TABLE 2

Rates for the Bohr effect in five vertebrates compared with the chemistry of H^+ generation

	Bohr Effect Factor Δ log p_{50}/Δ pH	Control		10^{-3} M Methazolamide	
		H^+ Gen.	O_2 Release	H^+ Gen.	O_2 Release
		millimoles per liter · sec^{-1}			
Frog	-0.25	498	0.55	0.064	0.053
Dogfish	-0.34	182	5.0	0.075	0.070
Goosefish	-	380	18.0	0.064	0.17
Duck	-0.53	150	13.0	0.212	0.19
Human	-0.48	3420	16.5	0.176	0.38

Frog, dogfish and goosefish blood were studied at 16°C, duck at 41°C and human at 37°C. The values in Column 2 are from the calculated V_{cat} of Table 1. Columns 3 and 5 from the observed data of Figure 2. The values in Column 4 are the sum of the uncatalyzed and residual catalyzed rates given in Table 1.

The rate of the Bohr effect is so diminished at 10^{-3} M methazolamide that the amount of oxygen exchange thus mediated cannot occur within capillary transit time (inset, Figure 2). At lesser concentrations of methazolamide, 10^{-4} and 10^{-5} M, the rate of the Bohr effect is still slowed, precluding the majority of its contribution in oxygen transfer (Swenson and Maren, Bull. MDIBL Vol. 16, 1976). These latter levels are readily achieved in vivo and yet no overt toxicity has been reported. The organism may compensate by increasing the blood flow, dilating and recruiting capillaries, increasing red cell 2,3-diphosphoglycerate, widening pO_2 and pCO_2 AV gradients or shifting to a greater degree of anaerobic metabolism. Whether this imposes any physiological disadvantage, especially in conditions of cardiovascular or pulmonary compromise, remains for further investigation.

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PERMSELECTIVE PROPERTIES OF THE SECRETORY EPITHELIAL BARRIER IN THE ISOLATED PERFUSED RECTAL GLAND OF THE SPINY DOGFISH (*Squalus acanthias* L.)

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The constraints upon passive fluxes of metabolically-inert, lipid-insoluble electrolytes across secretory epithelial barriers are difficult to evaluate in vivo owing to a complex interplay of local fixed or induced charges, potential differences generated during active transport of ions, Donnan effects, unstirred layer artefacts and diffusion potentials. Direct measurement of transmembrane potential gradients may be impossible in many tissues in which membrane surfaces are inaccessible, as in the hepatic canaliculi, or where introduction of micro-electrodes may produce functional disturbances that obscure interpretation.

A means of surmounting this difficulty has been suggested by the observation (Bradley and Herz, Diseases of the Liver and Biliary Tract, 5th Quatr. Meeting Int. Assoc. Study of the Liver, Acapulco 1974, pp. 113-116, Karger, Basel, 1976) that the biliary ^{14}C-ferrocyanide clearance (C_F) in rats is considerably less than the simultaneously determined biliary ^3H-sucrose clearance (C_S) despite similar molecular weights and diffusivity (MW 304 vs. 342; D_{25} 11.56 x 10^{-6} vs. 5.21 x 10^{-6}). The clearance of ^{14}C-carboxylated inulin (C_C) was also found to be significantly less than that of neutral ^{3}H-methoxy-inulin (C_H). It seeme reasonable to attribute this phenomenon largely to interference with anionic movement by negatively charge transmembrane channels, by a luminal charge secondary to an active transport potential (presumably associated with bile acid secretion) or by both in varied combination. Since the ratios of the clearances of the weight-matched charged and uncharged solutes are approximately equivalent to the ratios of the concentrations of the charged moiety on each side of the membrane, the bioelectric constraint may be tentatively evaluated quantitatively as an "equivalent potential difference" (EPD) by means of the Nernst equation. B solute pairs were found to yield comparable values for EPD and since EPD remained constant over a wide range of bile flow and bile acid secretion, it was inferred that the lower clearance of the charged member of each pair could be accounted for preponderantly by a charge effect rather than a transport potential. Unfortunately this inference could not be tested by direct measurement.

