These results do not provide evidence for any reflex vasomotor changes induced by spinal cord stimulation. The results are fully explainable by the effect of the cardiac inhibition and the contraction of the trunk skeletal muscle mass which exerted torsion and compression effects on the blood vessels. The cardiac inhibition cannot be explained by a vagal reflex since it occurred in the absence of the brain and observations on the stimulus strength to response indicate that the inhibition is not due to the spread of stimulus through electrolyte conducting medium.

This research was supported by a grant from the New Jersey Heart Association and its Warren County affiliate.

1971 #35

PURINE METABOLISM IN PRIMITIVE ERYTHROCYTES

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Over the past eight years this laboratory has examined various aspects of the purine metabolism of human erythrocytes. Several enzymes of purine nucleotide metabolism have been described in some detail and new methods have been developed employing high-pressure liquid chromatography for the examination of patterns of nucleotides in small amounts of tissues (J. of Chromatography, 52, 257, 1970). Recently we have undertaken a comparative study of the erythrocytes of various terrestrial vertebrates. The present report extends these studies to a preliminary examination of some aspects of purine metabolism of the nucleated erythrocytes of several of the marine species. Since the cyclostomes and elasmobranchs are among the most primitive vertebrates, particular attention was paid to erythrocytes from the hagfish *(Myxine glutinosa)* and the dogfish *(Squalus acanthias).*

Figure 1 shows the nucleotide patterns obtained by high-pressure liquid chromatography of neutralized trichloracetic acid extracts of erythrocytes from man *(Homo sapiens)*, saltwater eel *(Anguilla rostrata)*, sand dab *(Lophopsetta maculata)*, skate *(Raja erinacea)*, and the hagfish *(Myxine glutinosa)*. In comparison with other tissues examined by this laboratory the nucleotide pattern for

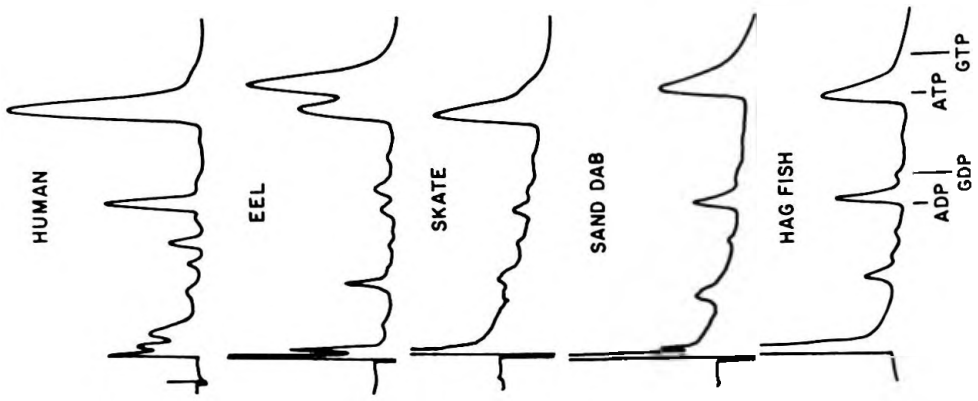

Figure 1: Washed freshly prepared erythrocytes were extracted by vortexing into 2 volumes of 12% TCA. The extracts were neutralized with Tris prior to analysis in a Varian LCS-1000 high-pressure liquid chromatography apparatus by the method described in Journal of Chromatography, 52, 257, 1970. 20 μl aliquots were examined.

Table 1

Table 1: The enzymic assays were performed by methods referred to in Biochemical Pharmacology, 20, 1341, 1971. Isoelectrofocusing for the identification of NDP kinase enzymes was performed as in Figure 2 by the method described in Biochemistry, 10, 2139, 1971.

ACTIVITIES OF ENZYMES OF PURINE METABOLISM IN VARIOUS SPECIES

Species	Enzyme Activity μmolar units/ml cells					NDP kinase Isozymes (Isoelectric points)
	PNPase	AMP kinase	GMP kinase	HGPRT	NDP kinase	
	(a)	(a)	(a)	(c)	(a)	(b)
Human	13.5	1.3	0.35	0.3	76.0	5.4, 5.8, 6.3, 6.8, 7.3, 8.3
Harbor Seal	N.D.	1.7	0.13	N.D.	25-45	4.5, 5.5, 6.1, 6.6, 7.3
Saltwater Eel	N.D.	0.6	0.4	N.D.	40	5.0, 5.8, 6.3, 7.0, 8.2
Dogfish	0.1	1.9	0.05	N.D.	25	5.1, 5.3, 5.5, 5.9, 6.5, 6.9, 8.2
Hagfish	0.5	0.5	N.D.	N.D.	19	5.2, 6.0, 6.3, 7.3

The abbreviations used are: PNPase, purine nucleoside phosphorylase (purine nucleoside: orthophosphate ribosyl transferase, E.C. 2.4.2.1.); AMP kinase, adenylate kinase (ATP:AMP phosphotransferase, E.C. 2.7.4.3.); GMP kinase, guanylate kinase (ATP:GMP phosphotransferase, E.C. 2.7.4.8.); HGPRT, hypoxanthine-guanine phosphoribosyl transferase, E.C. 2.4.2.8.; NDP kinase, nucleoside diphosphokinase (ATP:nucleoside diphosphate phosphotransferase, E.C. 2.7.4.6.)

