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.

1965 #8

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

and x-rays were taken at 2 to 3/sec. intervals using a Schonander rapid film changer. An electrocardiogram was used to demonstrate bradycardia during diving to ascertain a normal dive response. Out of water there was rapid filling of mesenteric, lumbar, splenic, renal, femoral and pelvic arteries. Before the end of the series of films all dye had left the aorta, venous patterns of blood flow were noted, and nephrograms had progressed to venous phase. During diving however, there was poor filling of the same arteries, which were smaller in diameter, with apparent complete occlusion of small arterial radicals. Dye remained in the aorta and no nephrogram was noted even at the end of the film series. In addition, the film series showed that the dye progressed cephalad during diving. The angiographic studies dramatically demonstrated the existence of an arterial constrictor response during diving, and revealed that the constriction was graded throughout medium and small arteries and not segmented in nature. Angiograms of the cerebral circulation revealed maintenance of cerebral perfusion during diving.

These studies directly document the existence of profound arterial constriction and loss of perfusion involving peripheral tissues during diving. As a result, body O_2 stores are available for the maintenance of O_2 dependent metabolism in the central nervous system.

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DIRECT MEASUREMENT OF DIODRAST-^{131}I TRANSPORT STEPS IN THE ISOLATED FLOUNDER TUBULE

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Flounder tubules in vitro have provided a useful preparation for the study of renal organic acid transport. From visualization of phenol red and chlorphenol red uptake under appropriate conditions it has been concluded that there are at least two concentration steps involved in organic acid secretion. The first step, into the tubule cells, ordinarily results in little, if any, elevation of dye concentration in the cells compared to the medium. The second step, from the cells to the lumen, ordinarily results in a much higher concentration in the lumen than in either the cells or the bathing medium (1,2). In the absence of calcium in the bathing medium, however, accumulation of the dye is limited to the cells, little or none appearing in the lumen (2,3).

It was considered of interest to re-examine this transport mechanism using the new method of in vitro tubule perfusion (4) to measure directly Diodrast-^{131}I concentration gradients and transport rates in single isolated flounder renal tubules.

METHODS:

1. Dissection: Fragments of flounder tubule were immersed in the physiological saline solution previously observed to support maximum chlorphenol red accumulation (3), and fragments of tubules 1 to 4 mm long were dissected free hand using fine forceps and needles under a stereoscopic microscope at 20 to 30 x magnification. During dissection the saline solution was oxygenated and cooled to 10-15°C.

2. Perfusion: Three concentric micropipets were used for perfusion. The outer pipet had an inside diameter at its tip approximately equal to the outside diameter of a flounder tubule. This pipet was attached to suction and served to pull the tubule over the inner pipets and seal the