sured with an eye-piece micrometer. The average slope for each droplet sequence was estimated to determine the half-time of reabsorption or influx ($t_{1/2}$), such that $t_{1/2} = \frac{\ln 0.5}{\text{average slope}}$. Water flux ($J_v$) was calculated from the formula $J_v = \frac{0.345r}{t_{1/2}}$, where r represents the radius of the droplet. The rate of net reabsorption or outflux was estimated from the $t_{1/2}$ of isolated droplets containing amphibian's Ringer's bicarbonate solution. Influx into the lumen was measured as the rate of expansion of isolated droplets containing raffinose (non-reabsorbable solute) in water (200 m Osm/L). Hydrostatic pressure in peritubular capillaries was determined using the Landis technique. The anatomical relationships of the renal vascular supply was studied from casts of the renal venous portal system and renal arterial system made with microfil (Canton Bio-Medical Products).

The mean hydrostatic pressure of peritubular capillaries, formed by the renal venous portal system, was 2.67 ± 0.15 cm HOH (mean ± S.E.) and the pressure in renal arterioles was 10.20 ± 0.52 cm HOH, during control conditions. After ligation of the vena cava, proximal to the kidneys, the pressure in peritubular capillaries rose significantly to 5.50 ± 0.36 cm HOH ($p < 0.001$), but was unchanged in renal arterioles, 9.25 ± 1.28 cm HOH. The control $t_{1/2}$ was 20.67 ± 2.15 min and J_v was 2.17 ± 0.28 x 10^{-5} mm^3/mm^2 sec. Net reabsorption was reduced when hydrostatic pressure was increased. The $t_{1/2}$ increased to 123.59 ± 34.23 min ($p < 0.005$) and J_v was reduced to 0.58 ± 0.10 x 10^{-5} ($p < 0.001$). Passive influx, measured as the rate of expansion of a droplet containing raffinose, increased during the experimental period. The control $t_{1/2}$ decreased from 6.43 ± 0.64 to 4.08 ± 0.48 min ($p < 0.01$) and J_v increased from 5.84 ± 0.69 x 10^{-5} to 8.63 ± 0.84 x 10^{-5} mm^3/mm^2 sec ($p < 0.02$).

This study shows that increased peritubular capillary pressure reduces net reabsorption from the proximal tubule in the amphibian, with a renal venous portal system, as well as in the mammal. The mechanism of this response is due, at least in part, to increased passive backflux into the lumen. Since active reabsorption is related to the electrical-chemical gradient across the tubular wall, the steady state sodium concentration and electrical gradient during control and experimental condition will be measured in order to determine the effect of increased hydrostatic pressure on active outflux.

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1970 #18

THE ELECTROPHYSIOLOGY OF THE GASTRIC MUCOSA OF A FRESHWATER ELASMOBRANCH

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It has previously been established that the isolated gastric mucosae of 3 species of marine elasmobranchs, so far examined, differ in electrical properties from those of other vertebrates (Science 129:1224-25, 1959; Sharks, Skates and Rays ed. P. W. Gilbert, R. F. Mathewson, and D. P. Rall, Johns Hopkins Press, Baltimore, Maryland, 1967, pp. 299-315). Since the electrolyte balance and the urea metabolism of a freshwater elasmobranch, *Potamotrygon* spp. (Science

158:375-77, 1967) is more similar to that of other vertebrates than to that of marine elasmobranchs, it was of interest to determine the electrophysiological characteristics of gastric mucosae of this species.

Four specimens of sting rays which come from the upper Amazon were received from a commercial supplier of tropical fish. Upon receipt they were placed in aerated tapwater at 25° C for several days. After sacrifice, the gastric mucosa was stripped from the serosal muscle coat. The mucosa was mounted in a flux chamber with a 1 cm aperture. The serosal surface of the mucosa was exposed to 12 ml of solution of Na 152, K 10, Ca 10, Mg 4, Cl 140, HCO$_3$ 30, HPO$_4$ 2, and SO$_4$ 4 mEq/L as well as 28 mM/L glucose. The solution was gassed by 95% O$_2$ 5% CO$_2$. A similar solution bathed the mucosal surface but HCO$_3$ and HPO$_4$ were replaced by Cl and it was gassed with 100% O$_2$. Experiments were conducted at room temperature, ca 25° C. Solutions in contact with the mucosal surface were titrated to pH 7. The transmucosal potential difference was sensed by a pair of calomel cells leading to a high impedance potentiometer. Observed readings were corrected for small asymmetry potentials between the combination of agar-saline bridges and calomel cells.

