OBSERVATIONS ON THE MECHANISM OF PHOSPHATE UPTAKE BY RABBIT ERYTHROCYTES*

PHOSPHATE ADSORPTION IN RELATION TO CELL SURFACE STRUCTURE; EQUILIBRIA OF PHOSPHATE ADSORPTION AND ABSORPTION

by

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INTRODUCTION

The hypothesis has been advanced in previous papers of this department[1-3] that orthophosphate ions (ph) are taken up by erythrocytes by means of cell membrane reactions involving the formation of adenosine triphosphate. The evidence for this hypothesis is based on a study of the relative distribution of ^{32}P in the inorganic phosphate and organic acid-soluble phosphate esters of whole blood. The results of the present investigation suggest that there are three distinct and separate stages in the uptake of phosphate by erythrocytes. The data for ^{32}P uptake from a series of ^{32}P concentrations over a range of 10^5 have been interpreted to describe two adsorption stages followed by adsorption of phosphate into the cell. The possible forms of combining phosphate with adsorbent were utilized to establish apparent equilibria for each stage of phosphate uptake.

METHODS

Blood of healthy rabbits was drawn by cardiac puncture into a 30 ml syringe, which contained about one milligram of sodium heparinate[1,4,5]. At least three weeks elapsed between samplings of each animal.

The following reaction mixture was prepared: 0.5 ml of $H_3{}^{32}PO_4$ with a radioactivity of $\sim 1.8 \cdot 10^{-4}$ mc/ml was placed into 10 cm Pyrex tubes which contained sufficient NaCl to make the final salinity of the mixture 0.9%. Solutions of ^{32}P concentrations were prepared by either diluting the original $H_3{}^{32}PO_4$ of the Oak Ridge National Laboratory or by adding $H_3{}^{32}PO_4$ to which the required amount of 0.1 *N* NaOH was added to adjust the pH to 7.40. Sufficient plasma was then added to the tubes to make the final hematocrit value (H″) 0.31, when 3.5 ml of whole blood of the same sample was subsequently added.

The dilution by the plasma alone reduced the original hematocrit (H) to 0.35 (H′). This uniform hematocrit was expected to eliminate variations of P uptake which had been observed by PERTZOFF AND GEMMILL and in other experiments of this investigation. This expectation did not materialize for reasons to be reported separately. The diluent plasma was obtained by centrifuging blood for

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5 min at 3,000 r.p.m. The required volume of plasma was removed in accordance with prior duplicate H-determinations in closed Winthrobe tubes for 30 min at 3,000 r.p.m. A buffer was dispensed with because the final blood pH remained close to 7.40, thus eliminating any extraneous ion effects. At this pH phosphate ions are proportioned between the forms HPO_4^{-2} and $H_2PO_4^-$ in the ratio 2.12 : 1.00. The final composition of the reacting blood mixture is enumerated in Table I.

The mixture was incubated in the stoppered tubes for 3 h at 37.4° C, cooled in an ice bath and centrifuged for 5 min at 3,000 r.p.m. at room temperature. Then the suspension was again chilled, and 0.20 ml of the supernatant fluid (f) were plated out on a one inch aluminum planchet. They had been sprayed previously with Krylon which prevented flaking of the sample and confined the fluid to a central drop.

The aliquot was dried under infrared light and then its radioactivity was counted taking into account instrument calibration. Reference activities were obtained from controls, in which ^{32}P was added to ice-cooled blood after incubation. Register counts were converted to moles (m) of labeled orthophosphate (^{32}ph) per unit volume of plasma (p), fluid (f), or erythrocytes (c).

Each experiment was run in duplicate, and two 0.2 ml aliquots of the supernatant of each tube were counted to 3 × 4,096 counts each. Thus, a total of 12 estimations of radio-activity of each experiment gave a mean value with a standard error $s_{\bar{x}} = \pm 2.75\%$ for the mean $\bar{x}$ of the sample, which corresponded to a standard error $\sigma_{\bar{m}} = 6.16\%$ for the mean $\bar{m}$ of a large number of samples[6]. Errors of this magnitude become insignificant in logarithmic expressions as shown in Fig. 1.

