of this dye occurred in the bile of the hagfish even after 24 hours. Further study is needed on this latter point, but it is interesting to speculate that there may be a molecular weight cut-off in the hagfish biliary system, because both phenol red and ICG are sulfonic acids but the molecular weight of the latter is more than two times that of the former. The bile to plasma ratio does suggest active transport of this material although this ratio is the smallest seen in 24 hours compared with the other two species. ICG was not looked for in the urine but careful inspection of the urinary ducts of the hagfish showed only clear fluid with no suggestion of a green tint.

These three species served very well to study a compound which can be metabolized like phenol red and a non-metabolized one like ICG. It is interesting that the dogfish, an elasobranch, has the capacity to convert phenol red to its glucuronide. It is not surprising that the primitive hagfish apparently does not have this capacity. Further studies on other species such as the skate are in progress.

1972 #22

STUDY OF THE RECTAL GLAND OF *Squalus acanthias* IN VITRO

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Burger and Hess (Science 131; 670, 1960) established that the rectal gland in *Squalus acanthias* is a salt-secreting organ which produces a hypertonic sodium chloride solution with a concentration nearly twice that of plasma. In order to examine the characteristics of ion transport in this unique tissue, studies were conducted on the *in vitro* perfused gland. Within five minutes after removal from freshly killed fish the solitary artery of glands weighing 1.5 and 2.5 gm was cannulated with polyethelene tubing (PE90) while similar tubing was secured into the duct. After rinsing with heparinized saline, a constant perfusion was begun using a Harvard peristaltic pump (Model 1201) with fluid containing in mM/L, NaCl 280, KCl 6, $CaCl_2$ 2.5, $MgCl_2$ 3, Na_2SO_4 0.5, NaH_2PO_4 1, Urea 350, glucose 5 and $NaHCO_3$ 8. The perfusion fluid was gassed with 95 percent O_2 and five percent CO_2 and the temperature of the gland maintained at 16 - 18°C. A bubble chamber and mercury manometer were placed on line between the pump and perfused gland. The secreted fluid was collected in timed aliquots under mineral oil for analysis. Perfusion rates varied between 2.0 and 4.0 ml/min, approximately four to eight times the estimated flow rate of whole blood *in vivo* (0.22 ± 0.15 ml/gm/min) (MDIBL Bulletin 11; 53, 1971). The perfusion pressure at 2.0 ml/min was 10.3 ± 1.7 mm Hg (mean ± SE) and 30.4 ± 3.2 at the rate of 4.0 ml/min.

The average Na and K concentrations in the rectal gland fluid from 12 glands were 405.7 ± 0.7 mEq/L and 11.5 ± 0.7, respectively, compared to the values in the perfusion fluid which averaged 281.2 ± 2.9, Na and 5.9 ± 0.4, K. These data indicated a gland fluid to plasma ratio of 1.45 ± 0.04 for Na^+ and 1.97 ± 0.14 for K^+ under free flow conditions. A steeper concentration gradient for sodium across the cell membrane facing the ductal system was demonstrated from measurements of intracellular ion concentration. The Na^+ concentration in 10 glands, expressed as mEq/L tissue water was 167.5 ± 17.3, while that of K^+ was 106.8 ± 10.6. From the estimated value of 0.24 for

the ECF volume (Comp. Biochem. Physiol. 42A, 195, 1972) and the serum Na^+, 254 ± 1.9 mEq/L and K^+, 1.8 ± 0.1 mEq/L, concentrations, the intracellular concentrations of Na^+ and K^+ were calculated to be 67.7 mEq/L and 87.9 respectively.

In order to determine whether function would remain stable under the conditions of this study, the rate of secretion of Na^+ and K^+ was measured in 30-minute intervals over periods of 90 to 120 minutes. As shown in Table 1, while secretion rate differed markedly between glands, there was reasonable constancy in individual preparations. In an additional five experiments the rate of sodium secretion was found to vary directly with perfusion rate, as perfusion rate was varied between 0.4 ml/min and 4.0 ml/min. Since the concentration of Na remained unchanged, enhancement of secretion was due to an increase in volume.