The maximum potential difference across these 4 mucosae ranged from 12.5 to 19 mv with the mucosal surface being negative to the serosal surface. A typical experiment is displayed in Figure 1. Because of expense, no attempt was made to establish the optimal conditions for the

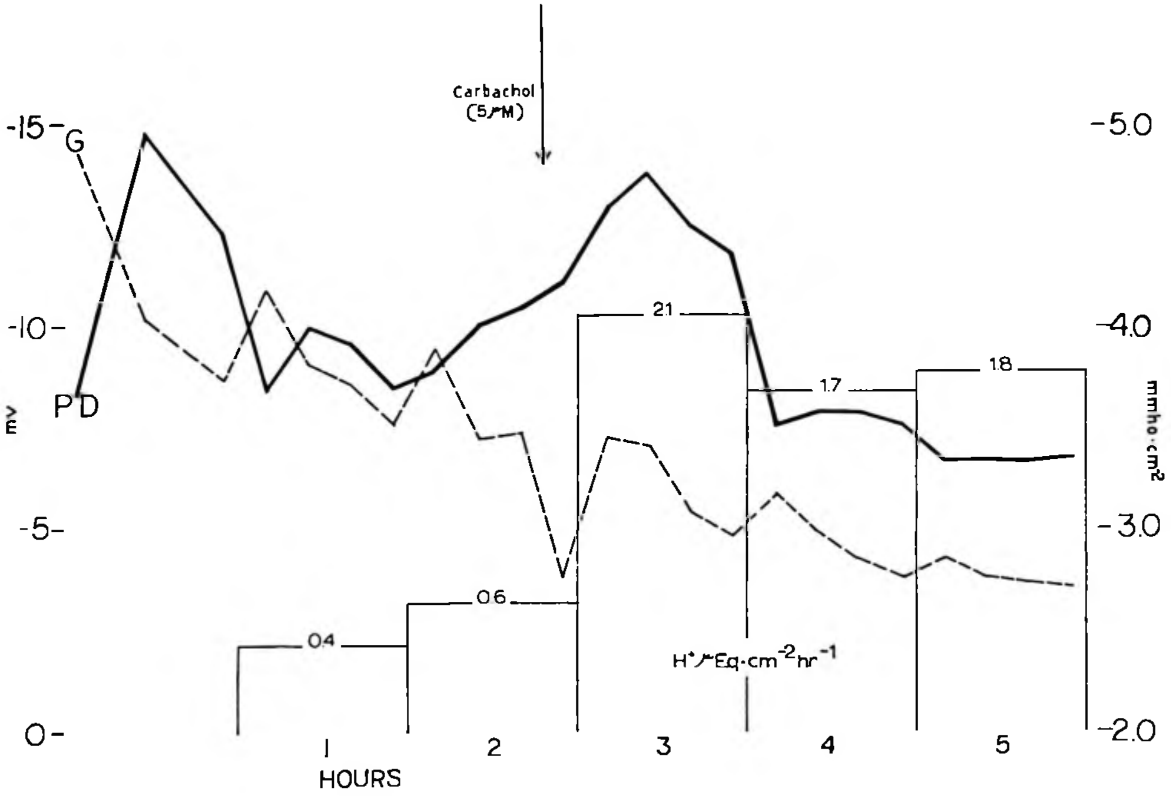

Figure 1. The time course of the transmucosal potential difference, mucosal surface negative to serosal surface, conductance and rate of hydrogen ion secretion (shown as bars). Wet weight 82 mg cm^{-2}.

maintenance of these mucosae. All deteriorated over a 4-6 hour experiment. Nevertheless it was possible to establish that they responded to carbachol by increasing the rate of hydrogen ion secretion, and when the submucosal surface was exposed to a solution containing a high concentration of potassium (110 mM/L) there was a sharp reversal of potential. This response to a high extracellular K was the same as that encountered in the isolated frog gastric mucosa. A micrograph of one of the mucosae is presented in Figure 2. By light microscopy it is not possible to distinguish the mucosa of Potamotrygon from that of Squalus acanthius, a marine elasmobranch, or from that of the bullfrog, Rana catesbiana.