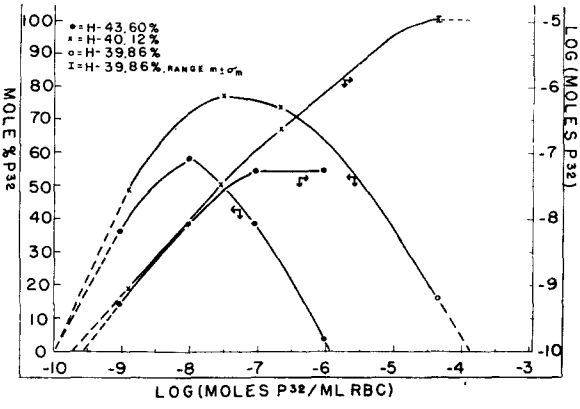

Fig. 1. Uptake of radio-active phosphate by rabbit erythrocytes from a series of ^{32}ph concentration at pH 7.4. Hematocrit fractions of 1) freshly drawn blood: H = 0.4363, ●; H = 0.401, ×; H = 0.399, O and I. 2) blood diluted with its own plasma: H′ = 0.350. 3) blood diluted with plasma and saline ^{32}ph: H″ = 0.315, ●; H″ = 0.314, ×; H″ = 0.311, O and I. Abscissa: Logarithm of initial ^{32}ph concentration as moles/ml RBC, C_{oc}. Ordinates: Right side, total ^{32}ph uptake by an equivalent of 3.5 ml of fresh blood as log moles ^{32}ph, log x/m. Left side, total ^{32}ph uptake by an equivalent of 3.5 ml. of fresh blood as per cent of total initial moles of ^{32}ph supply.

TABLE

COMPOSITION OF RABBIT BLOOD AND ITS

Function	*Units*	*Symbols*		*Values*
Original blood, hematocrit	vol. %	H		0.436 (A)
plasma fraction	vol. %	$1-H$		0.564
Blood after dilution with plasma				
hematocrit	vol. %	H'		0.350
plasma fraction	vol. %	$1-H'$		0.650
Blood after additional dilution with ^{32}ph solution				
hematocrit	vol. %	H''		0.310
fluid fraction	vol. %	$1-H''$		0.690
Supply of ^{32}ph, moles/ml fluid	m/ml f	C'_{of}	$5.205 \cdot 10^{-7}$	$5.091 \cdot 10^{-8}$
Supply of ^{32}ph, moles/ml RBC	m/ml c	C'_{oc}	$9.348 \cdot 10^{-7}$	$8.914 \cdot 10^{-8}$
Uptake of ^{32}ph, moles/ml fluid	m/ml f	x/m_f	$1.640 \cdot 10^{-8}$	$1.618 \cdot 10^{-8}$
Uptake of ^{32}ph, moles/ml RBC	m/ml c	x/m_c	$3.574 \cdot 10^{-8}$	$3.526 \cdot 10^{-8}$
Equilibrium ^{32}ph, moles/ml fluid	m/ml f	C'_f	$5.042 \cdot 10^{-7}$	$3.473 \cdot 10^{-8}$
Equilibrium $^{31+32}ph$, moles/ml fluid	m/ml f	C_f	$1.532 \cdot 10^{-6}$	$1.063 \cdot 10^{-6}$

Legends: c = erythrocytes; C = equilibrium concentration; f = extracellular fluid;

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RESULTS AND DISCUSSION

The experimental results are summarized in Table I and illustrated in Fig. 1, which shows that cells take up ^{32}ph from initial concentrations of as low as 10^{-9} *m* ^{32}ph/ml cells (C_{oc}). (Radioactivity in the 0.2 ml counting aliquots is at the lower threshold of resolution at this concentration.) While the greatest fraction of ^{32}ph is taken up from solutions with C_{oc} of 10^{-8} to 10^{-7} m/ml cells, uptake saturation is initiated at C_{oc} of approximately 10^{-7} and ends when the relative uptake approaches zero.

