1	7±1	5

$\bar{x}$ ±S.E. x 10^6 cm.sec^{-1}

[S→M = serosa to mucosa, M→S = mucosa to serosa]

TABLE 3 SCN and Cl Fluxes, Bullfrog Gastric Mucosa

mV	Flux	SCN	Cl
0	S→M	0.60±.04	1.01±.08
	M→S	0.31±.02	0.50±.04
60	S→M	0.14±.03	0.44±.07
	M→S	0.43±.01	0.56±.04
	n	8	6

$\bar{x}$ ±S.E. μEq.hr^{-1}cm^{-2}

[SCN] & [Cl] 15 mM, [NO_3] 62 mM.

TABLE 4 Intracellular Content. Ratio Mucosal Eluate Label to Bulk Solution

	Cell mM SCN	Cell mM Cl	Mucosal Label:Solution Label, ml/g	SCN	Cl
0 mV	12	5	S→M	.11±.03	.02±.03
			M→S	.12±.01	.08±.01
60 mV	16	10	S→M	.20±.03	.09±.04
			M→S	.09±.01	.08±.01

Table 1 also provides data for the bullfrog mucosa following the foregoing protocol but omitting the use of a 50 mM SCN-saline. It is noteworthy that the sign of the apparent bi-anionic PD is reversed, and is that expected for $U_{Cl} > U_{SCN}$.

An explanation for what might be considered an unexpected dependence has not been established. Given that the findings displayed in Figures 1a and 1b were conducted on paired mucosal segments, we must conclude that the difference is not accidental. A likely explanation might be sought in the kinetics of exchange from the serosal extracellular space (Hogben, J. Gen. Physiol. 51:240s-249s, 1968), with the $t_{\frac{1}{2}}$ for desaturation of the submucosal slab being about 8 minutes and that of intertubular interstices perhaps 60 minutes. By inference it is likely that much of the 'bi-anionic' PD

PD develops across the surface epithelial cells and that exposure of the mucosa to a lesser SCN:Cl gradient for longer than 20 minutes might evoke a greater change than 0.16 mV/mEq. The duration of exposure to these higher concentrations was intentionally limited to minimize irreversible deleterious changes induced by SCN.

The kinetics of SCN transfer through the bullfrog gastric mucosa have been studied when both surfaces were exposed to identical solutions but for the radionuclide. Over the range from 0.5 to 20 mEq/L, the transmucosal rate constants for SCN are relatively concentration independent, Table 2. With Cl and SCN both present in equal concentration, 15 mM, and NO_3 being the predominant anion, 62 mM, both Cl and SCN are actively transported across the short-circuited mucosa, 0 mV Table 3, though SCN less avidly than Cl. In qualitative terms the larger flux ratio for SCN at 60 mV indicates that a greater fraction of its flux occurs by passive diffusion of the ionic species than by active transport, 'exchange' diffusion, and a putative diffusion of HSCN.

The ratio of cell activities, Table 4, establish an upper limit for the SCN lumen into cell flux of 1.0 $\mu Eq.hr^{-1}cm^{-2}$ since any parallel intercellular shunting would reduce this value. This flux can be given as a rate constant of 18×10^{-6} $cm.sec.^{-1}$. Since 10 mM SCN suffices to inhibit a short-circuited bullfrog mucosa secreting H^+ at a rate of 1.2 $\mu Eq.hr^{-1}cm^{-2}$ and under circumstances where the mucosal "micro-environment" pH approaches that of the luminal bulk solution (Hogben, Circulation 26: 1179-1188, 1962), the inhibition of H^+ secretion cannot be accounted for by back-diffusion of HSCN. (Employing a more conventional saline, 2.5 mM SCN, 89.5 mM Cl and 20 mM HCO_3, the rate constant for the "lumen to cell" SCN flux was $25 \times 10^{-6}cm.sec.^{-1}$, 6 paired experiments.)

The postulate of SCN permeation as a nonionized acid moiety, HSCN, has been revived to explain the osmotic behavior of liposomes exposed to iso-osmotic NH_4SCN (Singer, Canad. J. Physiol. Pharm. 51:523-531, 778, 779-784, 1973). The experimental approach derives from the classical studies of erythrocytes but unfortunately a possible effect of SCN on the integrity of the liposome membrane has not been excluded. Similarly the mitochondrial swelling induced by SCN after addition of nigercin (Mitchell and Moyle, Europ. J. Biochem. 9:149-155, 1969) should be validated perhaps by use of the rapid flow tube technique.

The striking species difference between the apparent SCN:Cl bi-anionic PD developed across the dogfish and bullfrog gastric mucosae prompts scrutiny of the intracellular anion concentrations. Since, for the bullfrog, the intracellular concentrations of SCN are greater than those of Cl and approach those of the external media, Table 4, it would require for the bullfrog an unexpected and extraordinary reduction of the intracellular activity coefficient before the species difference could be assigned to this locus.

