terial constriction was capable of sustaining diving for periods longer than compatible with life when atropine has prevented normal response to diving.

The blood pressure during cardiac pacing was of interest. During diving the blood pressure remained normal or slightly elevated if the rate approximated non-diving rates, 130-150/minute. If the rate was increased to 180/minute, blood pressure and pulse pressure decreased progressively. The blood pressure could be increased or decreased at will by varying the heart rate with the pacer. During rapid pacing an occasional pacer induced stimulus failed to cause a ventricular response. When this occurred, blood pressure and pulse pressure were increased with the next ventricular contraction. These findings can be explained by a limited return of blood to the right side of the heart during diving.

With this restricted return of blood to the right heart, there would occur a decrease in diastolic ventricular filling if the heart rate remained fast. Bradycardia, however, would maintain diastolic ventricular filling by increasing the diastolic period, presumably sustaining ventricular stroke volume and ejection force.

The arterial constrictor response limits oxygen consumption by non-critical areas thus conserving the available oxygen stores for oxidative dependent cerebral metabolism during diving. The decrease in cardiac output serves to prevent an unduly high cerebral blood flow during the period of a restricted arterial perfusion bed. The bradycardia sustains an effective ventricular ejection force and aortic perfusion pressure during the period of limited return of blood to the heart.

1966 #10

PSEUDOCHOLINESTERASE ACTIVITY IN THE DOGFISH, Squalus acanthias

Carroll E. Cross, Eugene D. Robin, H. V. Murdaugh, J. B. L. Gee, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Pharmacogenetics is a relatively new area in biology which involves the study of genetic processes revealed by the use of newly synthesized pharmacologic agents. An important disorder which falls into this area is the heritable disorder, pseudocholinesterase deficiency. Patients with this disease manifest prolonged apnea when exposed to the pharmacological agent, succinyl choline. This contrasts with the brief duration of apnea found in normal subjects. The prolongation of paralysis is caused by a deficiency of the enzyme pseudocholinesterase which hydrolyzes succinyl choline. With enzyme deficiency, succinyl choline persists at neuromuscular junctions and paralysis persists.

This study was prompted by the known absence of serum albumin in Squalus acanthias, knowledge that mammalian pseudocholinesterase migrates with the albumin fraction in electrophoresis, and an oral communication suggesting absence of psuedocholinesterase activity in dogfish serum.

Dogfish were artificially ventilated with flowing sea water by means of cannulae inserted into the spiracles. Succinyl choline was administered intravascularly by a single dose using the same quantity per kilo body weight effective in man. The duration of paralysis was measured as the length of time the opercular reflex was lost (Nature 211:1187, 1966). Serum pseudocholinesterase activity was measured in dogfish serum by a modification of the technique of McArdle

(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

Carroll E. Cross, James Theodore, H. V. Murdaugh, and E. D. Robin, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

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: $\dfrac{\int_0^t Na^{22} \text{ as counts/min/gm RBC} \cdot dt}{[Na^+] \text{ in mEq/gm RBC}}$