Asterias belongs to the group with optimum at pH 10.0 -10.2. Considering the Mytilus-diet of these animals, experiments have been started to determine whether the enzyme is of external origin from the food, or is part of the mitochondrial complex of the starfish cells.

The activity of the enzyme is characterized by the formation of an enzyme-substrate complex with the thiosulfate. At optimal cyanide level. 50mM., the *Littorina* enzyme gives a  $K = 6 \times 10^{-3}$  at 25 °C. The association of the enzyme with its other substrate, the cyanide, seems more complicated. The activity at low cyanide level suggests a  $K_m$  below 10<sup>-3</sup>, but the picture at higher cyanide levels is complicated by an inhibitory effect. Indeed, the enzyme is very sensitive towards cyanide; in the abrence of thiosulfate, low cyanide concentrations render the enzyme irreversibly inactive. For protection and activity the optimal thiosulfate concentration is 100 - 120 mM. Under normal activity-measurement conditions there is a competitive balance between the protective effect of thiosulfate and the inactivation through cyanide. However,  $v_{max}$  is identical for both substrates if measured at optimal concentration of the other substrate.

### Respiration Of The Avian Salt Gland In Media Of Various Ionic Composition

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Respiration measurements of tissue slices from the salt gland of herring gulls was undertaken in order to investigate the correlation between active ion transport and oxygen consumption. The  $Qo_2$  (ul  $O_2$  per mg dry weight of tissue per hr.) of the salt gland in Krebs phosphate media (pH 7.4) and oxygen gas phase was about 9.2, declining slowly after an initial period of 30 to 40 minutes of constant respiration. Addition of glucose to the media had no effect. Metacholine, if added to the medium, increased the  $Qo_2$  by 55%. When metacholine was added while a respiration experiment was in progress there was an immediate increase of the respiration by at least 20%.

The salt gland slices showed remarkably little response to changes in the NaCl concentration in the media. Increase by 50% of the NaCl concentration, with or without metacholine, gave no increase in respiration, and doubling the amount caused inhibition. In substituted Krebs solutions the strongest effect was shown by Na-succinate. When Na-succinate substituted all the NaCl in the medium, the Qo<sub>2</sub> was 20 to 24, or more than twice the Qo<sub>2</sub> in the Krebs phosphate solution. This increase was probably due to a large amount of succinic dehydrogenase in the glands. Other substitutes such as Na-lactate or NaBr showed about the same activity as normal Krebs medium, Qo<sub>2</sub> being around 11. Media with isotonic sucrose, and without Na, or without Cl, showed lower activity than normal Krebs medium (Qo<sub>2</sub> about 7.5 for Na less media, and 6.4 for Cl less, values close to the respiration of strophanthidin inhibited slices -  $Qo_2 = 6.8$ ). 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

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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.