binding or an adsorption to the cell membrane because such processes would be expected to occur at considerably more rapid rates. Although it is possible that the nicotinamide adenylate passed the membrane barrier in an unchanged molecular form, the experiments do not provide evidence to this point. On the other hand, the assimilation can be accounted for by the phosphomonoesterase activity associated with this cell. This enzyme has been shown by several investigators to act on yeast adenylic acid to yield adenosine. Tsuboi and Hudson(8) have assayed the esterase activity of hemolysates of human red cells and found a value of 6.5 mmoles phosphorus liberated per hour per liter RBC at 37°C. This value was measured at pH 5.5, the optimum hydrogen-ion concentration reported for this enzyme. The data of these investigators and others(9,10) show that considerable enzyme activity is present at pH 7.0 particularly in the presence of magnesium which markedly increased enzyme activity at neutrality. In the present experiments the esterase activity, as indicated by assimilation of radioactivity, was somewhat lower and of a magnitude which might be expected at neutral pH. Therefore it is possible that by the action of this enzyme amounts of adenosine sufficient to contribute to ATP formation were made available to the cells. But, contrary to what might be expected through the action of this esterase, only minimal assimilation of the labelled compound occurred at the more optimal pH of 6.4. However, at this pH, membrane nucleoside phosphorylase activity is minimal (8), and consequently adenosine may not be able to enter the cell readily. At neutral pH, the activity of nucleoside phosphorylase is maximal and thereby by its action facilitates the entry of adenosine and its incorporation into cell substrates.

Summary. The addition of nicotinamide adenylate compounds was found to maintain, to a significant extent, the glycolytic capacity of fresh human erythrocytes incubated *in vitro* for 20 hours. The compounds appeared to act also to restore the metabolism of aged cells. Although it is not believed that nucleotides readily enter the intracellular compartment, evidence is presented that erythrocytes can slowly assimilate adenine nucleoside phosphates. The possible role of erythrocyte phosphomonoesterase is discussed.

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In vitro Inhibition of Na^+-K^+ and Mg^{2+} ATPases by Mono, Di and Trivalent Cations.* (30658)

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It is now generally accepted that mammalian cells maintain their ionic composition by pumping potassium inward and sodium outward. Evidence that active cation transport is intimately involved with the enzymatic hydrolysis of ATP is available(1). Observations that both the Na^+-K^+ ATPase and active cation transport systems share common characteristics, have led to the hypothesis that

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they are synonymous, or that the enzyme is a fractional part of a larger, as yet unresolved complex(2-5). Na^+-K^+ ATPase has been reported to be present on kidney cortex membranes(6). It has been proposed that this enzyme is concerned with the renal tubular reabsorption and excretion of sodium(7-9). Information on the effect of other ions upon sodium homeostasis both *in vivo* and *in vitro* remains largely unavailable.

This paper deals with the effects of various cations upon the isolated, solubilized Na^+-K^+ and Mg^{2+} ATPases derived from normal rat kidney.

Materials and methods. Fresh kidneys obtained from normal adult male albino rats were excised immediately following decapitation, and were chilled in cold .25 M sucrose-.001 M EDTA. Whole tissue homogenates, 10% w/v, were prepared with the aid of an all glass, hand powered Tenbroeck tissue grinder. The homogenizing medium consisted of .25 M sucrose-.001 M EDTA-.0025 M desoxycholic acid adjusted to pH 7.4 with solid tris. The clear yellow supernatant, containing the enzymes, was obtained by centrifugation at 37,000 $\times$ *g* for 40 minutes at 0°C. This fraction was dialyzed against cold .001 M GSH-.001 M EDTA, pH 7.5, for 5 hours, followed by continuously flowing cold demineralized water, pH 7.4, for an additional 16 hours.

