globin was present, presumably because of the hepatectomy. Furthermore, since donor-type haptoglobin was present, it seems clear that the liver is at least a site of storage or activation of haptoglobin which can be released under appropriate conditions.

Haptoglobin-hemoglobin complexes are cleared from the circulation by the liver(7), but the subsequent fate of the haptoglobin molecules is not known. The present investigation suggests that the haptoglobin is degraded after sequestration by the liver, since the haptoglobin which appeared following the transplantation was of donor specificity and not heterogeneous as would be expected if bound recipient haptoglobin and bound haptoglobin from the transfused blood were released by the transplanted liver.

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Effect of Na+ and K+ on the Adenylate Kinase of Human Erythrocytes.* (29364)

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The presence of a Na+, K+-activated adenosinetriphosphatase (ATPase) in erythrocyte membranes has been reported by several investigators(1-3). Evidence suggesting the participation of this enzyme in the process of monovalent cation selectivity of erythrocytes has been reviewed(4). The various enzyme preparations described thus far all release two equivalents of phosphate from each adenosinetriphosphate (ATP) molecule. A possibility which could account for the observed release of 2 phosphates is the presence of an adenylate kinase in the ATPase preparations. This investigation was undertaken to determine: (1) if adenylate kinase is present in the erythrocyte membrane preparations and (2) the effect of Na+ and K+ on whatever enzyme activity is responsible for release of the second phosphate of ATP. A present finding shows that contrary to a previous report(5), kinase activity can be detected in membranes prepared by several different methods and that this enzyme is also activated by monovalent cations. After the completion of this work Post and Sen(6) also reported the presence of adenylate kinase in erythrocyte membrane preparations used for the study of ATPase. They did not, however, study the effect of Na+ and K+ on the kinase activity.

Materials and methods. Sodium salts of ATP and adenosinediphosphate (ADP) were obtained from Sigma Chemical Co. (St. Louis), and converted to the tris salts by passage through columns of cation exchange resin Dowex 50 in tris form. Adenosine-5′-monophosphate (AMP) and yeast hexokinase, either crystalline or Type IV, were also purchased from Sigma. All other chemicals used were of reagent grade quality.

Adenylate kinase of various preparations was measured, according to Colowick and Kalckar(7), by incubating aliquots with 5 μmoles of ADP, 100 K.M. units of hexokinase, 5 μmoles of Mg^{++}, 100 μmoles of tris-

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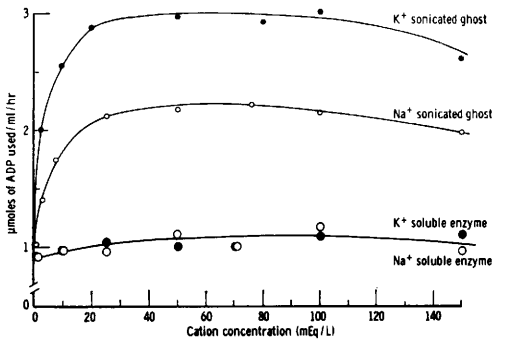

FIG. 1. Effect of varying concentrations of Na^+ and K^+ on soluble and membrane-bound adenylate kinase of human erythrocytes.

HCl (pH 7.4), and 9 mg of glucose, at 37°. Total volume was 2.5 ml. The reaction time was chosen so that no more than 30% of the ADP was used up in any experiment. During this phase of the reaction, the velocity was a linear function of time. The reaction mixtures were deproteinized by addition of 1.5 ml of 8% $HClO_4$. After centrifugation, aliquots of the supernatant were boiled in a water bath for 25 minutes. Preliminary experiments had shown this to be the optimal hydrolysis time for determination of the "easily hydrolyzable phosphate" of ADP under the conditions employed. Inorganic phosphate and ATPase activity were determined as previously described(3).

Membrane-free erythrocyte hemolysates were prepared as follows: Saline-washed human erythrocytes, from out-dated blood-bank blood, were hemolyzed in 7 volumes of cold distilled water. The hemolysate was centrifuged at 18000 $\times$ *g* for 30 minutes. The supernatant was used as the source of soluble adenylate kinase. Ghosts (intact erythrocyte membranes) and sonicated ghosts were prepared as reported before(3).

