were determined in arterial and mixed venous (duct of Cuvier) bloods and cardiac output was measured with the dye dilution technique. The study showed that there was little excretion of lactate across the gill membrane over a wide range of lactate concentrations. In approximately half of the animals, mixed venous blood contained lower lactate concentrations than arterial blood, indicating gill production of lactate. Infusion of buffered sodium lactate (pH 7.5) did not result in augmented gill excretion.

The renal mechanism for the handling of lactate was investigated by simultaneous measurements of lactate and C^{14} inulin clearances. Lactate to inulin clearance ratios averaged 0.31 over a wide range of arterial lactate concentrations indicating lactate reabsorption by the renal tubule. As a result of this avid net reabsorption total urinary excretion of lactate is low.

Lactate concentrations in coelemic fluid averaged approximately 1/20 of simultaneously determined arterial lactate levels indicating that little lactate is excreted into this comparment. It was also shown that $\frac{(H^+ \text{ blood}}{(H^+ \text{ coelomic fluid}}$ did not equal $\frac{(\text{Lactate -) coelomic fluid}}{(\text{Lactate -) blood}}$

Therefore lactate transport in coelomic fluid does not obey ionic diffusion.

Thus the normal resting lactate of the "wild" animal in the bay appears similar to that found in other vertebrates. Following capture, enhanced anaerobic glycolysis resulting from vigorous muscular exercise and an unfavorable environment leads to increased lactogenesis. Because gill and renal excretion are limited, lactate concentrations continue to be sharply elevated. Although not specifically measured in this study, the functional capacity of dogfish liver to metabolize lactate must likewise be sharply limited.

1965 #30

ROLE OF Na^+-K^+ ADENOSINE TRIPHOSPHATASE IN THE RECTAL GLAND OF Squalus acanthias*

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An adenosine triphosphatase located in the membrane fraction of many tissues has been implicated in active ion transport. This enzyme is activated by Na^+ and K^+ together, but by neither ion alone. That activity stimulated by Na^+ and K^+ is totally inhibited by the cardiac glycoside, ouabain. ATP-ase activity fitting the above definition has been found in the rectal gland of the dogfish, a sodium chloride secreting organ. Properties of this enzyme system in the dogfish rectal gland were defined. Methods of preparation and assay were identical to those previously described (Palmer and Nechay, J. Pharmacol. 146: 92, 1964). Activity in the whole homogenate is of the order of 1.2 μmoles of inorganic phosphorous formed per mg of wet tissue weight per hour. This value is threefold higher than avian salt gland homogenates prepared in a similar manner. Only small amounts of activity were found in the nuclear and mitochondrial centrifugal fraction, most of the activity was in the supernate after the mitochondria had been separated. Enzyme activity was maximal at 37°C. A Q_{10} was calculated between 22° and 37° of 1.46. Maximal ion activation occurred at concentration of 20-100 mM Na^+ and 4-20 mM K^+, values similar to mammalian systems previously studied in spite of the large difference in extracellular fluid

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composition of the two classes. The Na^+ and K^+ stimulated ATP-ase activity was inhibited by ouabain ($I_{50} = 10^{-6}$ M), para chloromercuribenzoate ($I_{50} = 3 \times 10^{-6}$ M), chlormerodrin ($I_{50} = 3 \times 10^{-6}$ M), and 20% glycerol. Previous work on enzyme from nerve tissue in the blue crab Callinectes sapidus showed that in the presence of 350 mM KCl (approximate intracellular concentration in axoplasm) addition of Na^+ ion gave maximal activation at 50 mM Na^+. This was not true in the rectal gland. Maximal activation (in the presence of 350 mM KCl) was not approached even at 250 mM Na^+. Thus a minor but perhaps important distinction has been made in ion activation properties between the two species.

1965 #31

IN VITRO PERFUSION OF THE ISOLATED RECTAL GLAND OF Squalus acanthias*

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In vivo the rectal gland secretes a neutral solution of NaCl containing very little urea and essentially no Mg^{++} or Ca^{++}. The anatomy of the gland, with a single afferent artery and a single excretory stoma, lends itself to available techniques for arterial perfusion and collection of the secretion in vitro. Glands obtained from freshly killed dogfish were placed in cold (10°C) perfusion medium. The artery was cannulated with P.E. #50 tubing attached to a #22 needle. The stoma was cannulated with P.E. #100 tubing and perfusion begun from a reservoir 10-50 cm above the gland. The perfusion fluid contained in meq/L NaCl 250, KCl 5, $CaCl_2$ 6, $MgCl_2$ 3, $NaHCO_3$ 12, Urea 350 mM, glucose 10 mM. The perfusion gluid was gassed with 95% O_2 and 5% CO_2, and maintained at 10-15°C. Perfusion rates varied from 25 to 150 ml/hr. The technique was developed to the extent that about three minutes elapsed from the time of removal to the time when perfusion was begun. Functional preparations began to secrete a fluid immediately and continued to do so for 3-4 hours. If the initial secretion contained Mg^{++} or Ca^{++} greater than 1 meq/L the preparation was discarded. Altogether ten glands had no Mg^{++} or Ca^{++} in the secretion during the observation periods. Secretory flows ranged between 0.2 and 2.0 ml/hr. Chloride concentrations were constant for any single gland and ranged from 250 to 520 meq/L. In six cases the secretory fluid had chloride significantly higher than that of the perfusate. It was the impression, although not systematically investigated, that glands being perfused at faster rates tended to have lower secretory chlorides, although Ca^{++} and Mg^{++} were excluded from the secretion at these faster rates. Acetazolamide (4×10^{-4} M) stopped secretion immediately and completely in the three preparations where it was tested. Ouabain (10^{-4} M) initially increased secretory flow 2-5-fold, then completely stopped secretion in three glands where it was given. Neither drug altered the arterial perfusion rate.

In this preliminary investigation the following conclusions were reached:

(1) The rectal gland is technically approachable for isolated perfusion, and gradients from perfusion fluid to secretion fluid for Cl^-, Mg^{++} and Ca^{++} can be achieved.

(2) The rate of perfusion and the perfusion pressure are critical determinants of gland function in vitro, and require further investigation and better control.

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