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All blood and urine samples were drawn anaerobically. All test substances were injected intravenously or intramuscularly.

The following analyses were performed: pH, total  $\text{CO}_2$ , urea, chloride, sodium, potassium, freezing point depression, phosphate, inulin, ammonia, and titratable acidity.

The pH of dogfish urine was unaffected by the injection of: sodium bicarbonate, alkaline phosphate, sodium phosphate, creatinine, p-aminohippuric acid, maleate, mercurhydrin, BAL, sodium fluoride, iodoacetic acid, beryllium sulfate, dinitrophenol, and potassium chloride. Variations in titratable acidity were directly related to variations in phosphate excretion.

In the dogfish and sculpin, the intravenous administration of "Diamox" failed to alter the composition of the urine with respect to  $\text{CO}_2$ , bicarbonate, sodium, potassium or volume. This lack of response to "Diamox" contrasted sharply with that elicited in the catfish, which responded as does the mammal: an increase in urine flow, an alkalization of the urine, and an increased excretion of bicarbonate, sodium and potassium. Both catfish and dogfish manifested a decrease in blood pH.

Despite the lack of renal response to a challenge with bicarbonate, the level of bicarbonate in the dogfish serum was not appreciably altered by the intravenous administration of the maximum tolerated doses. However, the combination of bicarbonate and "Diamox" resulted in a sustained rise in serum bicarbonate and pH.

The results of these experiments indicate that the acidification of dogfish and sculpin urine is independent of a "Diamox-sensitive" carbonic anhydrase system. They also suggest that there exists an extra-renal site which is susceptible to the action of "Diamox".

### Studies on the Regulation of Bicarbonate Concentration in the Coelomic Fluid of the Sea Urchin\*

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Many years ago it was noted (H. W. Smith) that the coelomic fluid of the sea urchin (*Strongylocentrotus*) had a bicarbonate concentration of approximately 4 millimoles/liter as compared with a bicarbonate concentration of 1.8 millimoles/liter in the surrounding sea.

Experiments were carried out in an effort to determine whether an active mechanism of acid-base balance was operating in maintaining this difference.

Coelomic fluid, drawn in anaerobic syringes, was obtained by

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puncturing the thick peri-oral surface of the sea urchin, and the sea water bathing the sea urchin were analyzed for total  $\text{CO}_2$ , pH, and  $\text{pCO}_2$ . Using the Henderson-Hasselbach equation, with an alpha value of 0.0545 and a  $\text{pK}'$  for sea water of 6.17, the concentration of carbonic acid in sea water and coelomic fluid was calculated.

Sea urchins were maintained in sea water baths of varying concentrations of bicarbonate (1-50 millimoles/liter) and under varying partial pressures of  $\text{CO}_2$  in sealed air-tight jars.

As the sea urchins were maintained in the baths for increasing time periods, up to 75 hours, it was noted that the bicarbonate concentration of the coelomic fluid slowly came into equilibrium with the bicarbonate concentration in the surrounding sea water. No relationship suggesting an active mechanism involving  $\text{pCO}_2$ , pH, or total  $\text{CO}_2$  could be observed.

It is believed that the bicarbonate concentration of the coelomic fluid reflects endogenously produced carbon dioxide. The small area of the coelomic membrane lining the body cavity of the sea urchin relative to the large volume of coelomic fluid, presents a limited diffusion surface for the bicarbonate; the quantity of sea water under natural conditions obviates the possibility of attaining bicarbonate equilibrium.

These considerations suggest that diffusion limitation rather than active transport is responsible for the bicarbonate concentration gradient across the sea urchin coelomic wall.

### Active Transport by Renal Tubule Cells

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Three different approaches were used to elucidate certain physico-chemical characteristics of divalent ion transport by renal tubules of glomerular and aglomerular fish kidneys. Transfer rates for magnesium ions were determined in intact fish using clearance techniques, in isolated and perfused kidneys, and in thin slices of fish kidneys maintained in synthetic media. Experiments were designed to determine whether the active transfer of magnesium ions is limited by a maximal rate ( $T_m$ ), whether transport is via a mechanism shared by other divalent cations, and whether the transfer process is dependent upon aerobic phosphorylation as an energy source. Also, preliminary experiments were conducted to characterize the mechanisms accountable for the active cellular transport of creatine and trimethylamine oxide.

Perfusion of the isolated aglomerular kidney of *Lophius* could provide a very useful method for studying the kinetics of the tubular transfer process because it obviates the complication of glomerular filtration and