The rectal gland of the dogfish (*Squalus acanthias L.*) appears to provide an unusually suitable system in which clearances of weight-matched charged-uncharged solute couples could be assessed in terms of known changes in transport potential. Since the demonstration of hypertonic sodium chloride secretion by the rectal gland in vivo by Burger and Hess (Science 131:670-671, 1960) it has been found that secretion may be maintained in vitro under appropriate conditions (Palmer, Clin. Res. 14:77, 1966). Secretion is associated with active chloride transport against an electrochemical gradient of 13.6 ± 1.2 mV (Hayslett et al. Am. J. Physiol. 226:1188-1192, 1974; Siegel, Schon and Hayslett Am. J. Physiol. 230:1250-1254, 1976), and is markedly stimulated by theophylline (Th) and dibutyryl cyclic AMP (DBcAMP) during in vitro perfusion (Stoff et al. J. Exp. Zool. 199:443-448, 1977). With the in vitro preparation it has been possible to measure C_F and C_S as C_C and C_H simultaneously over a wide range of flows induced by administration of Th and DBcAMP in order to determine if charged-uncharged ratios change in accord with electrical potential differences between duct lumen and perfusion fluid.

In each experiment, the rectal gland was removed after cord transection. Arterial perfusion with Shark Ringer (SR) solution (Forster, Goldstein and Rosen, Comp. Biochem. Physiol. 43A:3-12, 1972) bubbled with 95 percent O_2 and 5 percent CO_2 was started under hydrostatic pressure load at 85 cm water within 3 to 10 minutes after excision in most experiments. Flow rates were controlled by tubing clamp, monitored by constant collection of venous outflow and stabilized at 1 to 5 ml/min. Secretion was collected throughout study by means of a cannula (PE 50) tied in the duct. Perfusion fluid was maintained at a temperature of approximately 10°C by immersion of the tubing in an iced water bath. The gland, partially submerged in a Petri dish, was kept at 12 to 16°C by a thermoelectric heat-exchanger (Cambion). The electrical potential difference PD, was measured between the perfusate and the duct by salt bridges of polyethylene tubing, PE 240, filled with 275 mM NaCl and 3 percent agar. One bridge was placed between the perfusate reservoir and a reference calomel cell (Radiometer K 401) in 1 M KCl, a second bridge was placed between the secretion collection tube and another calomel half-cell. Asymmetry PD was determined at the beginning of each experiment (-0.31 ± 0.19 mV) and was not observed to change appreciably thereafter. Voltage measurements were made by a high input impedance battery powered electrometer (Keithley Model 602) off ground. Isotopically labeled sodium ^{14}C-ferrocyanide (NEN-200 µCi; together with unlabeled carrier, 75.7 mg) and ^{3}H-sucrose (NEN-260 µCi without carrier) or ^{14}C-carboxyl (NEN-250 µCi) and ^{3}H-methoxyl-inulin (NEN-100 µCi) in 4 to 5 SR solution were added to 400-500 ml of perfusate immediately after stabilization of venous outflow. Sample of secretion were obtained continuously over successive intervals of time that varied with rates of flow the need to collect more than 50 µl for purposes of analyses. Potential differences between duct and perfusate (PD) were measured frequently during the latter part of each period. Three or more control periods were collected during a progressive decline in secretion rate (V) prior to the response to theophylline (Merck) which was added to the perfusate (1 ml 25 mM per 100 ml) in combination with DBcAMP (Sigma Chem.)

on most occasions. Collections continued thereafter up to 3 hours. Radioactivities of ^{3}H and ^{14}C were measured in perfusate and secretion after diluting 25 to 50 µl aliquots in 2 ml of distilled water. Duplicate samples of 0.5 ml each were counted in a Packard Tri-Carb (Model 3003) liquid scintillation spectrometer after addition of 10 ml Instabray (Yorktown Research). An internal standard and the channel ratio were used to correct for quenching and discrimination between both isotopes respectively. Clearances were computed in the usual manner.

Satisfactory experiments were completed in 23 fish (7 male, 16 female) - in 16 using ^{3}H-sucrose and ^{14}C-ferrocyanide (F/S); in 7, ^{3}H-methoxy- and ^{14}C-carboxyl-inulins (C/H). Values for duct-perfusate PD were obtained in 11 of the former and in all of the latter. (On four occasions, the isotope concentrations in perfusate and secretion were approximately equal, presumably owing to direct entry of perfusate into the lumen of the gland through small lacerations of the ventral venous sinus or its tributaries during catheterization. These and two additional experiments in which very little or no secretion occurred have been excluded from consideration.) Isotope appeared in the secretory outflow very quickly - within less than one minute after entering the gland in the perfusate on four occasions and in less than three minutes in four more when reliable measurments could be made. To assure maximal stabilization of concentrations in secretion, clearance values during a single control period just prior to onset of the response to Th - DBcAMP, when V had reached its lowest value, were used in each experiment for comparison with values during a single period at the peak of the response. These data were summarized in Table 1 separately for C_F and C_S and for C_C and C_H. All clearances increased during Th - DBcAMP stimulation in every experiment while secretion-perfusate concentration ratios (S/P) failed to show any consistent change, falling somewhat more frequently

