Whether this is a manifestation of glomerular intermittence or of a flow-dependent mechanism for glucose reabsorption cannot be stated at this time. Supported by National Science Foundation Grant GB-2580.

1966 #3

ON THE COUPLING BETWEEN METABOLISM AND ANAEROBIC SODIUM TRANSPORT IN THE RED BLOOD CELLS OF THE DOGFISH SHARK (Squalus acanthias)

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The nucleated red blood cells of the dogfish shark transport sodium in vitro from cell water to extracellular fluid at a faster rate than mammalian red blood cells and at temperatures of 20 to 22° C. This transport occurs anaerobically as well as aerobically. During anaerobic transport, glucose is utilized as substrate and anaerobic glycolysis has been found to be approximately twice as great as aerobic glycolysis. Oligomycin, an inhibitor of oxidative phosphorylation in mitochondrial-linked energy producing systems, inhibits roughly 50% of anaerobic sodium transport via the erythrocytes. In oxidative systems, oligomycin depresses oxygen consumption; but in the anaerobic studies on the dogfish erythrocytes, glycolysis was markedly stimulated. The depression of sodium transport, all other things being equal, should result in an accumulation of cellular ATP stores in view of the fact utilization of energy for sodium transport is decreased. The increased rate of glycolysis, similarly should result in an increase in cellular ATP stores assuming that glycolysis occurs normally and that ATP is produced normally via the Embden-Meyerhof pathway. Yet the measured levels of ATP in the presence of oligomycin were moderately diminished rather than increased. Utilizing a cell membrane ATPase preparation from dogfish erythrocytes, it was found that oligomycin inhibited sodium, potassium stimulated ATPase activity, rather than stimulating it. Were stimulation to have occurred, the decrease in ATP concentrations despite the presence of inhibition of sodium transport and increased anaerobic metabolism might have been explicable. However hydrolysis of ATP by plasma membrane ATPase activity was decreased and this too should contribute to an accumulation of ATP in the cell water. The combination of effects suggests that glycolysis was "uncoupled" from phosphorylation. Moreover, the fact that sodium transport was inhibited in the presence of appreciable levels of ATP would provide evidence, albeit indirect, that ATP per se may not be the immediate source of energy for sodium transport. Electron micrographs of dogfish erythrocytes performed by Dr. W. L. Doyle of the University of Chicago have shown no discrete mitochondria in the cells. Thus, it seems unlikely that oligomycin acted conventionally on a high energy intermediate arising from glycolytic ATP recycled in a reversed order through mitochondria. We believe that these data provide phenomenologic evidence for a high energy intermediate of glycolysis; if such an intermediate exists, it presumably serves as the energy source for anaerobic erythrocyte sodium transport.

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EFFECTS OF CHANGES IN TEMPERATURE ON BODY FLUID pH IN THE DOGFISH, Squalus acanthias

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The operational definition of normal pH of biological fluids is of general interest. In particular, changes in temperature as these affect H^+ activity in poikilothermic forms is of fundamental importance.

The thermodynamic basis of pH measurement does not strictly permit the comparison of pH values obtained at different temperature since $E_T = E^o - \frac{RT}{F} 1n_a H^+$ and E^o must be arbitrarily defined at any temperature. However, biological systems present the problem of interpretation of pH measurements of various body fluids over a range of temperatures. The effects of varying body temperature from 2° C to 23° C was therefore investigated in the dogfish. pH measurements were performed on blood, celomic fluid, urine and sea H_2O. Measurements were made at the actual temperatures of the fish and compared to measurements made by using an electrode maintained at the other temperature to which the fish was ultimately subjected. The animal was maintained at each of the two temperatures for two hours before the body fluid samples were obtained:

1) Normal blood pH in the dogfish shark obtained in the wild state is 7.78 ± .06 (S.D.). This pH suggests that the pH of dogfish at ambient temperature is identical with other forms including amphibia and reptiles maintained at similar temperatures.
2) In vitro temperature coefficient of blood differs markedly from that of celomic fluid and urine. In blood there is approximately 0.013 ΔpH/° C change in temperature over the entire temperature range studied. In celomic fluid and urine the temperature coefficient depends on the temperature range being used. At relatively low temperature (2° - 5° C) these fluids have temperature coefficients similar to blood. At higher temperatures (>12° C) the temperature coefficient of these fluids is small (≅ 0.002). As a result, measurements of pH gradients based on in vitro measurements lead to gross errors.
3) In vivo modification of temperature leads to considerable changes in H^+ gradients between plasma and these two body fluids.

The implications of these findings for a fundamental understanding of the effect of temperature on H^+ are being further investigated.

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