The evident influence of original hematocrit on the level of ^{32}ph saturation will be the subject of a subsequent report. It enters this presentation solely to further ideas developed herein.

The initially almost linear plot of the logarithms of ^{32}ph uptake in moles/sample (x/m) as a function of added ^{32}ph, C_{oc}, suggests ^{32}ph adsorption on the cells. Conversion of the uptake data into the terms of a FREUNDLICH adsorption isotherm is presented in Tables I and II. A plot of the isotherm in Fig. 2 illustrates how an extremely narrow range of the total equilibrium concentration of original ^{31}ph plus added ^{32}ph (C_f) causes a significantly high ^{32}ph uptake per unit volume of fluid or of cells (x/m_f or x/m_c). Coincident slopes, $\frac{\Delta \log x/m_{f,c}}{\Delta \log C_f} = 845.1$, of the isotherms of both blood samples with H = 0.436 (A),

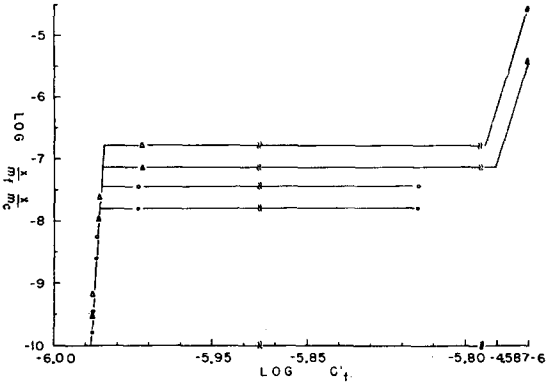

Fig. 2. Uptake of ^{32}ph by rabbit erythrocytes from a series of ^{32}ph concentrations at pH 7.4, expressed as a FREUNDLICH adsorption isotherm. Hematocrit fractions of freshly drawn blood before dilution with plasma and saline ^{32}ph: H = 0.4363, ● and O; H = 0.4006, ▲ and △. Abscissa: Logarithm of equilibrium ^{32}ph concentration as moles/ml extracellular fluid, $C'f$. Ordinate: Logarithm of ^{32}ph uptake as 1) moles/ml of extracellular fluid, x/m_f, ● and ▲; 2) moles/ml of RBC, x/m_c, O and △.

I

UPTAKE OF RADIOACTIVE PHOSPHATE

Values					
				0.401 (B)	
				0.599	
				0.350	
				0.650	
				0.310	
				0.680	
$5.125 \cdot 10^{-9}$	$5.192 \cdot 10^{-10}$	$2.529 \cdot 10^{-5}$	$1.125 \cdot 10^{-7}$	$1.552 \cdot 10^{-8}$	$6.306 \cdot 10^{-10}$
$9.203 \cdot 10^{-9}$	$9.324 \cdot 10^{-10}$	$4.995 \cdot 10^{-5}$	$2.200 \cdot 10^{-7}$	$3.034 \cdot 10^{-8}$	$1.233 \cdot 10^{-9}$
$2.450 \cdot 10^{-9}$	$1.544 \cdot 10^{-10}$	$3.721 \cdot 10^{-6}$	$7.427 \cdot 10^{-8}$	$1.087 \cdot 10^{-8}$	$2.892 \cdot 10^{-10}$
$5.341 \cdot 10^{-9}$	$3.366 \cdot 10^{-10}$	$8.244 \cdot 10^{-6}$	$1.626 \cdot 10^{-7}$	$2.380 \cdot 10^{-8}$	$6.332 \cdot 10^{-10}$
$2.674 \cdot 10^{-9}$	$3.648 \cdot 10^{-10}$	$2.157 \cdot 10^{-5}$	$3.825 \cdot 10^{-8}$	$4.652 \cdot 10^{-9}$	$3.414 \cdot 10^{-10}$
$1.031 \cdot 10^{-6}$	$1.028 \cdot 10^{-6}$	$22.594 \cdot 10^{-5}$	$1.066 \cdot 10^{-6}$	$1.033 \cdot 10^{-6}$	$1.028 \cdot 10^{-6}$

H = hematocrit; m = moles; s = saturation; x = moles removed from extracellar phase.