The assay system for testing Na^+-K^+ stimulation consisted of the following components, in μmoles: $MgCl_2$-5; tris ATP-5; tris-HCl buffer, pH 7.5-75; NaCl-120; and KCl-40, in a volume of 2.0 ml. All solutions were made up in demineralized water. The reactions were initiated by addition of .2 ml of enzyme preparation. Mg^{2+} ATPase was measured by deleting NaCl and KCl from the reaction mixtures and substituting an equivalent amount of choline chloride. Incubations were carried out at 27°C for 15 minutes, at which time the reactions were terminated and deproteinized by addition of TCA to a final concentration of 5%.

Inorganic phosphate, an indicator of ATP hydrolysis, was determined on an aliquot of the deproteinized reaction mixture using the procedure of Fiske and Subbarow(9). Protein was determined by the method of Lowry *et al*(10). Tris-ATP was prepared from the sodium salt as reported by Wheeler and Whittam(11). Additions of the various cations were as demineralized water solutions, replacing equal volumes of water.

Results. In Table I the effect of the various cations upon the Na^+-K^+ and Mg^{2+} ATPases is presented. Over the range of concentration tested, a number of cations do not appreciably alter the reaction rate of either activity, assuming that 2 discrete enzyme entities coexist in these preparations. Indeed, a recent report has raised the possibility that the Na^+-K^+ ATPase activity obtained from brain microsomes may be an isolation artifact(12).

Among those cations exhibiting a differential effect are: calcium, strontium, and barium, all of which exert an inhibitory action on the Na^+-K^+ stimulated species and a stimulatory effect on the Mg^{2+} ATPase portion. Enhancement of activity over the norm for the Na^+-K^+ ATPase fraction was not observed with any of the other cations listed. Cesium was found to depress minimally the Na^+-K^+ ATPase activity, but surprisingly increased the reaction rate of the Mg^{2+} dependent ATPase. Monovalent cation stimulated ATPase activity was observed to be inhibited to a greater degree by silver, vanadium, copper, zinc, cadmium, lead and mercury.

Discussion. Na^+-K^+ ATPase has been reported to contain sulfhydryl groups, which are apparently essential for optimum enzymatic activity(13-15). This may possibly explain the preferential inhibitory character upon the Na^+-K^+ ATPase of such cations as silver, and the other inorganic sulfhydryl reagents enumerated in the preceding section.

Aberrations concerning sodium accumulation and turnover have been postulated to be involved intrinsically in the development and progression of hypertensive disease(16). Experimental hypertension has been induced in rats by the administration of cadmium(17). This cation has been reported to enhance renal proximal tubular reabsorption of sodium(18,19).

In view of the reports that Na^+-K^+

TABLE I. Effect of Cations on Na^+-K^+ and Mg^{2+} ATPases of Normal Rat Kidney Preparations.

