EFFECT OF ADENOSINE TRIPHOSPHATE, MAGNESIUM, AND CALCIUM ON THE PHOSPHATE UPTAKE BY RABBIT ERYTHROCYTES

by

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INTRODUCTION

The results of previous investigations in this department support the hypothesis that orthophosphate ions enter erythrocytes by the formation of adenosine triphosphate (ATP) at the cell membrane[1]. It seemed of interest to investigate the influence of the addition of ATP on the entry of phosphate into red blood cells. It was also suggested previously[2] that phosphate may enter red cells by adsorption on the cell surface in two stages followed by absorption of orthophosphate and of phosphoesters. Since the literature provides additional observations of enzymatic reactions at cell peripheries, the assumption that exogenous ATP affects phosphate uptake by erythrocyte surface reactions may be used as a working hypothesis.

It has been well established that phosphorylation involving ATP requires Mg^{++}[3]. HERS[4] established that, in the absence of K^+, maximum activity of the fructokinase system of the liver was observed when the ratio of ATP to Mg^{+2} was two. The ratio decreased to unity in the presence of equal molar concentrations of Mg and K. To obtain maximum activity of the muscle hexokinase system LIÉBECQ[5] found that the optimal ratio of ATP to Mg was again one. The ratio of ATP to total Mg, including the dissociated and undissociated phases, may be considerably higher than two at sites of high concentrations of divalent alkaline metal phosphates. A localized accumulation of these phosphates may occur at adsorption sites on the cell surface. Precipitation seems to be unlikely if an excess of dissociated $ATP^{=}$ is present. MANDL, GRAUER, AND NEUBERG[6] found that precipitation of calcium phosphate can be prevented by an ATP concentration of 40 times the calcium phosphate concentration. Magnesium phosphate, the solubility of which is almost identical with that of calcium phosphate, might be influenced similarily by ATP. In view of the above considerations, the experiments described in the present paper were designed to answer the following questions: 1. Do Ca^{+2}, Mg^{+2}, and ATP separately or in combination affect the uptake of phosphate ions? 2. Do such effects vary with the proportion of each substance?

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Results will be presented which suggest strongly that ATP, Ca^{+2}, Mg^{+2}, and $HPO_4^{-2} \rightleftharpoons H_2PO_4^-$ can form various combinations which inhibit or stimulate phosphate uptake by erythrocytes. An attempt is made to correlate the experimental findings with hypothetical combinations or complexes of these constituents.

METHODS

The experimental methods are essentially identical with those described previously[2,7] in which 3.5 ml of whole rabbit blood were incubated with 0.5 ml of radioactive orthophosphate (^{32}ph) at pH 7.40. Preliminary experiments utilized 3.5 ml of blood with 0.5 ml of a mixture containing all or some of the following reagents: 0.05 ml $H_3{}^{32}PO_4$ + 0.10 ml NaCl (to make the final concentration of NaCl 0.9%) with $H_3{}^{31}PO_4$ to bring the phosphate concentration to $1.21 \cdot 10^{-8}$ moles per blood sample; 0.10 ml of $Ca(OH)_2$ or $Mg(OH)_2$ to make either concentration $1.00 \cdot 10^{-8}$ moles per sample; and 0.25 ml Na_2ATP providing $4.00 \cdot 10^{-7}$ moles per sample. The final concentration of ATP was 33.1 times the phosphate concentration and 40.0 times the concentrations of Mg^{+2} and Ca^{+2}. The volume of the mixture added was made up to 0.5 ml with water. The pH did not change significantly since the pK_2 of neutralization between phosphoric acid and calcium hydroxide is 7.2. For detailed studies of Mg^{+2} and ATP effects, further experiments were performed with dilution series of either ion in the presence and absence of the other two.

RESULTS

Exploratory experiments, which are summarized in Table I, sections 1 and 2, indicate an inhibition of the uptake of ^{32}ph by those concentrations of Ca^{+2} and Mg^{+2} which are approximately equal to that of ^{32}ph (col. d, e, i). Additions of ATP in the presence of either cation increased inhibition (col. a, e, f, j), while ATP alone in this and in other experiments stimulated uptake (col. f, i, j). Even Mg^{+2} at a concentration which was more than three orders of magnitude lower than that of ^{32}ph inhibited phosphate uptake severely as shown in Table I, section 3, col. e, i. Equimolar K^+ reversed this effect by producing a distinct stimulation (col. c, i, j), as was expected from HERS' observations[4].