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and H = 0.401 (B), indicate a common adsorption process. The adsorbing surface reaches phosphate saturation at initial concentrations of $C_{cos}' = \log^{-1} 6.78$ to 6.50 according to Table II. The mean phosphate concentration becomes in this case $C_{fs}' = 1.100 \cdot 10^{-6}$, which is in equilibrium with a mean of $4.83 \cdot 10^{-8}\ m$ ^{32}ph/ml of fluid (x/m_{fs}').

TABLE II

FREUNDLICH ADSORPTION ISOTHERM OF UPTAKE OF RADIO-ACTIVE PHOSPHATE BY RABBIT BLOOD

Function	*Units*	*Symbol*	*Values*			*Difference*
			H=0.436	0.401	*Mean*	
Isotherm			$x/m_f = x/m_c = 5{,}050\ C_f^{845.1}$			
Equilibrium $^{31+32}$ph at complete adsorption	m/ml $f \cdot 10^{-6}$	C_{fs}'	1.100	1.100	1.100	
Uptake of ^{32}ph at adsorption equilibrium	m/ml $f \cdot 10^{-8}$	x/m_{fs}'	1.628	7.427	4.828	78.08%
	m/ml $c \cdot 10^{-8}$	x/m_{cs}'	3.583	1.626	9.923	77.97%
Supply of ^{32}ph at adsorption equilibrium	log m/ml c	C_{ocs}'	—6.78	—6.50	—6.64	

It is of interest to relate the ^{32}ph uptake at adsorption equilibrium, x/m_{fs}', to the structure of the cell surface, for which purpose this value is converted to x/m_{cs}' in Table II. Pertinent data adapted from the literature are assembled in Table III, wherein the surface distributions of proteins, lipids, lecithin, Ca, and of effective electrons (e^-) are compared with the cell volume. A consideration of the adsorption of only ^{32}ph ions shows that they could conceivably be located on the monomolecular lipoid antisphering film. A mean of $3.65 \cdot 10^6$ ^{32}ph ions are adsorbed, or almost a sufficient number to satisfy $4.37 \cdot 10^6$ e^-, which occupy 0.40% of the membrane surface. This approximate equivalency satisfies the requirement, observed by ROBERTSON AND WILKINS[8], that one anion entering a cell corresponds to one e^- leaving a cell surface during respiration.

TABLE III

DIMENSIONS OF RABBIT ERYTHROCYTES

Dimension	*Radial thickness of membrane incl.specific substance in A*	*Value*	*Unit*	*Reference*
RBC/ml RBC		$1.64 \cdot 10^{10}$	cells	14
RBC surface		$1.10 \cdot 10^{-6}$	cm^2	14
RBC volume		$6.1 \cdot 10^{-11}$	ml	14
Volume of membrane antisphering layer	20–25	$3.76 \cdot 10^{-13}$	ml	9, 23, 25
Fraction of antisphering layer of RBC volume		0.65, 0.58	%	9, 23
Volume of cell membrane	81.5	$1.44 \cdot 10^{-12}$	ml	23
Fraction of membrane of RBC volume		2.36	%	23
Effective surface e^-		$4.37 \cdot 10^6$	e^-/cell surface	24
Fraction of surface area held by effective e^-		0.40	%	24
Lipoprotein-stromatin	81.5, 57.5, 60–65	$1.10 \cdot 10^8$	molecules/cell surface	22, 9, 25
Lipoprotein adsorption sites on cell surface				
lipid adsorbing		$5.75 \cdot 10^7$	sites/cell	23
lipoprotein adsorbing		$1.29 \cdot 10^8$	sites/cell	23
Lecithin-protein surface layer	53	$2.47 \cdot 10^8$	lecithin molecules/cell	9
Cross sectional area of lecithin molecule		9.96 A^2		
Cross sectional area assigned to lecithin molecules on RBC surface		156.25 A^2		
Ca	53	$1.10 \cdot 10^8$	ions/cell surface	9
Hemoglobin		$6.47 \cdot 10^8$	molecules/cell	14