Cation	Na^+-K^+ ATPase, cation concentration			Mg^{2+} ATPase, cation concentration		
	10^{-4} M	5×10^{-3} M	10^{-3} M	10^{-4} M	5×10^{-3} M	10^{-3} M
Li^{1+}	−00.3 ± .4*	−00.5 ± .2*	−00.8 ± .4*	−00.8 ± .7*	−01.0 ± 1.0*	−02.7±1.2*
Cs^{1+}	−01.3 ± .2	−01.0 ± .2	−00.6 ± .9	+03.2 ± 1.3	+10.2 ± 1.3	+08.5 ± 1.8
Ag^{1+}	−35.9 ± .8	−39.7 ± 1.5	−41.0 ± 2.5	−19.2 ± 1.9	−25.9 ± .7	−29.6 ± 1.5
Be^{1+}	−11.7 ± .6	−30.9 ± .8	−36.7 ± .3	−09.7 ± 1.4	−30.2 ± 1.2	−43.5 ± 1.0
Ca^{2+}	−05.3 ± .7	−14.8 ± .4	−17.1 ± .8	+04.2 ± 1.6	+10.7 ± 2.9	+08.7 ± 4.3
Sr^{2+}	−02.3 ± .8	−05.7 ± .9	−12.2 ± 2.2	+03.5 ± .5	+04.0 ± .8	+00.8 ± 1.6
Ba^{2+}	−00.7 ± .3	−04.4 ± .3	−04.5 ± 2.1	+02.0 ± .7	+07.0 ± .9	+06.6 ± 1.4
V^{2+}	−28.3 ± .4	−37.8 ± .2	−46.9 ± .6	−06.9 ± 1.5	−21.7 ± 1.5	−30.2 ± 1.7
Cr^{2+}	−02.3 ± 1.3	−05.8 ± 1.4	−06.4 ± 1.6	−02.1 ± 1.7	−03.2 ± 1.2	−10.7 ± 1.9
Mn^{2+}	−02.2 ± .7	−09.2 ± 1.8	−15.0 ± 2.5	−00.4 ± .6	−03.5 ± .8	−09.2 ± .4
Fe^{2+}	−06.5 ± .7	−12.5 ± 1.9	−12.4 ± .4	−02.5 ± .2	−04.9 ± 1.2	−14.5 ± 2.6
Co^{2+}	−02.0 ± .9	−10.8 ± 2.0	−17.2 ± 1.8	00.0 ± .1	−05.7 ± .7	−17.9 ± 2.0
Ni^{2+}	−09.9 ± 2.3	−37.2 ± 2.8	−45.0 ± 3.3	−09.6 ± 1.9	−33.6 ± 4.2	−48.3 ± 2.3
Cu^{2+}	−30.2 ± 1.0	−50.6 ± .6	−62.5 ± .6	−18.0 ± 3.3	−40.6 ± 1.1	−57.5 ± .3
Zn^{2+}	−22.8 ± .4	−56.4 ± .7	−67.3 ± 1.0	−14.4 ± 1.2	−50.5 ± 1.3	−61.6 ± 1.2
Cd^{2+}	−13.1 ± 1.1	−41.6 ± .3	−52.9 ± .7	−02.3 ± 2.4	−25.4 ± .8	−40.5 ± 1.0
Hg^{2+}	−38.1 ± .7	−56.6 ± .6	−66.0 ± .8	−22.2 ± .6	−67.9 ± 2.4	−76.8 ± 1.8
Pb^{2+}	−16.8 ± .9	−28.5 ± .8	−30.6 ± 1.4	−03.8 ± 1.9	−06.2 ± 1.9	−12.6 ± 1.4
Ti^{3+}	−02.9 ± .2	−07.9 ± 1.8	−11.6 ± 1.2	−01.3 ± 1.0	−02.3 ± .9	−11.3 ± 3.1
Al^{3+}	−02.7 ± 1.4	−05.5 ± 1.2	−07.7 ± .9	−04.1 ± 2.9	−06.9 ± 1.6	−14.0 ± 1.5
Bi^{3+}	−00.2 ± .2	−00.3 ± .4	−00.8 ± .7	−02.0 ± 1.8	−02.0 ± 1.6	−04.9 ± 1.3

* SE.

All cations were added as the chlorides, with the exception of silver which was added as the nitrate. Values reported are the means of 3 observations, represented as the percentage change from untreated aliquots of enzyme.

stimulated ATPase is implicated in the renal tubular reabsorption and excretion of sodium (7,8), and that cadmium facilitates this reabsorptive process(18,19), the prospect of a correlation between an ionic species and an enzyme, resulting in a biochemical anomaly becomes evident. This postulate may be operative in hypertensive disease states. It is therefore feasible that this proposed lesion may occur at the Na^+-K^+ ATPase locus.

Summary. Na^+-K^+ ATPase has been found to be strongly inhibited *in vitro* by addition of silver, vanadium, copper, zinc, cadmium, lead and mercury. Calcium, strontium and barium enhance the dephosphorylation of ATP in the presence of Mg^{2+} dependent ATPase, while exerting an inhibitory effect on the monovalent cation stimulated portion. Cesium acts as an activator of Mg^{2+} ATPase in the system studied.

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