Table 1

Effect of theophylline-dibutyryl cAMP on rectal gland clearances of water, sodium ferrocyanide, sucrose, carboxyl- and methoxy- inulins

	V	S/P_F	C_F	S/P_S	C_S	C_F/C_S	PD	EPD
Control $\bar{m}$	70.6	0.085	0.106	0.105	0.110	0.793	-2.95	-2.50
(16) SE	±12.1	±0.027	±0.045	±0.031	±0.039	±0.081	±0.79 (12)	±1.26 (11)
Th-DBcAMP $\bar{m}$	769.1	0.048	0.513	0.084	0.998	0.493	-6.58	-6.68
(16) SE	±165.6	±0.018	±0.158	±0.021	±0.246	±0.077	±1.22 (11)	±1.63 (11)

	V	S/P_C	C_C	S/P_H	C_H	C_C/C_H	PD	EPD
Control $\bar{m}$	133.0	0.024	0.052	0.031	0.061	0.750	-4.29	-7.90
(7) SE	±66.8	±0.009	±0.025	±0.012	0.026	0.058	±2.14	±2.07
Th-DBcAMP $\bar{m}$	1122	0.013	0.226	0.022	0.372	0.595	-5.87	-14.60
(7) SE	±246	±0.004	±0.066	±0.006	±0.076	±0.071	±2.53	±3.25

Abbreviations: V - secretory flow rate - µl/hour/g gland weight; S/P - secretory perfusate concentration ratio for ^{14}C-ferrocyanide (F), ^{3}H-sucrose (S), ^{14}C-carboxyl (C) and ^{3}H-methoxy inulins (H); C - clearance rates for each solute indicated - µl/min/g GW; PD - observed transglandular potential difference - mV, EPD - calculated from clearance ratio; number of experiments for each group in parentheses.

than rising. The clearance of the charged moiety was always less than the clearance of the uncharged member of each solute pair, the C_F/C_S ratio averaging 0.793 ± SE 0.081 and C_C/C_H, 0.750 ± 0.058 during the final control period (Table 1). The C_F/C_S ratio declined in nearly every experiment during maximal stimulation, remaining unchanged in 3 experiments and falling in 13, while C_C/C_H fell in 6 and rose in 1. The reductions in V, C_F, C_S, and C_F/C_S were highly significant ($p < 0.001$). The changes observed in the smaller series of fish in which C_C and C_H were measured were less significant but definite increments occurred (V, $p < 001$; C_C, $p < 0.01$; C_H, $p < 0.005$). The decline in C_C/C_H was of uncertain significance as was the change in observed PD (corrected for the asymmetry potential) associated with it. Control values for PD in the larger series in which C_F and C_S were followed, varied widely over a range and with an average value in agreement with those reported by Hayslett et al. (1974); i.e., -2.11 ± 0.83 as compared with -3.6 ± 1.0. Observed PD became significantly more negative in the F/S series ($p < 0.005$) during stimulation though the average change was not as large as that reported by Stoff et al. (1977).

These results are consistent with passive movement of sucrose, ferrocyanide, and inulin across the rectal gland epithelium. Since none of these substances appears to traverse cellular plasma membranes with ease or to be actively transported, the short appearance time suggests that movement occurs preponderantly by paracellular pathways from perfusate-to-secretion. The barrier between extracellular fluid and lumen (tight junction ?) is semi-permeable, restricting molecular movement in proportion to molecular dimensions as is evident in Figure 1, where mean clearances of urea (taken from the data of Siegel et al. Bull. MDIBL 13:113-115, 1973), sucrose and methoxy-inulin (± standard error) from this study are plotted (log-log) against the relevant molecular radii. The lower clearances of the charged member of each weight-matched pair with respect to the clearances of the neutral member bespeaks a bioelectrical constraint. The work of Hayslett (1974) and Stoff (1977) and their associates strongly supports the view that secretion of the rectal gland is determined by active chloride output which is regulated in some manner by cyclic AMP. The fall in clearance ratios with increasing secretion during stimulation of chloride secretion by Th and DBcA indicates an increasingly negative potential barrier which operates to restrict anionic movement - presumably resulting from an active transport potential linked to movement of chloride ion across the cells.