Some conceivable interactions of ATP, Mg and phosphate. Interactions of Mg^{+2}, HPO_4^{-2}, ATP, and a kinase (E) may exist which promote or retard ^{32}ph uptake by erythrocytes depending upon their proportional representation in the incubating medium. Some conceivable reactions are listed in Table II. Phosphate ions may combine with Mg^{+2} to form a combination of low ionic activity[8,9] (react. 2a) or even a precipitate on adsorption faces (react. 2b). However the formation of a precipitate of phosphate can be expected only if its solubility product (K_{sp}) with its cation exceeds $3.16 \cdot 10^{-5}$. The requisite bulk concentrations[8] would exceed those used in these experiments, while the concentrations at adsorption sites on and in cell membranes may be sufficient. The first alternative of undissociated combinations of magnesium and phosphate ions might exist in two forms: 1. undissociated magnesium phosphate with a pK' of 1.62 at an ionic activity of extracellular fluids[8] of $\mu = 0.160$, which would reduce the amount of available phosphate ion (Table II, react. 2a); or 2. a surface complex of magnesium phosphate (react. 2b) may neutralize a negative surface charge by the remaining positive charge of magnesium, which could prevent the exchange reaction of e^- with phosphate at the cell surface as described previously[2]. The inhibitory action of Mg^{+2} in any of these combinations with ^{32}ph is limited by the lower concentration of either ion which must exceed the concentration of ATP[6].

Considering the accepted mechanism by which ATP participates in phosphorylations, it is apparent that its effectiveness could be lowered by converting the

TABLE I

PRELIMINARY TESTS ON THE EFFECTS OF Ca^{+2}, Mg^{+2}, AND ATP ON THE UPTAKE OF RADIOACTIVE PHOSPHATE BY RABBIT ERYTHROCYTES

a Section	*b* *H* %	*c* *H″* %	*d* K^+	*e* Ca^{+2}	*f* Mg^{+2}	*g* *ATP*	*h* ^{32}ph	*i* *units*	*j* ^{32}ph *uptake as % of initial moles*	*k* *Probability P of mean activity, less than*
			$10^{-9}\times$	$10^{-9}\times$	$10^{-9}\times$	$10^{-7}\times$	$10^{-9}\times$			
				10.00	16.00	4.00	12.10	m/s		
				2.50	2.50	1.00	3.03	m/ml m		
1	33.8	33.0		7.57	7.57	3.03	9.16	m/ml c		
				+	—	—	+		3.82	0.7
				+	—	+	+		2.69	0.01
				—	+	—	+		22.79	0.01 0.01
				—	+	+	+		13.41	
2	39.7	34.8		7.20	7.20	2.88	8.71	m/ml c		
				—	+	—	+		11.32	0.001
				—	—	+	+		29.04	0.3 0.001
				—	—	—	+		27.22	
							$10^{-5}\times$			
			30.00		30.00		6.97	m/s		
			6.73		6.73		1.56	m/ml m		
3	39.9	31.1	19.40		19.4		4.50	m/ml c		
			—		—		+		16.49	
			—		+		+		0.95	
			+		+		+		25.53	

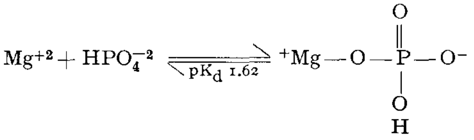
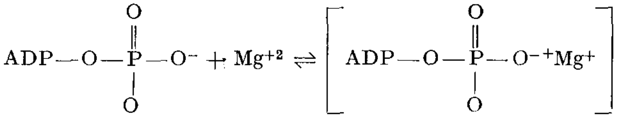
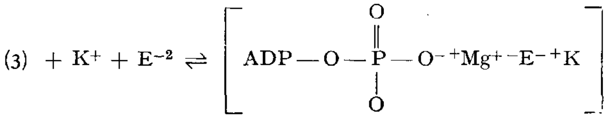
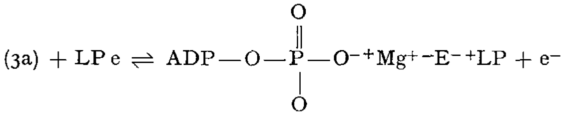
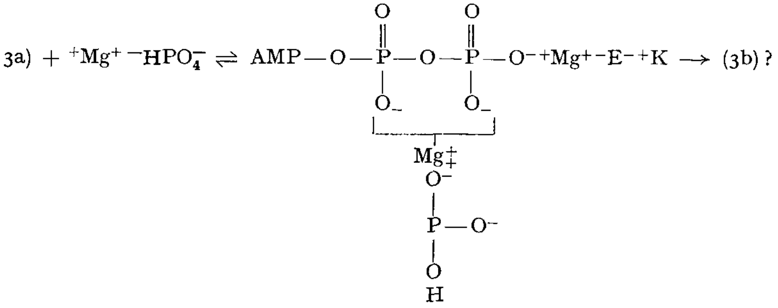
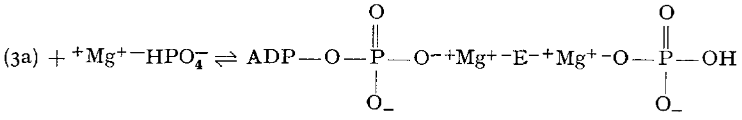
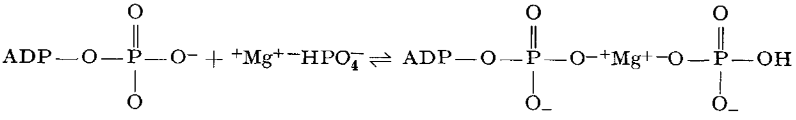
Symbols: *H* = hematocrit of whole blood; *H″* = hematocrit of incubating mixture; (m/s) = moles/sample of 3.5 ml of blood; (m/ml m) = moles/ml of mixture; (m/ml c) = moles/ml of erythrocytes; ^{32}ph = radioactive phosphate ion; P = maximum limit of statistical probability as given by the table of t-values[15]; all concentrations are initial quantities.

