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Sodium-Potassium Dependent Adenosine Triphosphatase of Mammalian Reticulocytes and Mature Red Blood Cells.* (30770)

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It is known that sodium is required for the active transport of certain aminoacids by intestinal mucosa(1), thymocytes(2), human leukocytes(3) and mammalian reticulocytes(4). In addition wherever sodium dependency is present potassium is required for maximal transport rates(3,4). These observations raise the fundamental question of whether active amino acid transport is linked directly or indirectly to Na-K-dependent strophanthin-inhibited, membrane adenosine triphosphatase (ATPase). Post has postulated a direct role of this enzyme in cationic transport(5). In a recent study in mammalian red blood cells it was found that, in contrast to the reticulocyte, the mature erythrocyte had a markedly reduced or absent capacity to concentrate aminoacids against a chemical gradient(4). Two possible explanations were considered for the altered transport pattern in the mature red cell: 1. The loss of capacity to concentrate aminoacids could be due to cessation of protein synthesis concomitant with red cell maturation. This possibility was examined by studying the effect of puromycin; this drug caused complete inhibition of protein synthesis in the reticulocyte without affecting its concentrative amino-acid uptake indicating that protein synthesis *per se* was not the responsible factor(4). 2. An alternative explanation could be a change in the level of activity or the distribution of Na-K-dependent ATPase in the mature red cell. While it has been demonstrated that the mature erythrocyte retains an active Na-K-dependent membrane ATPase(5) it is not known if the intacellular organelles of reticulocytes (which are lost upon maturation) contain an active Na-K-dependent ATPase which plays a key role in the transport and intracellular distribution of aminoacids. In the present study, this question has been examined by assaying the ATPase activity in mammalian reticulocytes and mature red blood cells. Both total and Na-K-dependent ATPase activities were found to be directly proportional to the percentage of reticulocytes in the various red cell preparations tested.

Materials and methods. Reticulocytosis was induced in New Zealand white rabbits and in Holtzman albino rats by repeated removal of 20-50 ml of blood from rabbits and 3-5 ml from rats by intracardiac puncture at 1-2 day intervals. Blood was collected in EDTA (final EDTA concentration 0.005 M). Each animal was given 10 mg of iron dextran (Imferon®) per kg body weight intramuscularly to compensate for iron loss. To obtain a reticulocyte-rich and reticulocyte-poor preparation from the same blood sample, the sample was centrifuged at 2000 × *g* for 2

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TABLE I. Total, Na-K-Dependent and Strophanthidin-Inhibited ATPase of Rabbit Erythrocyte Membranes.

Exp	Red cell layer	% reticulocytes	ATPase activity (mμmoles Pi/hr/mg dry wt) Control*	Na^+-K^+	Na^+-K^+-stroph.	Na^+-K^+ ATPase	Stroph-inhibited ATPase
1	Top	52	710	1277	782	567	495
	Bottom	5.5	196	327	222	131	105
2	Top	25	412	740	451	328	289
	Bottom	4	226	361	267	135	94

* Without Na^+ or K^+.

hours and the top one-third portion of the packed cells (reticulocyte-rich) and the bottom third (reticulocyte-poor) were saved.

Red cells were washed 3 times with 8 volumes of a solution containing 0.14 M NaCl, 0.0001 M EDTA and 0.005 M Tris-Cl, pH 7.4. Leukocytes were eliminated by removing the buffy coat after each centrifugation. Hemoglobin-free red cell membranes were prepared and their ATPase activity determined as described by Parker and Hoffman (6).‡ The substrate mixture contained: 0.002 M ATP, 0.00125 M $MgCl_2$, and 0.00025 M EDTA, 0.04 M NaCl, 0.02 M KCl and 0.01 M Tris, pH 7.4. The reaction was started by the addition of 1 ml of membrane suspension to 4 ml of substrate at 37°C. Aliquots of 2 ml were taken at 0 and 60 minutes and added to 2 ml of 6% perchloric acid. Inorganic phosphate was determined in the clear supernatant solutions by the method of Fiske and SubbaRow(7). ATPase activity was expressed as mμmoles Pi liberated per hour per mg dry weight.

ATP disodium salt was obtained from Sigma Chemical Co., St. Louis. Strophanthidin was purchased from Mann Research Laboratories, New York.

Results. APTase activity of rabbit erythrocytes. The ATPase activities of reticulocyte-rich and reticulocyte-poor portions obtained from 2 red cell preparations are shown in Table I. Both total ATPase (activity with Na^+ and K^+) as well as Na-K-dependent activity were considerably higher in the reticulocyte-rich red cell layers; the Na-K-dependent ATPase activity in a preparation containing 52% reticulocytes was approximately 4-fold that in a preparation with 5.5% reticulocytes. Addition of strophanthidin in a final concentration of 2.5×10^{-4} M resulted in 80-90% inhibition of the Na-K-dependent ATPase.

