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RED CELL PERMEABILITY TO ANIONS AND THE CARBONIC ANHYDRASE SYSTEM: JACOBS AND STEWARD REVISITED

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The phenomenon in which red cells suspended in NH_4Cl and $NaHCO_3$ undergo swelling and hemolysis (Jacobs and Stewart, J. Gen. Physiol. 25: 539, 1942) was reinvestigated with several goals in mind. The model is presumably one of exchange of external (medium) Cl^- for internal (red cell) HCO_3. The catalysis of $CO_2 \rightarrow HCO_3^-$ by carbonic anhydrase maintains the inner concentration of this ion, so that exchange continues, and NH_4Cl builds up within the cell. Thus the action of carbonic anhydrase and its inhibition at the very high concentrations in the living cell (some 10^5 greater than in the usual in vitro assay) could be studied, and some insight sought as to the role of the enzyme in HCO_3^- - Cl^- exchange. Finally, comparison of mammalian with elasmobranch erythrocytes was of interest.

Human red cells (0.025 ml) were suspended in 5 ml of 150 mM NH_4Cl containing concentrations of $NaHCO_3^-$ ranging from 1.5 mM to 25 mM. Over this range hemolysis time decreased from 5 minutes to 30 seconds. Solution containing the highest HCO_3^- (pH 7.6, CO_2 1mM) was chosen for further studies.

Two highly active carbonic anhydrase inhibitors were chosen, because of their rapidity of diffusion and chemical equilibrium with enzyme. Their properties are given in Acta Pharm. et Tox 17: 337, 1961. Methazolamide ($K_I = 15 \times 10^{-9}$M) and CL 13580 (2-o-chlorophenyl-thiadiazole-5-solfonamide; $K_I = 2 \times 10^{-9}$ M) gave graded increases in hemolysis time with dose. For each drug, a plateau of hemolysis time of 22-26 minutes was reached; further addition of inhibitor produced no effect. This "complete inhibition" was achieved with about 1600 μM methazolamide and 160 μM CL 13580. It is of interest that this latter figure is very close to the concentration of carbonic anhydrase in human red cells (Biochem Pharm. 6: 21, 1961), and it may be calculated that for a drug of this K_I essentially no excess is necessary for "complete inhibition," or strictly, inhibition greater than 99.9%. This experiment, then, yields the exchange rate in the absence of carbonic anhydrase, and it remains to be found whether this depends entirely on the uncatalyzed reaction $CO_2 \rightarrow HCO_3^-$, or whether other mechanisms may enter.

Similar experiments were done with dogfish red cells, except that 500 mM NH_4Cl was used, again with 25 mM $NaHCO_3$. Hemolysis occurred in 1 minute with 0.2 ml whole blood, the difference between this and the values for human blood reflecting in part the lower concentration of enzyme in the fish. Graded doses of CL 13580 increased the hemolysis time (16 μM yielded 10 min; 80 μM yielded 26 min) but in most experiments higher doses (160 μM to 800 μM) resulted in no hemolysis even after 80 minutes. Unlike human cells, this system could not operate in the absence of carbonic anhydrase.

The ultimate mechanisms of these phenomena are rather complex, but we have succeeded in measuring the rate in human cells in the absence of enzyme, and at the same time showing the stoichiometry between inhibitor and enzyme in the intact cell. It is surprising that the uncatalyzed or inhibited reaction proceeds much less readily (if at all) in the fish cells. The data suggest that in the human there is another receptor for CO_2 (perhaps hemoglobin) which provides some source for HCO_3^- in the absence of enzyme. If such a receptor is absent or much reduced

in the fish cells, and carbonic anhydrase is inhibited, the system cannot move, because conditions are the same inside and outside the cell. These speculations are set down as incentive to further study.

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UREA SPACE IN Squalus acanthias

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Urea is an important chemical factor in osmotic regulation in elasmobranchs. High concentrations of urea found in body fluids of these species are apparently related to very low extrarenal and renal loss of metabolically generated urea. In addition, certain elasmobranch tissues appear to have special mechanisms for urea transport. In mammals, it is generally accepted that cell membranes are freely permeable to urea, and that final volume distribution of urea is equal to total body water. Little information is available concerning the volume of distribution of urea in elasmobranchs.

The volume of distribution of C^{14} urea was measured in 9 animals and compared to total body water as measured by n-acetylantipyrine (NAAP), a substance known to distribute itself throughout total body water. The volume of distribution was also compared to the volume of distribution to chemical analogues of urea, methylurea and thiourea. The C^{14} urea space was found to be 64% of total body weight as compared to NAAP space of 66% total body weight. These values were not significantly different. Methylurea and thiourea spaces were essentially the same as urea space. The correspondence between urea and NAAP spaces suggests equality of urea concentration across most cell membranes of this animal at equilibrium.

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STUDIES OF LACTATE METABOLISM IN Squalus acanthias

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Measurements of blood lactate concentrations in the spiny dogfish, Squalus acanthias, have demonstrated concentrations that are substantially higher than those reported in other vertebrates. The mechanism of this high blood lactate concentration was investigated. In one phase of the study arterial lactate concentration was measured in 3 groups of animals: group A - 12 fish maintained in a salt water pen in the laboratory; group B - 12 fish maintained in live cars floating in the bay; and group C - 11 fish sampled on the boat within one minute of capture by hand line. The values were as follows: group A, 9.1 ± 5.3 mEg/L; group B, 6.9 ± 4.3 mEg/L; group C, 1.0 ± 0.2 mEg/L. The resting endogenous blood lactate concentrations of dogfish in their natural habitat appear to be similar to those of man.

The ability of elasmobranch gill to excrete lactate was investigated. Lactate concentrations