functional ATP-Mg-E-K unit[4] (Table II, react. 3a) into ATP-Mg-E-Mg-^{32}ph (react. 3d), ATP-Mg-^{32}ph (react. 4), or perhaps even into ATP (Mg-^{32}ph)-Mg-E-K units (react. 3c), where -K is exchangeable with a lipoprotein group. The first two cases (react. 3d, 4) would result in the exclusion of the kinase (E) or blocking of its second free bond, while the last case (react. 3c) might be inhibitory due to stearic interference by the -Mg-^{32}ph group on the first or second labile P atom of ATP. The observation by KOSHLAND[9] that Mg^{+2} can form a stable MgATP complex also infers a reduction of latent ATP activity.

Conversely, stimulation of phosphate uptake is conceivable if the formation of the complex ATP-Mg-E-K can be favoured by the accepted mechanism of the reactions (3a) and (3b). They might proceed best if the initial concentration of Mg^{+2} is less than the 40-fold ATP concentration in the plasma, in which circumstances the effects of solubili-

TABLE II

THEORETICAL COMBINATIONS OF ATP, Mg^{+2}, AND HPO_4^{-2} WHICH MAY AFFECT PHOSPHATE UPTAKE BY RABBIT ERYTHROCYTES

N	*E*	*Reaction*
1		$2.12\ HPO_4^{-2} \xrightleftharpoons[]{pH\ 7.40} H_2PO_4^-$
2a	I → S ←	 $Mg^{+2} + HPO_4^{-2} \xrightleftharpoons[pK_d\ 1.62]{} {}^{+}Mg-O-\underset{\underset{\underset{H}{O}}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^-$
2b	I	$(2a) \xrightleftharpoons[pK_{sp}\ 4.46]{} Mg\ HPO_{4\,(solid)}$
3		 $ADP-O-\underset{\underset{O}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^- + Mg^{+2} \rightleftharpoons \left[ADP-O-\underset{\underset{O}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^{-}\,{}^{+}Mg^{+}\right]$
3a		 $(3) + K^+ + E^{-2} \rightleftharpoons \left[ADP-O-\underset{\underset{O}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^{-}\,{}^{+}Mg^{+}\,{}^{-}E^{-}\,{}^{+}K\right]$
3b	S →	 $(3a) + LP\,e \rightleftharpoons ADP-O-\underset{\underset{O}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^{-}\,{}^{+}Mg^{+}\,{}^{-}E^{-}\,{}^{+}LP + e^-$
3c	?	 $3a) + {}^{+}Mg^{+}\,{}^{-}HPO_4^- \rightleftharpoons AMP-O-\underbrace{\underset{\underset{O_-}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O-\underset{\underset{O_-}{\vert}}{\overset{\overset{O}{\Vert}}{P}}}_{\underset{\underset{\underset{\underset{H}{O}}{\vert}}{\overset{\vert}{P}-O^-}}{\overset{Mg^{++}}{O^-}}}-O^{-}\,{}^{+}Mg^{+}\,{}^{-}E^{-}\,{}^{+}K \longrightarrow (3b)\,?$
3d	I →	 $(3a) + {}^{+}Mg^{+}\,{}^{-}HPO_4^- \rightleftharpoons ADP-O-\underset{\underset{O_-}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^{-}\,{}^{+}Mg^{+}\,{}^{-}E^{-}\,{}^{+}Mg^{+}\,{}^{-}O-\underset{\underset{O_-}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-OH$
4	I →	 $ADP-O-\underset{\underset{O}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^- + {}^{+}Mg^{+}\,{}^{-}HPO_4^- \rightleftharpoons ADP-O-\underset{\underset{O_-}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-O^{-}\,{}^{+}Mg^{+}\,{}^{-}O-\underset{\underset{O_-}{\vert}}{\overset{\overset{O}{\Vert}}{P}}-OH$

Left column, N = reaction number; center column, E = theoretical effects of reaction: I = inhibition, S = stimulation; right column: ADP = adenosine diphosphate, AMP = adenylic acid, e^- = electron, E = a kinase, LP^+ = lipoprotein complex in the cell membrane where $LP^+ \rightleftharpoons K^+$, pK_d = —log of the dissociation constant of magnesium phosphate, and pK_{sp} = —log of the solubility product of magnesium phosphate.