N.D. - Activity not detected at levels greater than 0.05 μmolar units per ml of erythrocytes

(a) - Values reported in Biochemical Pharmacology, 20, 1341, 1971

(b) - Values reported in Biochemistry, 10, 2139, 1971

(c) - Assayed by an isotopic procedure developed by C.M. Kong

human erythrocytes is relatively simple, containing principally the adenine nucleotides and only relatively lesser amounts of nucleotides of guanine, uracil, or cytosine. As seen in Figure 1, the erythrocytic nucleotide patterns for most of the marine species examined are also relatively uncomlicated. However, a most unusual nucleotide pattern was observed with eel erythrocytes. The pattern shown in Figure 1 is from erythrocytes of a saltwater eel, but essentially identical patterns were seen with erythrocytes from freshwater eels. Here, there is a strikingly large peak of GTP; in fact, the concentration of GTP in the eel erythrocytes is the greatest of any tissue that we have examined to date. Similar patterns were obtained with whole blood or with washed erythrocytes carefully freed of plasma and buffy coat. An intriguing possibility suggested by this observation is that the eel erythrocyte may use GTP rather than ATP in ion pumping and has a GTPase rather than ATPase activity.

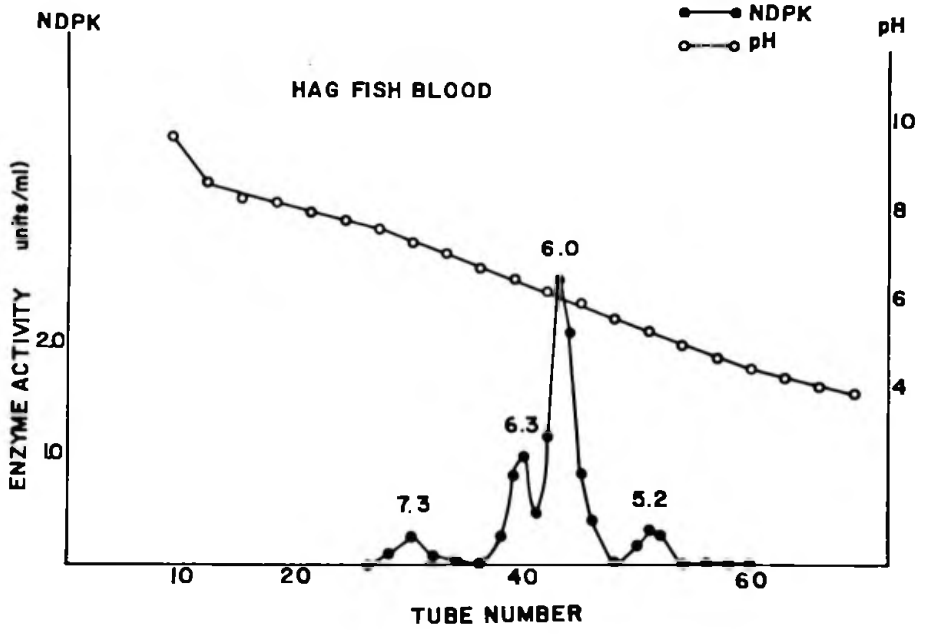

Figure 2: Isoelectrofocusing profile of nucleoside diphosphokinase of hagfish hemolysate. Dialyzed, centrifuged hemolysate from about 0.6 ml of hagfish erythrocyte containing about 12 units of nucleoside diphosphokinase were electrofocused for 60 hours at 600 V in a 110 ml isoelectrofocusing column containing 1% Ampholine (pH 3-10) in a sucrose gradient. The contents of the column were collected in fractions of 1 ml and assayed for enzymic activity by the coupled pyruvate kinase-lactate dehydrogenase method (Journal of Biological Chemistry, 241, 271, 1966).

Table 1 is a compilation of some of the enzymic activities measured in the nucleated erythrocytes of several marine species in comparison with activities of related enzymes in human and harbor seal *(Phoca vitullina)* erythrocytes. Particularly noteworthy is the high activity of nucleoside diphosphokinase (NDP kinase) found in the erythrocytes of all of the species examined. Strict comparison of these relative enzymic activities is not possible because assays with human erythrocytes were measured at 30° whereas some of the determinations in other species were made at room temperature. This laboratory has recently reported the occurrence of marked heterogeneity of NDP kinase in the human erythrocyte and has identified at least six isozymes with isoelectric points varying from 5.4 to 8.3 (Biochemistry 10, 2139, 1971). It was of considerable interest to find that similar marked electrophoretic heterogeneity occurs in the erythrocytes of all of the species examined.

Figure 2 presents the results of an isoelectric focusing experiment with the centrifuged, dialyzed supernatant fluid of hemolyzed hagfish erythrocytes. Here it was found that four distinct isozymes of NDP kinase occur with isoelectric points of 5.2, 6.0, 6.3 and 7.3. The true function in erythrocytic metabolism of these isozymes of NDP kinase is not apparent, but it seems possible that more detailed comparative studies with a number of species may provide useful insights.

This work was supported by USPHS Grant CA 07340 and ACS Grant T-94.

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UREOSMOTIC REGULATION BY THE KIDNEYS AND GILLS OF THE LITTLE SKATE, *Raja erinacea*

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The renal and branchial excretion of urea were studied under various experimental conditions: in skates adapted to sea water or 50 percent sea water and in skates acutely transferred to 75 percent sea water. Urine collections were made by means of a cloacal catheter attached to a toy balloon. The catheter was generally sewn in place the day before the experiment. Branchial efflux of urea was studied with ^{14}C-urea. The skates (with cloacal catheters) were placed in aerated aquaria containing a known volume of external medium (about 4 liters). The branchial flux was calculated by dividing the cpm appearing in the external medium by the specific radioactivity in plasma of fish. The results are shown in Table 1. Two groups of fish were used. One group was studied in sea water and again after acute transfer to 75 percent sea water. The second group was gradually (5 days) adapted to and studied in 50 percent sea water. Osmotic pressures of the external media were