(Quart. J. Med. 9:107, 1940). Following a single dose of succinyl choline, apnea in the dogfish lasts 45-75 minutes. This corresponds to the prolongation of apnea found in pseudocholinesterase deficient patients and contrasts with the maximum 4 minute apnea seen in normal man. Dogfish serum contains approximately 1/15 the activity of pseudocholinesterase per ml of serum found in normal man which correlates well with the prolongation of apnea in this species.

These studies indicate that the dogfish is an excellent model for the study of the mechanism of action and therapy of pseudocholinesterase deficiency. In addition, it offers that comparative pharmacogenetics is potentially a useful new discipline.

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METHODOLOGIC PROBLEMS IN THE STUDY OF CATION TRANSPORT IN ERYTHROCYTES OF THE DOGFISH, Squalus acanthias

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The use of dogfish erythrocytes in metabolic and transport studies presents a number of technical and biological problems. These include the following:

1. The dogfish erythrocyte is prone to rapid hemolysis with time. Hemolysis is increased markedly at temperatures greater than 20° C. In addition, mechanical agitation may cause cells to lose nuclei and to gragment. This problem can be met by rapid processing of cells, utilizing fairly short-term studies, minimal agitation and careful temperature control.

2. The number of cells available for a given study is limited by the low hematocrit of the animal and the intense hemolysis that may result when erythrocytes obtained from more than one animal are mixed. This hemolysis presumably occurs as a result of blood group incompatibility. This problem may be met by designing individual studies in which the upper limit of blood required is approximately 20 ml of packed red cells.

3. The high plasma $[Na^+]$ of approximately 250 mEq/L makes the calculation of intracellular sodium concentration sensitive to minor variations in the hematocrit of red cell suspensions. Since an estimate of intracellular Na^+ is required for the determination of specific activity in the calculation of Na^+ efflux, this becomes a major problem when efflux is measured by determining the rate of increase of isotopic Na in bathing media. This problem cannot be solved by washing the cells in Na^+ free media using $MgCl_2$ or $CaCl_2$ since these media appear to affect the structural integrity of the dogfish erythrocyte, causing them to adhere in a gelatinous fashion and producing sequential loss of Na^+.

This problem may be met by measuring the rate of decline of counts from radio labeled cells rather than the rate of increase of counts in the suspending media. Unlike mammalian cells, the rate of Na efflux is sufficiently high so that accurate measurements are possible and the problem of hematocrit is minimized.

4. The calculation of Na flux requires a determination of specific activity. Specific activity may be defined as: $\dfrac{\int_0^t Na^{22} \text{ as counts/min/gm RBC} \cdot dt}{[Na^+] \text{ in mEq/gm RBC}}$

The numerator of the equation initially changes rapidly in a non-linear fashion with time presumably because of:

a) exchange diffusion; and

b) high rate of Na^+ efflux.

However, between the second and fourth hour, the change in specific activity becomes more or less linear so that the mean of specific activities at 2 hours and 4 hours may be used as an acceptable approximation for calculating specific activity. Under these circumstances, back diffusion of Na^{22} (Na influx) may be fairly substantial so that efflux measurements represent net efflux and the measured transport must be considered as an estimate of the minimum flux rate.

It is clear that studies involving metabolism and cation transport in this erythrocyte must be performed under carefully controlled and specified conditions and that an absolute flux rate cannot be determined.

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REGULATION OF VENTRICULAR FLUID POTASSIUM CONCENTRATION IN Squalus acanthias

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Potassium concentration, $[K^+]$, of mammalian cerebrospinal fluid is regulated at 2.8 ± 0.2 meq/L, independent of plasma $[K^+]$, by active transport systems located in membranes separating cerebrospinal fluid and blood (Cserr, Am. J. Physiol. 209:1219-26, 1965). Considerably higher values than 2.8 meq/L have recently been obtained for $[K^+]$ of ventricular fluid (VF) in three species of elasmobranchii. Mean $[K^+]$ (in meq/L) reported for VF and plasma, respectively, were 7.8 and 6.6 in the spiny dogfish (Maren, Comp. Biochem. Physiol. 5:193-200, 1962), 5.2 and 5.5 in the lemon shark, and 5.5 and 4.2 in the nurse shark (Oppelt, Adamson, Subrod and Rall, Comp. Biochem. Physiol. 17:857-66, 1966). Similarity between $[K^+]$ of VF and plasma in each of the three species suggests that elasmobranchii may lack active transport systems for regulating K^+ exchange between VF and plasma. Our experiments were designed to test this hypothesis. Results indicate that $[K^+]$ obtained previously for dogfish VF were in error and that VF $[K^+]$ is regulated close to 3 me[/L in the dogfish as it is in mammals.

Mean (±SE) normal $[K^+]$ (in meq/kg H_2O) of dogfish plasma, VF and extradural fluid (EDF) were 4.1 ± .12 (N = 23), 3.5 ± .08 (N = 5) and 3.4 ± .12 (N = 6), respectively. Only samples obtained within two to three minutes after removal of the fish from water were used in determining mean values. In contrast, mean $[K^+]$ (in meq/kg H_2O) of plasma, VF and EDF obtained from fish kept out of water for seven to fifteen minutes before sampling were 6.0 ± .11 (N = 4), 5.8 (4.8, 6.8) (N = 2), and 3.2 ± .09 (N = 3), respectively. Mean $[K^+]$ of control plasma, drawn before the fish were taken out of water, was normal, 4.1 ± .09 (N = 4). These results illustrate the importance of rapid sampling and suggest that failure to collect samples immediately after fish were removed from the water may explain high potassium concentrations of VF and plasma obtained by Maren and Oppelt et al. Murdaugh et al. (this Bulletin 5(1):14-15, 1962) also reported a low value (3.3 meq/kg H_2O) for plasma $[K^+]$.