References p. 345.

zation by ATP are reduced[2,6,12]. A previously mentioned modification of this complex (react. 3c), ATP (Mg-^{32}ph)-Mg-E-K, may also be stimulating if the hypothetical Mg-^{32}ph side chain does not interfere with the activity of the -Mg-E-K group.

Experimental observations. The results of two series of experiments will be described, in which 1. the concentration of ATP was varied while the approximately equal concentrations of Mg^{+2} and of ^{32}ph remained constant; and 2. the concentration of Mg^{+2} was varied while the unequal concentrations of ATP and of ^{32}ph remained constant.

1. *To study the effect of extracellular ATP on the uptake of phosphate* ATP concentrations ranging from $4.00 \cdot 10^{-19}$ to $8.75 \cdot 10^{-6}$ moles/sample of 3.50 ml of whole blood were utilized. Also, $1.0 \cdot 10^{-8}$ moles of Mg^{+2} and $1.21 \cdot 10^{-8}$ moles of ^{32}ph were added in all cases. The effective concentrations of these three constituents is expressed by the initial concentration (*o*) of the reagent (*R*) in moles/ml of extracellular fluid (*f*),

$$R_{of} = \frac{R_o}{V'' (1\text{-}H'')} \qquad (1)$$

where R_o = concentration of reagent in moles/blood sample, V'' = final volume of the incubating mixture[2,7], and H'' = its hematocrit. Hence, the range of the effective concentrations of ATP of five experiments was between $1.5 \cdot 10^{-19} \pm$* to $3.4 \cdot 10^{-6} \pm$ moles/ml of fluid[2]. The concentrations of Mg^{+2} and of ^{32}ph were 3.9 and $4.7 \cdot 10^{-9} \pm$ moles/ml of fluid, respectively, in 4 experiments. A fifth experiment had $6.94 \cdot 10^{-9}$ moles of ^{32}ph/ml of fluid, thus raising the concentration of ^{32}ph by 58%.

The uptake of ^{32}ph can be expressed as moles of ^{32}ph taken up per 3.50 ml of blood. These values were converted into moles/ml of extracellular fluid according to equation (1) and might be plotted as a function of the concentration of ATP. However, since a plot of this relation is rather impractical for the present dimensions and without sufficient resolution for detailed analyses, a plot of the deviation of ^{32}ph uptake from control experiments as a function of the logarithm of the initial ATP concentration is more instructive ($\Delta x/m_f$ versus log A'_{of}). Such a graphical description showed individual curves for each experiment with a different animal. Yet, all curves resembled each other in shape and slope and differed only in the origin of their ordinates. A shift of the ordinate $\Delta x/m_f$ to a common origin ought to eliminate the spatial differences between each curve. When this was done a continuous, smooth curve was obtained (Fig. 1).

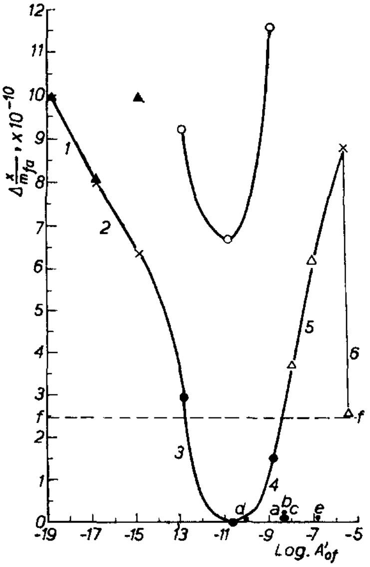


Fig. 1. Deviation of ^{32}ph uptake from the lowest experimental uptake as a function of added ATP. Abscissa: Log of the initial ATP concentration in moles/ml of extracellular fluid (Log A'_{of}). Ordinate: Adjusted deviation of ^{32}ph uptake in moles/ml of fluid ($\triangle x/m_{fa}$); initial ^{32}ph concentration = ph$'_{of}$ (see text). Symbols: Blood sample A = ●, B = ○, C = ▲, D = △, E = ×. Numbers on curve segments were used for ordinate shift as described in the text. Proportion of the initial concentration of ATP to that of Mg and of ^{32}ph at point *a*, where A'_{of} = Mg$'_{of}$; at *b*, where A'_{of} = ph$'_{of} \approx$ Mg$'_{of}$ for A, C to E; at *c*, where A'_{of} = ph$'_{of}$ for B = 158% ph$'_{of}$ for A, C to E; at *d*, where A'_{of} = 1/40 Mg$'_{of}$; and at *e*, where A'_{of} = 40 Mg$'_{of}$. The average level of the deviation of ^{32}ph uptake by the controls from the lowest experimental uptake = *f*–*f*.

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* The sign ± in this and subsequent concentration values is used to indicate that there is a range of R_{of} of equation (1) corresponding to the range of H''.

To implement the ordinate shift the minimum uptake of ^{32}ph/ml of fluid of all experiments of the ATP series is defined as the origin of the ordinate, where the deviation of uptake from control is defined as equalling zero. A reference point is hereby established which is common to all experiments of the ATP series. Each individual experimental curve as a whole is shifted along the new ordinate ($\Delta x/m_{fa}$) to the position that integrates the individual curve into a continuous curve representing all experiments of the series.