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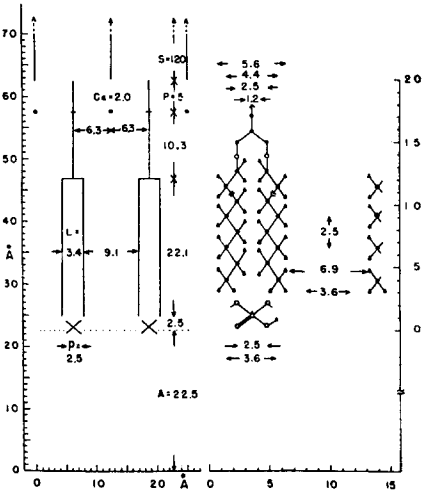
These effective adsorption sites are probably on the outer face of the antisphering film, which represents from 0.58 to 0.65% of the total cell volume, if the cell dimensions of WINKLER AND BUNGENBERG DE JONG[9], PONDER[23] and MITCHISON[25] are accepted. An estimate of one-half per cent for the occupied cell surface and cell volume fractions appears reasonable.

Table IV illustrates the relative proportion of adsorption sites per $^{31+32}$ph ion in the subantisphering layer of the membrane, on which an average of $3 \cdot 10^7$ $^{31+32}$ph ions are located. (Ten times more $^{31+32}$ph ions than ^{32}ph ions are adsorbed on the cells. Therefore a consideration of $^{31+32}$ph adsorption can neglect the disposition of ^{32}P.) The surface structure of the membrane appears to be a three-partite layer[9] with a mosaic structure of alternating lecithin molecules (Le) and Ca^{+2}, each one subtended by a protein molecule as illustrated in Fig. 3. The phosphorylated end of a lecithin molecule (32.4 A long) is based on a short protein molecule (Pr), and one Ca^{+2} is subtended by a stromatin protein chain (S) which is thought to lie in the same plane with the other



Tri-partite external part of cell membrane in schematic form

Terminal configuration of lecithin molecules with adsorbed phosphate ions

Fig. 3. Proposed phosphate adsorption complex on the erythrocyte surface[9,23].

Coordinates in Angström units·

Symbols:

A = antisphering lipoid film
Ca = calcium ion
L = lecithin molecule
P = short protein molecule
p = phosphate ion
S = stromatin chain atoms of:
● = carbon
○ = oxygen
▲ = hydrogen
△ = phosphorus

(Modified after A. FREY-WYSSLING, *Submicroscopic Morphology of Protoplasm and its Derivatives* (1953) p. 266, Elsevier Publishing Company, Inc., New York and Amsterdam).

molecules and ions. LINDEMANN[10] located by electron-microscopy two membrane layers in the human RBC ghost, in which a lipoid surface film is superimposed on a proteinaceous "stromatin" network, which has projections extending into the space of the lipoid film. They may be interpreted as being protein chains, which have coacervated during the preparatory removal of antisphering and lecithin lipid molecules. The total quantity of both lipids amounts to a bimolecular layer around the erythrocytes, which had been observed as early as 1925 by GORTER AND GRENDEL[11]. Phosphate ions of the fluid, in this case principally ^{31}ph, are actually faced by only one dense adsorption surface, the palisade-like array of lecithin molecules inside the relatively less dense antisphering film. Table IV shows that 2.1 Ca^{+2} ions and 4.5 proteinaceous adsorption points per phosphate ion combine with 2.13 Le molecules to form an adsorption complex of the relative composition $(Ca_{2.1} \cdot Le_{2.1} \cdot Pr_{4.5} \cdot ph)$. Its apparent molecular weight may be estimated as approximately 80,745 if the molecular weight of protein is set at one Svedberg unit.