The total and strophanthidin-inhibited ATPase activities in red cell preparations with varying reticulocyte concentrations are shown in Fig. 1. A clearcut direct relationship is noted between degree of activity and percentage of reticulocytes.

ATPase activity of rat erythrocytes. A simi-

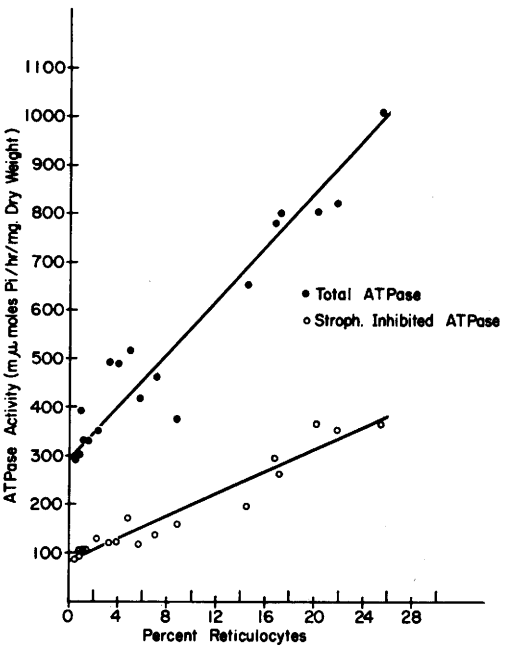

FIG. 1. Relation of total and strophanthidin-inhibited ATPase activity to percentage of reticulocytes in rabbit erythrocyte preparations.

‡ When reticulocyte membranes, prepared in this manner, are suspended in isotonic medium and stained with brilliant cresyl blue reticulum is found in the hemoglobin-free ghosts.

TABLE II. Total, Na-K-Dependent, and Strophanthidin-Inhibited ATPase of Rat Erythrocyte Membranes.

		ATPase activity (mμmoles Pi/hr/mg dry wt)				
Red cell layer	% reticulocytes	Control*	Na^+-K^+	Na^+-K^+-stroph.	Na^+-K^+ ATPase	Stroph-inhibited ATPase
Top	20.2	520	1196	919	676	277
Bottom	6.2	345	785	640	440	145
Mature RBC	1.2	306	680	566	374	114

* Without Na^+ or K^+.

lar relationship was observed between ATPase activity and reticulocyte concentration in red cell preparations obtained from the rat (Table II). On a per unit weight basis rat erythrocytes had significantly greater ATPase activity than rabbit erythrocytes. It is of interest also that Na-K-dependent ATPase of rat erythrocytes was inhibited only 30-40% by 2.5×10^{-4} M strophanthidin. This degree of inhibition could not be magnified by varying the concentrations of Na^+ or K^+.

Discussion. The role of Na-K-dependent strophanthin-inhibited membrane adenosine triphosphatase in the transport of nonelectrolytes has not been ascertained. At least two observations suggest than an intimate relationship exists between this enzyme and the transport of aminoacids: 1. The active transport of some aminoacids is sodium-dependent and requires both Na^+ and K^+ for maximal stimulation(3,4). 2. The active transport of sodium-responsive aminoacids is also markedly inhibited by strophanthin(3). The loss of Na-K-dependent ATPase from the red cell upon maturation concomitant with loss of capacity in the mature red cell to concentrate aminoacids(4) lends further support to this hypothesis.

The greater level of ATPase in the reticulocyte could reflect a higher concentration of enzyme in the membrane of this cell as compared to that of mature erythrocyte or the presence of ATPase in the intracellular reticulum of the reticulocyte which is lost upon maturation. Attempts to separate the reticulum from the membranes, in order to assay for ATPase separately, have not been successful.

The relative resistance of Na-K-dependent ATPase of rat red cells to strophanthidin is of interest. Duggan(8) has recently described an ATPase from frog sartorius microsomes which was stimulated by K^+ alone as well as Na^+ alone without synergistic effect from both cations and the increase in activity was ouabain-insensitive. While Na-K-dependent ATPase of rat red cells is much less sensitive to strophanthidin than the enzyme from rabbit red cells, the enzyme from both sources is stimulated only about 15% by sodium alone, 60-80% by potassium alone and 200-250% by both cations. These findings and those of Duggan indicate that inhibition by strophanthin is not a uniform property of cation-stimulated ATPase from all sources but that both the cationic specificity as well as the sensitivity to strophanthin may vary with the source of enzyme.

Summary. The level of Na-K-dependent ATPase was found to be considerably higher in reticulocytes than in mature erythrocytes. The fall in enzyme activity in the red cell upon maturation occurring concomitantly with loss of capacity to concentrate aminoacids lends support to the hypothesis that Na-K-dependent ATPase plays an important role in the active transport of aminoacids.

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