A proof of the permissibility of this procedure required a replotting of the experimental data as the deviations of the logarithm of ^{32}ph uptake/ml of fluid from the controls as a function of the deviation of the logarithm of the final concentration of radioactive plus stable phosphate from control. Such a graph showed individual curves for each experimental run, where all curves approximated lines with almost identical slopes. The validity of the relationship

$$\log x/m_f = a\ (\log C_f) + b \tag{2}$$

was suggested, where x/m_f = uptake of ^{32}ph in moles/ml of fluid, and C_f = the final concentration of $^{31+32}$ph in the extracellular fluid in moles/ml. The ordinate intercept b was a variable and dependent on some unknown factor which may be intrinsic in the particular blood sample. Hence, a shift of the ordinate of each curve of this graph eliminated this variable and produced a straight line. This could be expected from the theorem that a plot of uptake against loss is linear if both coordinates are expressed in the same units.

A distinct stimulation of ^{32}ph uptake in the presence of an excess of Mg^{+2} appears to exist even at extremely low ATP concentrations ($\Delta x/m_{fa}$ vs. $\log A'_{of}$ in Fig. 1). This stimulation decreases approximately exponentially with increasing A'_{of}. But if A'_{of} approximates 1/40 of the Mg^{+2} concentration at point d, maximum inhibition is observed. Further increases of A'_{of} revert this decreasing trend, and $\Delta x/m_{fa}$ reaches the control level f — f if A'_{of} approximates the concentration of Mg^{+2} at points a and b. If the concentration of ATP exceeds that of Mg^{+2} and of ^{32}ph, $\Delta x/m_{fa}$ is increased up to that value of A'_{of} which just causes hemolysis of rabbit erythrocytes.

The fact that the uptake of ^{32}ph was inhibited by ATP under conditions of almost equal initial concentrations of ^{32}ph and Mg^{+2} (Fig. 1, point b) suggested that Mg^{+2} could bind ^{32}ph in the presence of ATP. However, when the initial ^{32}ph concentration was raised by 58% as in sample B (from point b to c) to provide an excess of available

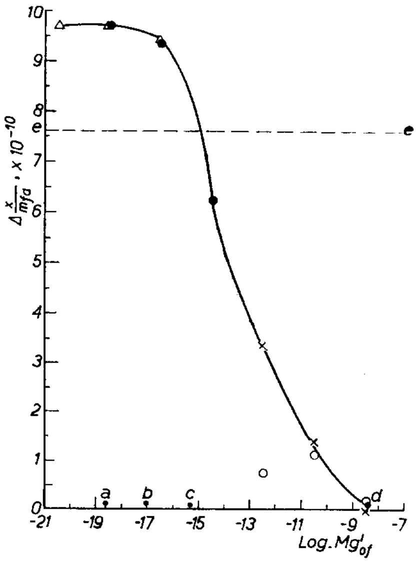

Fig. 2. Deviation of uptake of ^{32}ph from the lowest experimental uptake as a function of added Mg^{+2}. Abscissa: Log of the initial concentration of Mg^{+2} in moles/ml of fluid (Log Mg$'_{of}$). Adjusted deviations of ^{32}ph uptake in moles/ml of fluid after shifting of ordinates (see text). Symbols: Blood sample G = ●, H = ○, I = ×, J = △. Numbers on curve segments were used for the described ordinate shift. Proportion of the initial concentration of Mg^{+2} to that of ATP and of ^{32}ph at point a, where Mg$'_{of}$ = 1/40 A$'_{of}$; at b, where Mg$'_{of}$ = A$'_{of}$; at c, where Mg$'_{of}$ = 40 A$'_{of}$; and at d, where Mg$'_{of}$ = ph$'_{of}$. The average level of the deviations of ^{32}ph uptake by the controls from the lowest experimental uptake = e — e.

phosphate, the uptake of ^{32}ph was increased above the control level (f—f) without materially disturbing the shape of this segment of the curve. The minimum uptake still appears at $A'_{of} = 1/40$ the Mg^{+2} concentration.

2. *The data for the uptake of ^{32}ph as a function of a series of concentrations of Mg^{+2}* are treated like those of the experiments with the series of ATP concentrations. The range of the effective concentrations of Mg^{+2} of four experiments was $3.3 \cdot 10^{-21} \pm$ to $3.3 \cdot 10^{-9} \pm$ moles/ml of fluid. The concentrations of ATP and of ^{32}ph were $1.0 \cdot 10^{-17} \pm$ and $4.0 \cdot 10^{-9} \pm$ moles/ml of fluid, respectively. Fig. 2 shows a plot of $\Delta x/m_{fa}$ versus log Mg'_{of}. It indicates that Mg^{+2} inhibit the uptake of ^{32}ph approximately exponentially if Mg'_{of} exceeds A'_{of} to the right of point c. The uptake is stimulated if ATP is in excess (point a). Both effects balance each other if Mg'_{of} approximates $40 \times A'_{of}$ (point c at control level e—e). Hemolysis interferes with the measurement of ^{32}ph uptake when Mg'_{of} approximates the ^{32}ph concentration (point d).