The proportions of constituents of the complex have been derived from the fact that between $5.8 \cdot 10^7$ and $7.0 \cdot 10^7$ lipoid molecules or adsorption sites exist on the

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TABLE IV

DISPOSITION OF ADSORBED TOTAL PHOSPHATE ON THE SURFACE OF RABBIT ERYTHROCYTES

Function	*Mean values of blood samples A and B*	*Units or derivation*
$\frac{^{32}\text{ph uptake}}{^{32}\text{ph adsorption}}$ at equilibria	25.85	$\frac{x/m_{cs}}{x/m_{cs}'}$
Fraction of original ^{31}ph adsorbed	$7.18 \cdot 10^{-7}$	m
Total phosphate ions/cell ($^{31+32}$ph)	$3.00 \cdot 10^{7}$	ions/cell
Ratios of Ca^{+2}: lecithin:protein:$^{31+32}$ph	2.1:2.1:4.5:1	
Proposed M. Wt. of membrane complex	80,745	Protein = 1 Svedberg unit = 17,600
Minimum surface space/phosphate ion	$1.56 \cdot 10^{-14}$	cm^2
Calc. maximum number of phosphate ions/RBC surface	$7.04 \cdot 10^{7}$	
Fraction of $^{31+32}$ph ions adsorbed on complex of calculated maximum no. of ions/cell surface	42.7	%
Volume of phosphate-membrane complex	$3.92 \cdot 10^{-12}$	ml
Fraction of complex volume of cell volume	6.42	%

membrane[9, 23, 26]. The mean value of $6.4 \cdot 10^{7}$ molecules may be considered to be coordinated with $3.0 \cdot 10^{7}$ phosphate ions with a ratio of 2.1. Since one Ca^{+2} is coordinated with each lecithin molecule[9] according to Fig. 3, the same ratio is valid for the Ca–ph proportion. The number of protein molecules or adsorption sites per cell surface[9, 23] has been estimated at 12.9 to $14.1 \cdot 10^{7}$ which gives a mean ratio of 4.5 protein molecules per phosphate ion. The proposed membrane structure of Fig. 3 permits a HPO_4^{-2} tetrahedron with a diameter of 3.6 A to be positioned between and level with the two external and terminal methyl groups of the two C_{16} lecithin chains. Their nth carbon-atoms are 4.4 A apart and their $(1-n)$th atoms 2.5 A. The two negative oxygen bonds are positioned just opposite the two terminal and internal H-atoms of both chains, thus providing an opportunity for shared H atoms between C_1 and phosphate. The observation that approximately 2 lecithin molecules are coordinated with each phosphate ion may be due to the possibility that a phosphate ion may be attracted alternately by the two external methyl groups of a lecithin molecule and by the tangential $-CH_2$ groups of two adjoining molecules. There is ample space for a phosphate ion of 3.6 A to be attracted by these $-CH_2$ groups which are 6.9 A apart. This distance is subject to variation according to the exact location and angle of attachment of the two internal methyl groups of the lecithin molecule to its substrate.

The required tangential area to accommodate such a phosphate complex is estimated at $1.56 \cdot 10^{-14}$ cm^2, and there is sufficient space on the cell surface for $7.04 \cdot 10^{7}$ of such complexes. The phosphate ion population was found by experiments to be only $3.00 \cdot 10^{7}$. The discrepancy can be credited to hematocrit irregularities which will be discussed in another paper. But the agreement of the population values is sufficiently close to warrant a calculation of the volume occupied by the complex. Assuming that the complex has a mean density of 1.029 and a molecular weight of 80,745, $3.92 \cdot 10^{-12}$ ml or 6.42% of the erythrocyte volume contains the complex (Table IV). Utilization of PONDER's data for cell-membrane thickness indicates that 2.36% of the cell volume appears as a lipoidal protein membrane (Table III). Again, the discrepancy is due to hematocrit effects.