Results and discussion. The presence of a soluble adenylate kinase in erythrocytes was reported by Cerletti and Bucci(5). With the assay conditions used by these investigators, the erythrocyte membranes did not show any kinase activity. We first prepared membranes by 6 different methods(1-3, 8). All contained some adenylate kinase activity. Further studies were done only with the two preparations referred to in *Methods*.

Since it was possible that the activity of the membrane preparations was due to some soluble enzyme trapped with them, ghosts and sonicated ghosts were repeatedly washed with 0.2 M tris buffer. In several experiments buffers at different pH values ranging between 7.0 and 8.2 were used. Most of the kinase activity remained with the membranes after repeated washings. This difficulty of the removal of a portion of the enzyme suggested the possibility that the kinase bound to the membrane may have different properties from the soluble enzyme. The possibility of the activation of the membrane-bound kinase by monovalent cations was of immediate interest and was, therefore, studied. Fig. 1 shows the effects of various concentrations of Na+ and K+ on the soluble kinase and the kinase of the sonicated ghosts. Appropriate dilutions of the two preparations were made to obtain the same range of activity per unit volume. It is evident that the cations have no effect on the soluble enzyme. Either Na+ or K+ activate the bound enzyme. At equal concentrations K+ is a better activator than Na+.

Since we had already shown(3) that the ATPase of the sonicated ghosts could also be activated by either Na+ or K+, the question arose as to whether the apparent activation of the ATPase was not due to activation of the kinase. It could be argued that in a system where both ATPase and adenylate kinase are present, if ATPase is inhibited by ADP (which is not unexpected), the activation of kinase by Na+ or K+ would enhance the removal of ADP and thus result in an apparent stimulation of the ATPase activity. This possibility was ruled out when the effects of Na+ and K+ on the ATPase and adenylate kinase of the sonicated ghosts and intact ghosts were compared. The results are shown in Table I. It can be seen that while the kinase of both the sonicated and intact ghosts are activated by either Na+ or K+, the ATPase of the intact ghosts is not affected by the presence of either ion alone. If the activation of the ATPase were indeed due to that of kinase, it would be expected that the ATPase of the intact ghosts would also be stimulated by

TABLE I. Effect of Na+ and K+ on Adenylate Kinase and ATPase of Intact and Sonicated Erythrocyte Membranes.

Enzyme source	mM Na+	mM K+	Adenylate kinase, μmoles of ADP used/mg dry wt/hr	ATPase, μmoles Pi/mg/hr
Intact ghosts	0	0	.50	.080
	80	0	.71	.082
	0	30	.79	.078
	80	30	.78	.142
Sonicated ghosts	0	0	.46	.18
	80	0	.70	.24
	0	30	.77	.26
	80	30	.77	.34

either Na+ or K+. Comparison of the effects of the simultaneous presence of Na+ and K+ on various activities, also shown in Table I, further strengthen the point that the activation of the kinase is not responsible for the stimulation of ATPase. While a synergistic effect of Na+ and K+ on the ATPase of both intact and sonicated ghosts is evident, no such effect on the activity of the kinase is obtained.

The data presented here demonstrate that monovalent cations in addition to affecting the ATPase also activate the adenylate kinase of the membranes. This adds another to the list of enzymes from erythrocyte membranes which are activated by the monovalent cations(9-11). Of these, adenylic acid deaminase is of special interest in relation to the present work. In studies on the adenylate kinase of muscle it has been shown(7) that AMP, which is one of the substrates of the enzyme, can also act as its inhibitor. Since the erythrocyte membrane preparations used by us for the study of adenylate kinase also contain an Na+ or K+ requiring AMP deaminase(11), it seemed possible that the stimulation of the kinase activity by Na+ or K+ was due to conversion of AMP, the inhibitor of kinase, to inosinemonophosphate. To test this possibility, we examined the effect of AMP on the adenylate kinase of the sonicated membranes under the assay conditions used in our experiments. AMP at concentrations as high as 1 mM had no effect on kinase activity. It, therefore, seems that the 3 enzymic activities, ATPase, adenylate kinase, and adenylic acid deaminase, are independently stimulated by the monovalent cations. It is, however, interesting that the pattern of activation of the 3 enzymes is similar(11). K+ is a better activator than Na+ in all 3 cases, and the ion concentrations at which maximum and half-maximum reaction velocities are obtained are also the same. At this point, it is not possible to decide whether adenylate kinase or the adenylic acid deaminase are in any way related to the membrane ATPase and the transport of Na+ and K+ across the cell membrane. Such possible relationships are now being studied.

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