DISCUSSION

Taking up earlier remarks about solubilization effects of ATP and about various combinations of ATP, Mg and radioactive phosphate ions[6] (Table II) the experimental results of Figs. 1 and 2 can be used to estimate the degree of dissociation of these ions at certain strategic concentrations. A study of dissociation with changing concentrations of ATP and Mg, respectively, may be a means of elucidating some of the reasons for the shapes of ^{32}ph uptake curves of Figs. 1 and 2. The percentages of free ions of ATP, Mg, and phosphate are calculated in Table III for their minimum and maximum initial concentrations and for those concentrations of ATP and Mg which are equal to the initial concentration of ^{32}ph, $1/40 \times Mg'_{of}$, Mg'_{of}, $40 \times Mg'_{of}$, and equal to $1/40 \times A'_{of}$, A'_{of}, and $40 \times A'_{of}$, respectively (col. f, h, j). These series of concentrations include those that may cause solubilization[6] and complexing reactions[4,5,8,10,11].

Effects of ATP. Table III, (col. l to o, r, and s) and Fig. 1 show that ATP favors ^{32}ph uptake if all ATP is combined with Mg in the presence of an excess of free Mg^{+2} and also if all Mg is combined with ATP in the presence of an excess of free ATP^{-2}. Uptake is reduced if both Mg and ATP remain uncombined with each other. The initial and almost equal concentrations of Mg and phosphate ions are sufficient to form undissociated $MgHPO_4$[8] in the extracellular fluid and to form deposited $MgHPO_4$ in the cell antisphering layer[2,8] in competition with the Mg-ATP complex (Table III, col. o, q). This line of thought suggests that these forms of $MgHPO_4$ might exist only in the absence of the Mg-ATP complex (col. b, footnote 7). In turn, deposited $MgHPO_4$ might only exist to a significant extent on the antisphering layer if its undissociated and precursory form is present.

Since the dissociation constant for the Mg-ATP complex, which has been used in this discussion, is based on an 1:1 proportion of both components, there is reason to believe that the ATP-Mg-E-K unit of reaction 3b (Table II) is active in stimulating ^{32}ph uptake. Its retardation in the absence of this complex might be due either to the formation of undissociated $MgHPO_4$ (react. 2a and 2b) or to the formation of inhibiting Mg_{1+n}-ATP complexes (react. 3c and 3d) similar to the complexes proposed by HERS[4] and LIÉBECQ[5]. They demonstrated the blocking of the second hexokinase bond by a second Mg atom whereby phosphorylation of hexoses was halted. Their observations suggest in this connection that one step in the uptake of ^{32}ph is mediated by hexokinase.

TABLI

PROBABLY COMBINED AND FREE ATP, Mg, AND PHOSPHATE IN RABBI

Exp. with var. conc. of	K_{sp}[b]	K_d[f, g]	μ	*Ref.*	*Initial concentrations* *ATP* *Order*	*Initial concentrations* *ATP* M[a]	*Initial concentrations* *Mg* *Order*	*Initial concentrations* *Mg* M[a, b]
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*
	$MgHPO_4$							
	$3.160 \cdot 10^{-5}$		0.16	8				
Mg	$1.945 \cdot 10^{-17}$[c]		0.16				minimum	$1.202 \cdot 10^{-15}$[d]
	$1.945 \cdot 10^{-4}$[c]		0.16				maximum	$1.202 \cdot 10^{-2}$
	$2.618 \cdot 10^{-4}$[c]		0.16					$1.619 \cdot 10^{-2}$
ATP	$2.248 \cdot 10^{-4}$[c]		0.16					$1.388 \cdot 10^{-2}$
	$2.618 \cdot 10^{-4}$[c]		0.16					$1.619 \cdot 10^{-2}$
		$MgHPO_4$[f]						
Mg		$2.398 \cdot 10^{-2}$	0.16	8			minimum	$3.330 \cdot 10^{-18}$
							maximum	$3.330 \cdot 10^{-6}$
								$4.027 \cdot 10^{-6}$
ATP		$2.398 \cdot 10^{-2}$	0.16	10				$3.845 \cdot 10^{-6}$
		MgATP[g]						
		$8.7 \cdot 10^{-5}$	0.1					
Mg		$1.9 \cdot 10^{-4}$[h]	0.16			$1.001 \cdot 10^{-14}$	minimum	$3.330 \cdot 10^{-18}$
						$1.001 \cdot 10^{-14}$	1/40 of (ATP)	$2.504 \cdot 10^{-16}$
						$1.001 \cdot 10^{-14}$	=	$1.001 \cdot 10^{-14}$
						$1.001 \cdot 10^{-14}$	maximum	$3.330 \cdot 10^{-6}$
ATP		$1.9 \cdot 10^{-4}$[h]	0.16		minimum	$1.536 \cdot 10^{-16}$		$3.845 \cdot 10^{-6}$
					1/40 of (Mg)	$9.601 \cdot 10^{-8}$		$3.845 \cdot 10^{-6}$
						$3.845 \cdot 10^{-6}$	=	$3.845 \cdot 10^{-6}$
					40 × (Mg)	$1.538 \cdot 10^{-4}$		$3.845 \cdot 10^{-6}$
					maximum	$3.365 \cdot 10^{-3}$		$3.845 \cdot 10^{-6}$

[a] Concentrations expressed as moles/liter of extracellular fluid of the incubating mixture.