TABLE 1

The Rate of Na and K Secretion by the *In Vitro* Perfused Rectal Gland

	$\underline{GF_{Na} \cdot V}$ μEq/min/g				$\underline{GF_{K} \cdot V}$ μEq/min/g			
Minutes	0-30′	30′-60′	60′-90′	90′-120′	0-30′	30′-60′	60′-90′	90′-120′
1.	1.76	3.13	4.01	4.55	0.04	0.07	0.09	0.10
2.	0.58	0.20	0.17	0.11	0.01	0.01	0.01	0.01
3.	0.15	0.17	0.23		0.01	0.01		
4.	0.30	0.29	0.16	0.19	0.01	0.01	0.01	0.01
5.	0.13	0.08	0.08	0.08	0.01	0.01	0.01	0.01
6.	0.20	0.32	0.32		0.01	0.01	0.01	
7.	0.94	0.78	0.99	1.40	0.02	0.01	0.02	0.03

Further studies were performed to characterize the properties of the mechanism responsible for Na transport. In three glands the concentration of Na was reduced in the perfusion fluid while osmolarity was maintained with mannitol. When the concentration was decreased from 281 mEq/L to 220 the rate of sodium secretion was unchanged, while the ratio of Na concentration in glandular fluid to perfusate (RGF/P) rose to 1.8. Further reduction of Na concentration to 131 mEq/L resulted in a ratio of 2.8. When Na was decreased to 70 mEq/L, one-fourth of the normal value, the rate of secretion rose from 0.10 μEq/min/gm to 1.2 and the RGF/P ratio fell to 1.0. In four experiments ouabain, 1 x 10^{-3}M, was added to the perfusate. The rate of sodium excretion in experimental vs control periods was unchanged in two glands, with ratios of 1.0 and 0.78, declined in one to 0.34 and rose to a ratio of 2.1 in the fourth. It was of interest that RGF/P Na concentration fell from the average control value of 1.50 to less than 1.2 in three of four glands during the experimental period.

These preliminary studies indicate that sodium secretion by the rectal gland continues under conditions of *in vitro* perfusion against a steep concentration gradient. Because of the variation in the spontaneous rate of secretion further improvement in the method of study will be required in order to detect modest experimentally induced changes.

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ANOMALOUS BEHAVIOR OF ^{3}H- AND ^{14}C-LABELLED INULIN AND ^{3}H-LABELLED POLYETHYLENE GLYCOL IN INCUBATED KIDNEY TISSUE OF THE WINTER FLOUNDER, *Pseudopleuronectes americanus*.

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Renal function studies in the southern flounder, *Paralichthys lethostigma*, have revealed significant discrepancies between the measured clearances of ^{3}H-methoxy inulin and ^{14}C-polyethylene glycol: the ^{14}C-PEG clearance always exceeded, and was sometimes double, the clearance of ^{3}H-inulin (Hickman, unpublished observations, 1972). The present series of experiments was carried out to establish whether or not ^{3}H-methoxy inulin and other labelled carbohydrates used in renal function studies exhibit transtubular transport, binding, cellular penetration or other behavior that would render them unsuitable for estimates of glomerular filtration rate.

Small, freshly trawled winter flounder, *Pseudopleuronectes americanus*, 75-220 g in weight, were used in two series of experiments. In each experiment of the first series, the kidney of a freshly-killed flounder was teased into 2-5 mg fragments, rinsed in Forster balanced saline (Forster, R.P. and S.K. Hong, Jour. Cell. Comp. Physiol., 51; 259-272, 1958), and transferred to a 20 ml glass vial containing 2 ml of Forster saline to which had been added 5-15 μCi of a ^{3}H-labelled carbohydrate polymer (^{3}H-methoxy inulin, ^{3}H-PEG 1000 MW, or ^{3}H-PEG 4000 MW) and a ^{14}C-labelled carbohydrate (^{14}C-carboxyl inulin, ^{14}C-PEG 4000 MW or ^{14}C-dextran 24,000 number av. MW). The tritium and ^{14}C-labelled compounds were used in randomized combinations in different experiments. The vial was incubated at 15°C with gentle agitation for three hours.

At intervals samples of tissue were removed, weighed, dissolved in a tissue solubilizer, and prepared for liquid scintillation counting. These *in vitro* experiments measured the maximum penetration of the labelled compound into kidney tissue in the absence of glomerular filtration. Since the broken ends of tubules in this preparation close off by constriction (Kinter, W.B., Amer. Jour. Physiol., 211: 1152-1164, 1966), the distribution of the label is presumably entirely extracellular. The results of 11 *in vitro* experiments, summarized in Table 1, show that the "tissue space" or volume of distribution of ^{14}C-PEG (averaging 23.95 percent) was consistently and significantly smaller than that of ^{3}H-methoxy inulin, ^{14}C-inulin, and ^{3}H-PEG which ranged between 34.6 and 44.2 percent. A single experiment with ^{14}C-dextran yielded a space estimate of 24.2 percent almost identical to ^{14}C-PEG. In all experiments maximum space values were reached in about 20 minutes with little or no enlargement with time. Entry was rapid and the difference between the smaller tissue spaces of ^{14}C-PEG and ^{14}C-dextran on the one hand, and the larger tissue spaces of ^{3}H-inulin, ^{14}C-inulin, and ^{3}H-PEG on the other, was established before the first sample was taken at five minutes of incubation.