In view of the established role of –SH groups in the maintenance of the structure and function of the erythrocyte membrane, it seems possible that the repair process involving membrane –SH groups may be of importance to the erythrocyte under various physiological and pathological conditions.

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1 B. SHAPIRO, G. KOLLMAN AND J. ASNEN, *Radiation Res.*, 27 (1966) 139.
2 R. M. SUTHERLAND, Ph. D. Thesis, University of Rochester, 1966.
3 R. M. SUTHERLAND, A. ROTHSTEIN AND R. I. WEED, *J. Cell. Physiol.*, 69 (1967) 185.
4 D. K. MYERS AND L. LEVY, *Nature*, 204 (1964) 1324.
5 D. K. MYERS AND R. W. BIDE, *Radiation Res.*, 27 (1966) 250.
6 R. I. WEED, C. F. REED AND G. BERG, *J. Clin. Invest.*, 42 (1963) 581.
7 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
8 G. L. ELLMAN, *Arch. Biochem. Biophys.*, 82 (1959) 70.

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The inhibition of (Na^+-K^+)-activated ATPase by beryllium

It has been reported that beryllium inhibits alkaline phosphatase (EC 3.1.3.1) by competing with Mg^{2+} (refs. 1–4). Phosphoglucomutase (EC 2.7.5.1) is found to be irreversibly inhibited by binding a mole of beryllium per mole of enzyme, presumably to Mg^{2+} site[5].

Since many divalent cations are known to be inhibitory to Na^+-K^+ ATPase[6], we planned to study the effect of beryllium on this enzyme. During the course of our study, THOMAS AND ALDRIDGE[7] reported on the inhibition of brain microsomal ATPase by rather high concentration of $BeSO_4$ (0.64 mM). They described the inhibition to be due to a combination of beryllium with ATP, thereby depleting the enzyme of its usual Mg^{2+}–ATP complex. He did not consider the inhibition to be due to the direct action of beryllium on the enzyme. We found, however, beryllium inhibition of Na^+-K^+ ATPase prepared from microsomal fraction of guinea-pig kidney cortex to be additionally dependent on the presence of cations such as Na^+, K^+, and Mg^{2+}. The data described below suggest that the inhibition is due to the direct action of beryllium on the enzyme and that Na^+, K^+ and Mg^{2+} change the state of the enzyme in the absence of ATP.

Microsomal ATPase was prepared from guinea-pig kidney cortex[8] and care-

fully washed with 50 mM Tris (pH 7.0) just before use, since inhibition by beryllium was markedly affected by cations as described later. The enzyme was preincubated with $2 \cdot 10^{-5}$ M $BeCl_2$ under the various conditions indicated. After preincubation, enzyme activity was determined by transferring into an assay mixture of 1.0 ml final volume containing 40 mM triethanolamine–HCl (pH 7.4), 80 mM NaCl, 16 mM KCl, 2 mM $MgCl_2$ and 2 mM Tris–ATP. After incubation at 37° for 3 min, reactions were terminated by addition of 1.0 ml of 10% trichloroacetic acid. Inorganic phosphate released during the incubation was determined by the method of FISKE AND SUBBAROW. ATPase activity was expressed as μmoles of inorganic phosphate released per min per mg protein. Na^+-K^+ ATPase was determined as the activity inhibited by $2 \cdot 10^{-4}$ M ouabain and Mg^{2+} ATPase as activity insensitive to ouabain.

When $BeCl_2$ was added at the start of the assay, no inhibition was observed over a period of 30 min, but on preincubation of the enzyme with $BeCl_2$ in the presence of Mg^{2+} or K^+ *plus* Mg^{2+}, marked inhibition was observed at the initial stage of incubation in the standard assay medium, followed by gradual recovery from inhibition. For complete recovery, incubation for 30 min or longer was necessary. Since the activity measured within the first 3 min following the preincubation was dependent on the conditions of preincubation, activity measurements were limited to this period of incubation. As shown in Table I, the presence of Mg^{2+} in the preincubation medium was necessary for the inhibitory effect of beryllium. The inhibitory effect was observed to increase by the presence of K^+ in the preincubation medium, and to decrease by the presence of Na^+.

At a constant concentration of $BeCl_2$ and Mg^{2+}, K^+ increased the rate of inhibition as shown in Fig. 1. The effect of Na^+ on the enzyme inhibition by beryllium is quite opposite to K^+ as shown in Fig. 2. Na^+ appears to decrease both the rate and extent of inhibition. Na^+ not only antagonizes the inhibition, but also reverses the inhibition.