[b] K_{sp} = solubility product of solid $MgHPO_4$ in biological fluids with an ionic activity $\mu = 0.16$.

[c] Calculated products of the initial experimental concentrations of Mg and ^{32}ph.

[d] Calculated concentrations to be expected within the erythrocyte antisphering layer[12] of $3.76 \cdot 10^{-13}$ ml.

[e] " + " ≈ 100%.

Stimulation of ^{32}ph uptake by a very great excess of Mg^{+2} over minute concentrations of ATP apparently proceeds by a different pathway which is relatively independent of ATP.

The solubilization of $MgHPO_4$ by ATP, which might be expected from observations with tricalcium phosphate[6], does not seem to be apparent when the concentration of ATP equals 40 times the Mg concentration (Fig. 1; Table III, col. f, l to o). But the inverse relation, A'_{of} 1/40 × Mg'_{of}, almost coincides with the minimum ^{32}ph uptake, in

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OOD PLASMA AS CALCULATED WITH CONSTANTS OF CITED AUTHORS

Initial concentrations		*Distribution in per cent*[e]						*Refer to*	
^{32}ph		*ATP*		*Mg*		^{32}ph			
Order	$M^{a,\,d}$	*free*	*combined with Mg*	*free*	*combined with ATP or* ^{32}ph	*free*	*combined with Mg*	*figure*	*point*
j	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*	*s*
	$1.619 \cdot 10^{-2\,d}$			+		+			
	$1.619 \cdot 10^{-2}$				+		+		
=	$1.619 \cdot 10^{-2}$				+		+		
	$1.619 \cdot 10^{-2}$				+		+		
=	$1.619 \cdot 10^{-2}$				+		+	1	a
	$4.027 \cdot 10^{-6}$				+	+			
	$4.027 \cdot 10^{-6}$				+	17	83		
=	$4.027 \cdot 10^{-6}$				+		+	2	d
	$4.717 \cdot 10^{-6}$				+		+		
	$4.027 \cdot 10^{-6}$	97	3		+				
	$4.027 \cdot 10^{-6}$	98	2		+				
	$4.027 \cdot 10^{-6}$	+		+				2	a
	$4.027 \cdot 10^{-6}$	+		+				2	b
	$4.717 \cdot 10^{-6}$		+	+					
	$4.717 \cdot 10^{-6}$	+		+				1	d
	$4.717 \cdot 10^{-6}$	+		+				1	b
	$4.717 \cdot 10^{-6}$	97	3		+			1	e
	$4.717 \cdot 10^{-6}$	+			+				

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[f] K_d = dissociation constant of liquid $MgHPO_4$ in biological fluids with an ionic activity $\mu = 0.16$.

[g] K_d = dissociation constant for the Ca-ATP complex and used in lieu of the K_d for the Mg-ATP complex. It is assumed that both values do not differ appreciably because the respective solubilities of various calcium and magnesium phosphates agree sufficiently for the purpose of this estimate.

[h] K_d of the Mg-ATP complex adjusted to a μ of 0.16.

which case both free ATP and Mg ions exist in the extracellular fluid. The free ATP may be thought of as being available for the solubilization and dissociation of undissociated $MgHPO_4$ similar to observations on insoluble alkaline metal phosphates by NEUBERG AND MANDL[12]. It seems therefore that solubilization and dissociation of phosphates are primarily dependent on the degree of dissociation of ATP rather than on the proportion of ATP to the metal ion.

Effects of Mg^{+2}. Table III and Fig. 2 present a situation which is similar to that for varying concentrations of ATP in Fig. 1. Magnesium favours ^{32}ph uptake if almost all Mg is combined with ATP in the presence of an excess of free ATP, even if the initial concentration of ATP is less than 10^{-14} moles per liter of extracellular fluid (Table III, col. g, l to o). Equal concentrations of ATP and Mg (col. h, r, s) and an excess of Mg over ATP induce sharp inhibition of ^{32}ph uptake because both remain uncombined with each other under the conditions of this experiment (col. e, n). A balance between inhibition and stimulation occurs when Mg'_{of} is approximately 40 times greater than A'_{of} (Fig. 2, point *c*). Furthermore, ^{32}ph uptake is stimulated by the dissociation pressure of Mg in the form of undissociated $MgHPO_4$ (Table III, col. o, q, r, s). The required Mg apparently may be supplied from any Mg which is not already bound as a Mg-ATP complex with a much lower dissociation constant. The ^{32}ph uptake was sharply reduced by hemolysis when Mg'_{of} became equal to the initial ^{32}ph concentration (col. i, k), in which case all ^{32}ph approaches a complete combination with Mg as undissociated $MgHPO_4$ (col. q). This, in turn, may form deposited $MgHPO_4$ on the cell antisphering layer, for which the solubility product is three orders of magnitudes below the dissociation constant of $MgHPO_{4\,(\text{liquid})}$ (col. b).