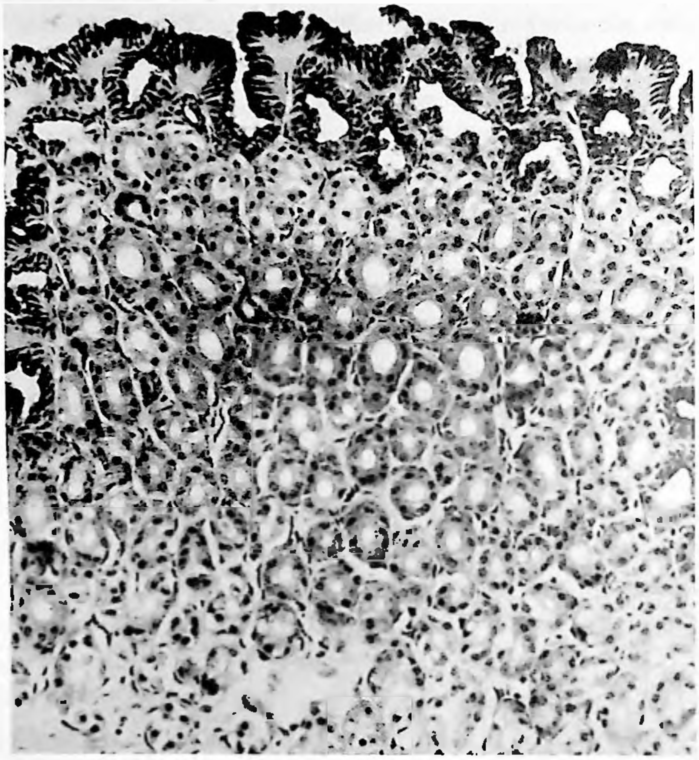

Figure 2. Light micrograph of sting ray gastric mucosa stained with the periodic acid Schiff reagent. It is quite similar to the mucosa of the dogfish though the mass of surface epithelial (mucous) cells may be somewhat less prominent. Magnification x 108.

Thus the isolated gastric mucosa from this freshwater elasmobranch does generate a significant transmucosal potential difference, which though not as large as generated by other vertebrates (30-40 mv). This smaller value may reflect less than optimal conditions for support in our preparation. In any event it is reasonable to anticipate that the active transport of chloride is dissociated from hydrogen ion transport. Concurrent with these experiments on the Amazon sting ray, we conducted experiments on the isolated gastric mucosa of the dogfish stimulated by carbachol and consistently encountered transmucosal potentials of several millivolts but oriented in the opposite direction to that encountered in other species; i.e. the PDs were oriented such that the mucosal surface was positive with respect to the serosal surface. It would be premature at this time to conclude that there is a clear separation between the marine and freshwater elasmobranchs with respect to the electrophysiology of the gastric mucosa because of the clouded experiments reported for southern marine elasmobranchs (Am. J. Physiol. 203, 1091-93, 1962). In this study a potential as high as 12 mv, mucosa negative with respect to serosa, was recorded but the average potential in 6 elasmobranchs secreting acid was only -2.2 mv.

1970 #19

EFFECT OF VASOPRESSIN AND OSMOTIC GRADIENT ON INTESTINAL MEMBRANE OF *Pseudopleuronectes americanus*

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The small intestine of winter flounder mounted in a lucite chamber and bathed on both sides with Forster teleost Ringer, was used to study the effects of vasopressin and osmotic gradient on membrane permeability. In Forster Ringer solution a potential difference (PD) ranging from 1-5 mV was observed with the serosa being electronegative to the mucosa. On the addition of vasopressin to the bathing solution, the PD and short circuit current (I_{sc}) became less negative, suggesting an increased cation flux from mucosal to serosal surface. The action of vasopressin was further studied by determining its effect on the permeability coefficient of the intestinal membrane for H_2), urea, thiourea and Na across the intestinal membrane. All K_{tran} values measured from mucosal-to-serosal side were greater than the values measured from serosal-to-mucosal. Vasopressin increased the permeability coefficient of each compound studied.

Table 1

EFFECT OF VASOPRESSIN ON INTESTINAL PERMEABILITY

Compound		No. of period/fish	Permeability coefficients $K_{trans.}$ (10^{-7} cm sec^{-1})	
			M→S	S→M
H_2O	control	16/10	350.5 ± 27	235.6 ± 20
	vasopressin	24/8	433.2 ± 19.4	351.5 ± 17
Urea	control	17/10	97.9 ± 9.0	66.1 ± 7.4
	vasopressin	24/8	149.5 ± 8.0	103.3 ± 6.8
Thiourea	control	14/10	94.9 ± 10.5	81.0 ± 10
	vasopressin	23/8	130.0 ± 8.3	94.4 ± 5.4
Na	control	8/6	145.2 ± 9.0	102.9 ± 6.4
	vasopressin	15/4	175.1 ± 7.0	133.3 ± 4.3

The effect of osmotic gradient on I_{sc} across the intestinal membrane is presented in Figure 1. When glucose or mannitol was added to the mucosal bathing fluid, the PD and I_{sc} became more negative. The increase of negativity was in the order of NaCl Ringer > Na_2SO_4 Ringer > LiCl Ringer > Choline Chloride Ringer. When glucose was added to the serosal side, both PD