

addition to chemical sodium measurements, chloride and potassium concentrations were measured in red cell water and in the Ringer's solutions.

If chloride is in thermodynamic equilibrium across the dogfish red blood cell membrane, both sodium and potassium are maintained in a state removed from equilibrium. Presumably therefore both of these cations are actively transported against an electrochemical gradient.

In contrast to non-nucleated mammalian red blood cells, the sodium concentrations of cell water were lower after prolonged incubation at 4°C without substrate than after incubation with substrate at approximately 20°C. When the cells were incubated in an oxygenated environment, Na²² efflux rates were greater by several fold than values reported for mammalian red blood cells. Sodium transport was maintained under anaerobic conditions although the efflux rates were approximately half of those observed in the aerobic system. Nevertheless, anaerobic sodium transport in the dogfish red blood cells at 16-22°C still appreciably exceeded values reported for mammalian red blood cells at 37°C. Strophanthin (1×10^{-5} mM) inhibited sodium transport under both aerobic and anaerobic conditions. After overnight incubation without substrate, lactate formation measured during flux studies ranged from approximately 50 to 250 μ moles/Kg red blood cells per hour in an oxygenated environment. In the anaerobic system, lactate formation was increased by over 100%; hence a brisk Pasteur effect was demonstrable.

The present studies define certain of the characteristics of sodium transport by the dogfish red blood cell. The data indicate that brisk transport occurs in these nucleated cells under aerobic conditions very much as it does in the nucleated red blood cells of the duck. In contrast to the duck cells however the rate of anaerobic transport by the dogfish red blood cells is somewhat less than aerobic transport rates. Nevertheless the dogfish appears equipped to sustain active cation transport by means of glycolytic metabolism. It therefore could serve as a valuable model for the study of the inter-relationships between glycolysis and a key endergonic biologic process.

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THE EFFECTS OF OLIGOMYCIN AND 2-4 DINITROPHENOL ON ANAEROBIC SODIUM TRANSPORT BY THE RED BLOOD CELLS OF THE DOGFISH SHARK (*Squalus acanthias*)*

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The red blood cells of the dogfish shark have been found to transport sodium from cell water to extracellular fluid under anaerobic as well as aerobic conditions. This cell type therefore could serve as a model for the investigation of certain of the inter-relationships between metabolism and transport in the elasmobranch under conditions wherein metabolic energy production is provided via the Embden-Meyerhof pathway. We previously have studied the coupling between anaerobic metabolism and anaerobic sodium transport in the isolated urinary bladder of the turtle. From these studies evidence emerged that suggested the possible existence of a high energy intermediate of glycolysis (Bricker, N. S., and Klahr, S. J. Gen. Physiol. To be published January 1966). This evidence consisted of the fact that 2-4 dinitrophenol, a classic un-

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coupler of oxidative phosphorylation which presumably acts to hydrolyze a high energy intermediate of oxidative phosphorylation, resulted in a prompt and marked inhibition of anaerobic sodium transport at the same time that it markedly stimulated anaerobic glycolysis. Moreover tissue ATP concentrations were decreased, but only modestly.

In the present studies Na^{22} efflux, glycolysis (measured as lactate formation) and ATP concentrations of dogfish red blood cells were examined in vitro. Glucose was used as substrate. Dinitrophenol (1×10^{-5} M) tended to inhibit sodium transport, but the results were inconsistent. A modest stimulation of glycolysis occurred, and ATP levels did not differ significantly from control cells. In contrast to these inconclusive effects of DNP, oligomycin had striking and to our knowledge previously undescribed effects. Na^{22} efflux was inhibited both aerobically and anaerobically. Glycolysis was stimulated profoundly. Under aerobic conditions, rates of glycolysis increased by as much as fivefold; under anaerobic conditions, when the spontaneous rate of glycolysis already was increased over the aerobic rate by more than twofold, additional increments of greater than 100% occurred in the majority of experiments. ATP concentrations were decreased slightly rather than being increased as might be expected on the basis of the stimulation of glycolysis and/or the inhibition of transport. Alcohol, used as the solvent for the oligomycin, did not reproduce the oligomycin effects.

In oxidative systems, oligomycin inhibits energy production, but it does not dissociate oxidation from phosphorylation. In mammalian red blood cells, Whittam has found that oligomycin decreases transport and decreases glycolysis. He has suggested that this antibiotic has an ATPase inhibitory effect similar to that of strophanthin. The patterns observed in the dogfish red blood cells thus differ from those described in human red blood cells and are reminiscent of the DNP effect described for the turtle bladder. The possibility exists therefore that oligomycin inhibits a high energy compound produced by glycolysis that is involved in energy provision for active sodium transport.

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ANGIOGRAPHIC STUDIES OF THE ARTERIAL CONSTRICTOR RESPONSE IN THE HARBOR SEAL Phoca Vitulina

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An arterial constrictor response to diving could functionally serve the primary role in conservation of oxygen during diving in the seal. The precise role of arterial constriction as well as its degree has not been clear, since its existence has only been demonstrated indirectly. Absence of urine formation, stable blood lactic acid concentrations, failure of venous dye injection to reach the aorta, and a decrease in web artery pressure during diving support the existence of an arterial constrictor response. In order to unequivocally establish the existence of arterial constrictor response, visual demonstration would be ideal. Four young female harbor seals were therefore trained to dive under laboratory conditions, and arterial angiography was performed out of water and during diving. A Teflon catheter was inserted into a femoral artery using procaine anesthesia. The catheter was then advanced into the aorta using a wire guide, and positioned under fluoroscopic visualization. Renografin ^R, 15 to 25 ml, was injected by an automatic injector