The specific conditions favouring ^{32}ph uptake point again to the activity of the ATP-Mg-E-K complex (Table II, react. 3b) which may be reduced if ATP and Mg remain uncombined with each other and permit the formation of undissociated $MgHPO_4$ (Table III, col. l, n, o, q, r, s). Dissociated phosphate apparently is necessary for phosphate uptake according to previously discussed schemes[2]. The ADP $\rightleftharpoons$ ATP system seems to be required to act as mediator for the transfer of inorganic phosphate ions to hexose-6-phosphate with the help of hexokinase which requires equimolar concentrations of ATP and Mg^{+2} [4,5]. Since the dissociation constant of the hexokinase reaction is in the neighbourhood of 10^{-3} [3], some hexose-6-phosphate might be transformed into hexose-1-phosphate by phosphoglucomutase with an equilibrium constant of 0.05 to 0.06[3]. SACKS[13] has presented evidence that when rat liver was incubated with plasma containing ^{32}P glucose-1-phosphate appeared labelled with ^{32}P.

Furthermore, GOURLEY[1] has shown that ^{32}ph may be incorporated rapidly into the labile P of ATP presumably at the surface of human erythrocytes. Support for the utilization of ^{32}ph by ATP for phosphate transfer into hexoses comes from a study by SCHILD AND MAURER of ^{32}ph utilization by human erythrocytes with the aid of paper electrophoresis[14]. They showed that ADP forms $AT^{32}P$ with ^{32}P and apparently releases it to creatine after only 10 min of incubation, while the formation of glucose-1-phosphate is not apparent until after an incubation time of approximately 4 hours. The results of both experimental approaches are substantially in agreement with the conclusion reached in the present investigation that ^{32}P can be incorporated into the ADP $\rightleftharpoons$ ATP system, from whence it may be transferred to a hexose.

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SUMMARY

1. The addition of adenosine triphosphate (ATP) to rabbit erythrocytes *in vitro* stimulated the uptake of labelled orthophosphate ions.

2. The addition of calcium or magnesium ions alone inhibited phosphate uptake when the concentrations of Ca, Mg, and phosphate were approximately equal.

3. The addition of both magnesium ions and ATP had a variable effect on phosphate uptake ranging from inhibition, when the concentrations of phosphate and magnesium were approximately equal, to stimulation when the concentration of ATP was greater or less than that of Mg.

4. An attempt has been made to explain these observations on the basis of the known effects of ATP on the solubility of divalent alkaline metal phosphates and the possible interactions which might occur in a system including ATP, Mg and phosphate ions.

RÉSUMÉ

1. L'addition d'adénosine triphosphate (ATP) à des érythrocytes de lapin *in vitro* stimule la fixation d'ions orthophosphates marqués.

2. L'addition d'ions calcium ou magnésium seuls inhibe la fixation du phosphate lorsque les concentrations en Ca, Mg et phosphate sont à peu près égales.

3. L'addition simultanée d'ions Mg et d'ATP a des effets variables sur la fixation des phosphates, allant de l'inhibition, si les concentrations en phosphate et en magnésium sont sensiblement égales, à la stimulation, si la concentration en ATP est inférieure ou supérieure à celle des ions Mg.

4. Les auteurs tentent d'expliquer ces observations à l'aide des effets bien connus de l'ATP sur la solubilité des phosphates des métaux alcalins divalents et des interactions possibles qui peuvent prendre naissance dans un système qui renferme de l'ATP et des ions Mg et phosphate.

ZUSAMMENFASSUNG

1. Die Zugabe von ATP zu Kaninchenerythrocyten *in vitro* fördert die Aufnahme von markierten Orthophosphationen.

2. Die Zugabe von Calcium- oder Magnesiumionen allein hindert die Phosphataufnahme, wenn die Konzentrationen von Ca, Mg und Phosphat ungefähr gleich sind.

3. Die Phosphataufnahme in Gegenwart von Magnesiumionen und ATP wird gehemmt, wenn die Konzentrationen von Phosphat und Mg ungefähr gleich gross sind. Sie wird begünstigt, wenn die ATP-Konzentration grösser oder kleiner als die Mg-Konzentration ist.

4. Es wird versucht die Beobachtungen auf Grund des Einflusses von ATP auf die Löslichkeit der Erdalkaliphosphate und der im System Mg, ATP, Phosphat möglichen Reaktionen zu erklären.

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