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.

1965 #9

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

inner pipets within the lumen of the tubule. The middle pipet was filled with physiological saline identical to that in the outside bath and the inner pipet was filled with "Kel-F" low viscosity oil. Regulation of fluid delivery into the tubule from either of the two inner pipets was by air pressure from syringes controlled by hand. For collections the distal end of the perfused tubule was pulled into an oil-filled pipet. Droplets of perfusion fluid emerging from the tubule were removed from the oil-filled pipet with a calibrated constriction pipet which was also filled with oil. Stop-flow perfusion experiments were carried out by connecting the pipets to the tubule in this manner, and isolating a column of physiological saline solution in the lumen between columns of oil. After 10 minutes the oil columns were advanced, forcing the saline solution out of the tubule into the oil-filled collecting pipet. The oil-filled tubule was then removed, blotted, and prepared for analysis.

Some tubules were also incubated without perfusion in 0.3 to 0.5 ml of physiological saline in covered 2 ml beakers.

3. Measurement of radioactivity: Tubules were extracted in TCA in the center of a stainless steel planchet for one hour and were then removed, dried, and stored for later weighing. These fluid samples and aliquots of tubule fluid and medium were dried and counted using a low background (<1 CPM) Geiger Counter.

4. Tubule volume was estimated from the length and diameter of individual tubules. The dried tubules have been saved and will be weighed later using a quartz fiber balance for more precise calculations.

5. Incubation conditions: All incubations were at room temperature with Diodrast^{131} I (3×10^{-5} M) added to the incubation fluids.

RESULTS:

In the initial studies it was found that Diodrast^{131} I concentration in tubules incubated for one hour without perfusion was 46 (range 19 to 107, 23 tubules) times as great as in the medium. When the tubule lumens were filled with oil and the tubules incubated for the same period of time, the diodrast concentration was approximately the same (mean 61, 5 tubules). Further, when the fluid contained in a tubule lumen was collected under oil using the stop-flow microperfusion technique (after 10 minutes of incubation in medium containing Diodrast I^{131}), the concentration of radioactivity in the luminal fluid was no higher than in the tubule cells (mean 70%, 4 measurements) and similar to the concentration found in the tubules after 60 minutes of incubation without perfusion. Thus, no evidence was found that there was a concentration gradient for diodrast higher in luminal fluid than in cells.

This result seemed inconsistent with previous observations using organic dyes (1-3), therefore experiments were carried out observing chlorphenol red uptake under the conditions of tubule perfusion. It was found that dye appeared in approximately equal concentration ("++") in both the cells and lumen, and that all the tubules observed had approximately the same dye concentration. In these experiments 95% O_2, 5% CO_2 had been used as the gas mixture. It was found that when 100% O_2 was used as the gas mixture, dye accumulation was generally more intense in the lumen ("+" to "++++") than in the cells ("O" to "++"). As noted in earlier studies (1), the concentration of dye in the lumen varied greatly between tubules, some tubules having no apparent uptake. The apparent difference from earlier studies (1) as regards the effect of CO_2 in the gas mixture is probably because in the earlier studies the incubation dishes were not covered and

the saline solutions were not fully equilibrated with the gas mixtures.

Because of these findings additional experiments were carried out, incubating single tubule fragments with 100% O_2 as the gas mixture. When tubules were incubated without perfusion, the Diobrast^{131} I content after 1 hour was more variable than in the earlier studies, some tubules having a concentration of radioactivity only 1 to 2 times as great as the medium. Mean concentration ratio was 30 (range 1.-100., 21 tubules). Experiments incubating tubules with oil in the lumen and with stop-flow microperfusion were carried out but have not been completely analyzed yet.

Kidneys from several other species were dissected (fundulus, sculpin, pollack, skate, and dogfish) and all appeared to be satisfactory for use in similar experiments.

CONCLUSION:

A method has been developed for measuring directly concentration gradients of radioactive substrates between cells, lumen, and outside bathing solutions in fragments of flounder renal tubule in vitro. It seems likely that the same method can be applied to other marine species. Using this method it should be possible to investigate the kinetics of organic acid transport, correlating transport rates with the transport steps at the cell borders.

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1965 #10

RENAL CAPACITIES TO MANIPULATE ELECTROLYTE IN THE SPINY DOGFISH, Squalus acanthias*

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The capacity of the kidney of the spiny dogfish to handle various electrolytes has been poorly explored, but is obviously important in understanding the pattern of this fish's adjustment to its marine environment. Simultaneous inulin-electrolyte clearances were run on normal dogfish and on fish loaded with magnesium chloride and sulphate; calcium chloride; and monosodium phosphate. Inulin urine-plasma ratios ranged from 2-15 with urine flows of 0.12-1.2 ml/kg/hr. The higher ratios (8-15) are uncommon. Thus there is always a reabsorption of filtered fluid, but a hypotonic urine. Chloride-inulin clearance ratios ranged from 0.038-0.455 (12% over 0.29). Evidence indicates that the supra-plasma urine chlorides which occur frequently result from no capacity of the kidney to concentrate chloride, but from an obligatory binding to other excreted

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