$Qo_2 = 6.8$ ). In Krebs solutions where the Na ion was substituted by Li the  $Qo_2$  rose to about 14, which is in the same magnitude as the stimulation by metacholine. The similarity of the Li effect to the activation by metacholine was more striking when the Li was added during the progress of an experiment. Moreover, the addition of metacholine and LiC1 together did not result in summation of effects.

If the Na ion was exchanged by K ion a marked inhibition resulted, the Qo<sub>2</sub> for KC1 medium being about 5.4 and for K-succinate 14-15.

Some of the outlined effects have since been clarified by investigating the Na and K movements inside the slices during the respiration experiment, and finding that 1) the Na and K content of the cells is very labile, changing rapidly in response to different amounts of these ions in the external media. 2) The cellular respiration is very sensitive to the amount of K ion in the cells (and not the Na content). Lowering the K content of the cell to about 1/10 the normal concentration by conducting the respiration experiments in K less medium is enough to increase the  $Qo_2$  to around 14. 3) Metacholine does not affect the respiration through a lowering of K content, the amount of K ions in the affected tissue being only a little less than in a normal Krebs solution.

This work was supported in part by a fellowship-in-aid from the American Philosophical Society and by grants No. Nonr 1181 (08) from the Office of Naval Research and No. H 2228 from the National Institutes of Health.

## Blood-Sea Water Barrier to Urea at the Dogfish Gill; Effect of Hypothermia and Urea-loading

## John W. Boylan and D. E. Antkowiak University of Buffalo

Blood perfusing the dogfish gills maintains its urea concentration of 2-2.5% in the presence of sea water which is virtually urea-free. Nothing is known concerning the (presumably) active mechanism which permits this gradient to exist.

Preliminary studies were directed toward (1) establishing the normal rate of urea excretion from the gills, (2) determining whether a threshold for urea exists at the gill, and (3) observing the effect of low gill temperature on the rate of urea excretion.

The following conclusions must be considered tentative, the data being numerically insufficient for statistical certainty. In sea water of  $9-10^{\circ}$ C loss of urea from the gills was of the order of 3.4 mgs./kg./hr. With intravenous urea-loading of .5 to 5 grams/kg. urea excretion at the gills was found to increase sharply at the highest load (5 gms./kg.). Sustained plasma levels in excess of 4000 mgs.% were associated with a more than twenty-fold increase in urea excretion at the gills. In one fish examined two days following a urea load of 5 gms/kg. the blood urea level had returned to normal.

The effect of temperature appears to be such that the least urea excretion at the gill occurs in water of 9-10°C. This is the temperature of water in which the fish is found in greatest number. At higher or lower temperatures more urea is lost from the gill, the greatest temperature effect being seen at 1-3°C. Sustained temperatures of 0°C or lower were not tolerated. Temperatures above 14°C were not tested. At 1-3°C a distinct increase in urea loss is seen despite a profound bradycardia (4-10/min.) and consequent reduction in gill perfusion. We intend to repeat the hypothermia study with controlled perfusion rates.

## Urea and Thiourea Excretion by Dogfish Kidney and Gill: Effect of Temperature

## John W. Boylan and Marilyn Lockwood University of Buffalo

Simultaneous renal clearances of urea, thiourea and inulin were determined in three adult, female dogfish. Reabsorption of filtered urea averaged 97% whereas on the average only 37% of filtered thiourea was reabsorbed.

The surface area of the dogfish gill was computed by micromeasurement, treating the lamellae as rectangles. There were 13-14 lamellae/mm gill filament and we estimated a total surface area of 370 mm<sup>2</sup>/gm body weight.

A preparation for studying excretion at the gill of the intact dogfish was developed in which sea water from a cooled reservoir was pumped to the spiracles and returned by gravity through the gills to the reservoir. The fish is suspended in air by a plastic sling. In a successful preparation normal gill movements are continued for several hours. The temperature and composition of the perfusate can be varied at will.

In our most successful preliminary experiments urea excretion at the dogfish gill averaged 3, 4 mgs/kg B. W./hr. when the temperature of the perfusate was 13-14°C. Excretion rises markedly with increasing temperature of the perfusate and averaged 46 mgs/kg B.W./hr in two fish perfused at 21 and 22 °C. At perfusate temperatures of 2-5°C, in spite of the known effect of cold in reducing the physical diffusion of urea, excretion was maintained at normal or slightly increased values- average 10, 5 mgs/kg B.W./hr in three fish.

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Gill excretion of injected thiourea seems to be of the same order as that of urea when each is calculated per unit difference in concentration gradient.

Thiourea excretion increases about twofold at perfusate temperatures of  $21 \cdot 22^{\circ}$ C but, unlike urea, it is markedly reduced by cold. At prefusate temperatures of 5 - 2°C thiourea excretion was 1/3 and 1/4 respectively of its average normal (13-14°C).