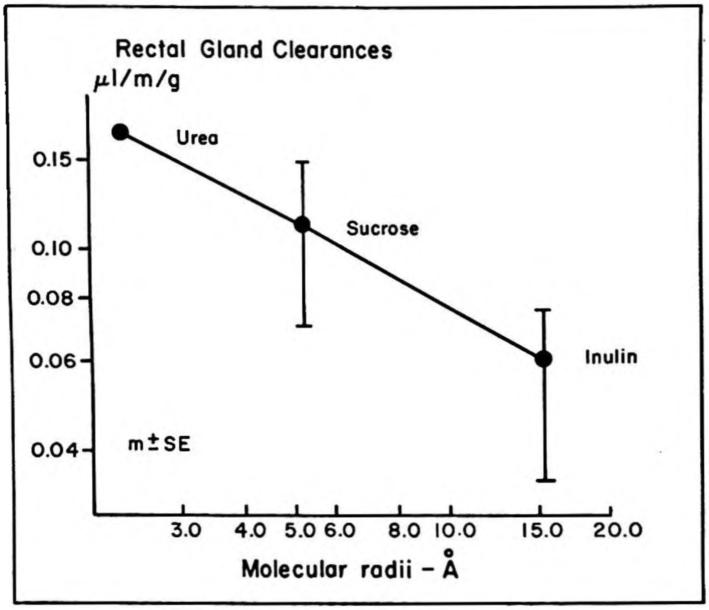

Figure 1. Relationship of rectal gland urea, sucrose, and methoxy-inulin clearances (μl/min/g gland weight) to their respective molecular radii. (Å).

The use of the Nernst equation as a means of evaluating a theoretical potential difference (EPD) that could retard movement of the charged relative to the uncharged member of each solute pair is truly appropriate only under ideal equilibrium conditions that cannot prevail in studies such as these. It must be assumed that the two members of each pair move through identical channels so that the neutral member may serve as a marker for water flow. The luminal concentration of the anion may then be computed by dividing the rate of anion secretion by the clearance of the neutral moiety. Since the anion concentration at the counter-luminal surface is equal to the perfusate concentration, the inner-outer anion concentration ratio is equal to the anion-neutral clearance ratio and EPD = (RT/zF) ln C_i/C_n where C_i is the clearance of the charged and C_n the clearance of the neutral solute, R, T, z, F having their usual significance. The higher EPD (Table 1) for $^{C}C/C_{H}$ than for $^{C}F/C_{S}$ suggests that the larger monovalent molecule may encounter a greater bioelectrical resistance possibly because it more closely approximates fixed negative charges during transmembrane movement or because it moves by a different route. In both sets of experiments, observed (PD) and calculated (EPD) values changed in the same direction duirng Th-DBcAMP stimulation. Comparison of EPD and PD must obviously be made with considerable caution owing to the uncertain validity of both measurements as guides to bioelectric phenomena at the epithelial surfaces in these studies. Certainly the assumptions upon which the Nernst equation is used are not wholly acceptable for the experimental situation. The direct measurement of the potential difference between the duct and the perfusate at some distance from the secreting epithelium, may be markedly affected by short-circuits and inputs of various kinds that are not easily controlled or identified at present. Nevertheless, the observation that both approaches yielded comparable values and changed similarly during stimulation of rectal gland secretion suggests that the clearance probe may be a useful tool for the evaluation of bioelectric potentials in situations where direct measurements are impossible.

LACK OF EFFECT OF ANGIOTENSIN II IN DOGFISH

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INTRODUCTION. In mammals, the administration of angiotensin II can cause diuresis or antidiuresis or have no effect at all on urine flow. As a general rule, small doses of angiotensin cause antidiuresis and large doses, diuresis. The response depends on many factors. First, angiotensin increases arterial blood pressure and this may influence renal function. Second, angiotensin constricts both afferent and efferent arterioles. Depending upon the relative sensitivities of these vascular beds, glomerular filtration could decrease or increase, ultimately influencing urine flow rate in parallel. Moreover, angiotensin might be involved in redistribution phenomena and thereby alter urine flow. Finally, angiotensin II is thought to have direct tubular effects; both a stimulatory and an inhibitory effect on water and solute reabsorption have been reported. Thus, with respect to water and solute excretion the tubular effects of angiotensin might either accentuate or attenuate the vascular effects.

In the experiments described below, angiotensin II was infused intravenously into *Squalus acanthias*, an animal which lacks the renin-angiotensin system. A wide range of rate of administration was used, 15-36 ng/min/Kg body weight. Arterial blood pressure, plasma composition, and renal functions were measured before, during, and after the infusion.

METHODS. Female dogfish were kept in live cars until they were used, usually 2-3 days after capture. At least 24 hours before the acute experiments, inulin was injected i.m. at several sites (10 gm %, 1 ml/Kg body weight). On the experimental day, the fish was restrained in a tank filled with rapidly running sea water. A small ventral incision was made in an area previously infiltrated with Lidocaine and an hepatic vein and a celiac artery were catheterized with polyethylene tubing. The arterial catheter was connected