and beta blocking agents, Dibenzyline (phenoxybenzamine) and Inderal (propranolol), 0.5-4 mg/kg, were given IV and the response to epinephrine was tested at times ranging from ten minutes to three days after blockade with one or both of the blocking agents. It was found that the fish given blocking agents did not differ in their response to epinephrine from the fish given saline controls regardless of variation in dosage, time of challenge with epinephrine or blocking agent(s) used.

In a further attempt to define alpha and beta receptors, phenylephrine, isoproteronol, norepinephrine and dopamine, 10-100 μg/kg were given IV to female dogfish. The dogfish gut was unresponsive to isoproterenol, norepinephrine, and dopamine. Phenylephrine had a stimulatory action on the distal stomach similar to that of epinephrine and this response was not blocked by the alpha blocking agent, phenoxybenzemine. From these experiments, it seems that the excitatory action of epinephrine on the GI tract of Squalus acanthias is not mediated by receptors analogous to the alpha and beta receptors which mediate the inhibitory response of epinephrine in the mammal.

An interesting aspect of the action of epinephrine on the dogfish gastrointestinal tract is that if a second equivalent dose of epinephrine is given 30 to 45 minutes after the initial dose, the second dose elicits a much smaller or at times, a negative response. One hypothesis which could explain this phenomenon is that after the initial stimulation, the smooth muscle may become hyperpolarized and therefore refractory to excitation by epinephrine. The energy for this "hyperpolarization" may come from an epinephrine-induced increase in the rate of glycogenolysis. Since theophylline also acts to increase glycogenolysis by its phosphodiesterase inhibition, one would expect that the gastrointestinal tract would become "hyperpolarized" or refractory to epinephrine after theophylline.

To test this hypothesis, aminophylline (25 mg/kg) was given to eight dogfish both male and female. Epinephrine (100 ug/kg) was given IV 30 to 50 minutes later. Aminophylline had no excitatory action itself on the gastrointestinal tract but it did totally inhibit the excitatory response to epinephrine.

From these preliminary experiments, we would like to suggest that the tachyphylactic response to epinephrine described above may be due to a hyperpolarization of the smooth muscle. The energy for the hyperpolarization may be derived from the increased glucose oxidation and ATP production secondary to an epinephrine-induced increase in the rate of glycogenolysis.

1967 #24

CATION TRANSPORT IN THE ERYTHROCYTE OF THE HARBOR SEAL (Phoca vitulina)

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Eadie and Kirk (Austral. J. Sc. 15:26, 1953) reported that potassium concentration in the seal erythrocyte is low. A preliminary study in three harbor seals confirmed this finding showing little chemical concentration difference for K^+ or Na^+ between red cell water and plasma water and stimulated a more detailed study of cation transport in the erythrocyte of this species. The small differences in red cell and plasma Na^+ and K^+ concentrations minimizes the error produced by trapping and provides a model that can be studied with less artifact produced by

errors in determined red cell electrolyte concentrations. In addition, the possibility of passive distribution of K^+ in this red cell was considered. Blood was obtained from six seals, and plasma and erythrocytes were processed for the determination of H_2O, Na^+, K^+, and Cl^- contents (J. Cell. & Comp. Physiol. 64:409, 1964). These values are presented in Table 1. Water content of the red cells was 62.6 ± 0.5 (S.D.)%. Plasma water content was 91.6 ± 0.3 (S.D.)%.

Table 1

mEq/L plasma H_2O	mEq/L red cell water	Concentration ratio
Na^+ 161 ± 4 (S.D.)	147 ± 8 (S.D.)	$\frac{Na^t_e}{Na^t_i}$ 1.10 ± 0.04 (S.D.)
K^+ 4.2 ± 0.3 (S.D.)	8.3 ± 0.6 (S.D.)	$\frac{K^t_e}{K^t_i}$ 0.50 ± 0.03 (S.D.)
Cl^- 116 ± 3 (S.D.)	70 ± 3 (S.D.)	$\frac{Cl^-_i}{Cl^-_e}$ 0.61 ± 0.02 (S.D.)

Assuming that Cl^- is in thermodynamic equilibrium across the plasma membrane, both Na^+ and K^+ appear to be distributed against electrochemical gradients. However the narrowness of these gradients suggested low energy requirements for Na^+ and K^+ transport.

Accordingly Na^+ efflux studies using Na^{22} and K^+ influx studies using K^{42} were performed at 39° C using washed seal erythrocytes. Sodium efflux averaged approximately 18.9 mEq/Kg red cells hr and potassium influx was approximately 0.6 mEq/Kg red cells/hr. In addition, ouabain 10^{-4}M did not measurably alter Na^+ efflux or K^+ influx in seal erythrocytes.

The seal erythrocyte appears to be an important model for studying the coupling of energy generation to cation transport in a cell with relatively low energy requirements for this function.

1967 #25

BLOOD VOLUME DURING DIVING IN THE HARBOR SEAL (Phoca vitulina)

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The oxygen dependency of the central nervous system of the seal determines that the duration of diving is limited by the oxygen stores available at the time of diving. The magnitude of the blood volume, with its contained red cell mass, is an important determinant of available oxygen stores. In addition, sequestration of blood during diving could limit the available oxygen stores by preventing the oxygen in the sequestered volume from reaching the oxygen dependent central nervous system.

Studies were designed to determine the blood volume of the seal and to determine if a major fraction of this volume is sequestered from the circulation during diving. Blood volume during and following diving was determined in four young male harbor seals. Blood was obtained