Given the structural complexity of the gastric epithelium and perhaps more importantly the side effects of SCN, the observed bi-anionic PD is being qualified as an *apparent* bi-anionic PD to stress that the phenomenon cannot be readily likened to a bi-ionic PD developed across a simple membrane.

It is not asserted that all the PD change encountered after an exposure of the dogfish mucosa to SCN asymmetrically should be attributed to the apparent bi-anionic PD. Nevertheless, a potential artifact may be eliminated by maintaining symmetry since the response to H_2-inhibitors is anomalous (Hogben et al., Bull. Mt. Desert Island Biol. Lab. 16:53-55, 1976). Nor is it asserted that hyperbaric oxygenation generates unique findings (Kidder, Am. J. Physiol. 231:1240-1245, 1976; Biophys. J. 17:90a; Bull. Mt. Desert Biol. Lab. 17:21-27, 1978). Not cited is adequate documentation that the secretagogue, carbachol, induces the isolated dogfish gastric mucosa, at sea level and 13°C, to develop a significant reversal of its spontaneous potential with the luminal surface becoming positive (Hogben et al., Comp. Biochem. Physiol. 42A:153-168, 1972). In addition to a significant temperature difference of more than 5°C, features of chamber design are germane to operation at 1 atmosphere (Hogben,

Gastric Secretion, Ed. Sachs, G. et al., Academic Press, N.Y., pp. 111-130, 1972). "Stat" titration to a pH of 4.5 underestimates the H^+ ion secretory rate but whether it does so consistently merits study. (Supported by USPHS grant AM 19020.)

NEUROGENIC CONTRIBUTIONS TO VASOMOTOR CONTROL IN *Squalus acanthias*

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Previous work from our laboratory suggested that vasomotor tone is present in the dogfish, *Squalus acanthias* (Opdyke et al., Comp. Biochem. Physiol. 42A:611-620, 1972). Further evidence is presented here with an analysis of possible neurogenic contributions to overall peripheral vasomotor control. Experiments were conducted to determine the level of neurogenic control by observing changes in blood pressure and heart rate under the following circumstances: 1) Freezing the brain to eliminate contributions to vasomotor tone by the higher central nervous system; 2) Administration of atropine, a cholinergic neuro-effector junction blocking agent; 3) Administration of the general ganglionic blocking agent, hexamethonium; and 4) Administration of the alpha receptor blocking agent, phentolamine.

It was also of interest to study the effect of angiotensin II (AII) under each of the above conditions. AII has been shown previously to elicit a pressor response in dogfish (Opdyke and Holcombe, Am. J. Physiol. 231:1750-1753, 1976). The possibility that a neural mechanism is involved in AII action has been explored here.

Male and female dogfish, weighing 1-6 kg were caught by trawl line in Frenchman Bay, Maine and kept in live cars 2-5 days before use. An indwelling Intramedic PE 60 catheter was placed in the caudal artery to monitor phasic dorsal aortic pressure (DAP) and heart rate (HR) with a P23AA Statham transducer and an Electronics-for-Medicine IR 4 recorder. All injections of AII, atropine, hexamethonium and phentolamine were made through this catheter. Fish undergoing brain-freeze operations were first injected with 15 mg/kg pentobarbital sodium intravenously which rendered the fish quiescent during the surgical procedures. In addition, procaine was infiltrated generously at the site of the cranial operation. Dogfish receiving only atropine, hexamethonium or phentolamine injections were unanesthetized.

All experiments were performed with the fish belly down in a V-shaped trough with the gills bathed in cold running seawater through tubes inserted in each spiracle. In the brain-freeze series, the brain was completely exposed from the anterior edge of the forebrain to the spino-medullary junction. Liquid nitrogen was applied liberally to all exposed surfaces of the brain and freezing occurred instantaneously. The brain was kept frozen throughout the remainder of the experiment by frequent applications of liquid N_2.

Data from experiments dealing with the maintenance of vasomotor tone in the dogfish were summarized in Table 1. In 5 fish there was no significant lowering of either systolic or diastolic pressure nor any change in heart rate as a result of freezing the brain. The previously reported effect of atropine on heart rate (Burger and Bradley, J. Cell. Comp. Physiol. 37-38:389-402, 1951) was again confirmed in 5 fish. Hexamethonium mimicked the effect of atropine by giving a highly significant elevation of heart rate from 21.6 ± 5.0 to 33.4 ± 2.2 beats/min.

Phentolamine injections were carried out on 13 fish following injection of either atropine or hexamethonium, or as a single initial injection. Phentolamine caused no significant change in HR in any case, but it did induce a highly significant decrease in both systolic pressure (from 31.3 ± 5.6 to 16.6 ± 2.5 Torr and diastolic pressure (from 26.3 ± 5.6 to 14.4 ± 2.8 Torr) in all trials.

Table 2 summarizes results of Angiotensin II (Beckman Instrument, Palo Alto, California) injections (20-40 µg) in the brain-freeze experiments. AII gave a highly significant elevation of both