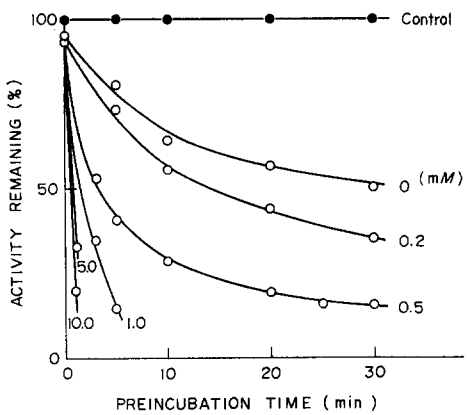
From the present observations on the effect of cations on inhibition by beryllium, it may be concluded that cations interact with Na^+-K^+ ATPase even in the absence of ATP. Our conclusion is supported by findings of other investigators that

TABLE I

THE EFFECT OF Na^+, K^+ AND Mg^{2+} ON THE INHIBITION OF Na^+-K^+ ATPASE AND Mg^{2+} ATPASE BY BERYLLIUM

The activities were determined as described in text after preincubation with $2 \cdot 10^{-5}$ M $BeCl_2$ for 30 min at 37° under various conditions indicated below.

Preincubation media (mM)			*μmoles P_i per min per mg protein*			
NaCl	*KCl*	*$MgCl_2$*	*Na^+-K^+ ATPase*		*Mg^{2+} ATPase*	
			Control	*+$BeCl_2$*	*Control*	*+$BeCl_2$*
0	0	0	1.30	1.27	0.28	0.24
32	0	0	1.32	1.28	0.29	0.24
0	32	0	1.23	1.28	0.29	0.22
0	0	4	1.25	0.68	0.28	0.22
0	32	4	1.32	0.13	0.29	0.25
32	0	4	1.33	0.93	0.29	0.29



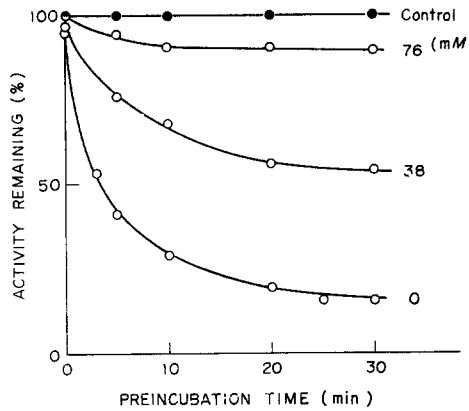


Fig. 1. The effect of K^+ on beryllium inhibition of Na^+-K^+ ATPase. The ATPase activity was determined as described in text, after the various preincubation periods with $BeCl_2$ ($2 \cdot 10^{-5}$ M) in the media containing 4 mM $MgCl_2$ and indicated concentrations of KCl. Control is the activity after the preincubation without beryllium.

Fig. 2. The effect of Na^+ on beryllium inhibition of Na^+-K^+ ATPase. The ATPase activity was determined as described for Fig. 1 except that preincubation media contained $2 \cdot 10^{-5}$ M $BeCl_2$, 0.5 mM KCl, 4 mM $MgCl_2$ and the indicated concentration of NaCl. Control is the activity after preincubation without beryllium.

inhibition of Na^+-K^+ ATPase by diisopropylfluorophosphate[9] and sulfhydryl-blocking reagents[10] was affected by cations in the preincubation medium without ATP.

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1 R. S. GRIER, M. B. HOOD AND M. B. HOAGLAND, *J. Biol. Chem.*, 180 (1949) 289.
2 W. N. ALDRIDGE, *Nature*, 165 (1950) 772.
3 F. W. KLEMPERER, J. N. MILLER AND M. MAZUR, *J. Biol. Chem.*, 180 (1949) 281.
4 K. P. DUBOIS, K. W. COCHRAN AND C. J. HILL, *Science*, 110 (1949) 420.
5 T. HASHIMOTO AND P. HANDLER, *Federation Proc.*, 24 (1965) 284.
6 R. RENDI AND M. L. UHR, *Biochim. Biophys. Acta*, 89 (1964) 525.
7 M. THOMAS AND W. N. ALDRIDGE, *Biochem. J.*, 98 (1966) 94.
8 J. S. CHARNOCK AND R. L. POST, *Australian J. Exptl. Biol.*, 41 (1963) 548.
9 L. E. HOKIN AND A. YODA, *Proc. Natl. Acad. Sci. U.S.*, 52 (1964) 454.
10 J. C. SKOU AND C. HILBERG, *Biochim. Biophys. Acta*, 110 (1965) 359.

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