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The question arises of how phosphate adsorption by cells compares with its adsorption by inorganic units of similar size. Clays free of exchangeable bases, like bentonite and kaolinite, serve as convenient means of comparison. They also adsorb orthophosphate according to a Freundlich adsorption isotherm[12], the adsorbed ions being exchanged for hydroxyl or other anions on the clay particle. GOHRING AND BARTHOLOMEW[13] found that bentonite clay of an average particle diameter of 2.75 μ adsorbs a maximum of $1.73 \cdot 10^{17}$ ions/cm^2 of surface area at a pH of 7.4. This is several orders of magnitude more than the adsorption of $2.73 \cdot 10^{13}$ ions/cm^2 by rabbit erythrocytes with a diameter of 7.5 μ of these experiments. It is therefore apparent that adsorption by erythrocytes is limited not by their surface area, but rather by their surface configuration and by their transfer equilibrium between cell surface and interior.

Adsorption of $^{31+32}$ph accounts for only a fraction of total uptake. Table V compares concentrations of phosphate in the fluid (C_{fs}) and in the cells (x/m_{cs}) at uptake saturation. (Uptake is defined as the sum of adsorption and absorption). Close to 83% of the average phosphate uptake is in the cell lumen under the conditions of this experiment.

TABLE V

UPTAKE AND ABSORPTION OF TOTAL PHOSPHATE BY RABBIT ERYTHROCYTES

Function	*Symbols*	*Mean values of blood samples A and B*	*Units*
Equilibrium $^{31+32}$ph at complete uptake	C_{fs}	$1.206 \cdot 10^{-5}$	m/ml
Uptake of $^{31+32}$ph at total uptake	x/m_{fs}	$2.171 \cdot 10^{-6}$	m/ml
Uptake of $^{31+32}$ph at total uptake	x/m_{cs}	$4.724 \cdot 10^{-6}$	m/ml
Equilibrium $^{31+32}$ph at complete absorption	$C_{fs}'' = C_{fs}-C_{fs}'$	$1.096 \cdot 10^{-5}$	m/ml
Absorption $^{31+32}$ph at complete absorption	$x/m_{cs}'' = x/m_{cs} = x/m_{cs}'$	$3.906 \cdot 10^{-5}$	m/ml
Ions $^{31+32}$ph/cell interior		$1.434 \cdot 10^{8}$	
Fraction of adsorption of total uptake by cells at complete adsorption		17.3	%
Fraction of absorption of total uptake by cells at complete absorption		82.7	%

It seems hardly a coincidence, that out of a total uptake of $4.72 \cdot 10^{-6}$ moles of phosphate, $3.91 \cdot 10^{-6}$ moles or $1.43 \cdot 10^{8}$ phosphate ions are absorbed by the cell interio- containing approximately $6.47 \cdot 10^{8}$ molecules of hemoglobin[14] (Table III). A possible equilibration between phosphate and hemoglobin may exist because their concentrations fall within the same order of magnitude, even though the hemoglobin concentration is a rather general estimate[14]. Observations of hemolysis by initial ^{32}ph concentrations beyond total uptake favour this concept.

It is possible now to state in Table VI absolute mean phosphate concentrations and apparent equilibria for the 3 uptake stages. The absolute mean concentration is defined as the average concentration of $^{31+32}$ph of both blood samples *A* and *B* in the 3 phases with respect to unit volume of their compartments, which have been defined above as the antisphering layer, the phosphate complex, and the cell interior.

A positive concentration gradient exists between the outermost phosphate compartments, C_{fs}, and $(Ca_{2.1} \cdot Le_{2.1} \cdot Pr_{4.5} \cdot ph)$. But the gradient becomes less than unity for the movement of phosphate from this complex into the cell. The values for the apparent equilibrium constants K_e', K_e'', and K_e''' express these gradients quanti-

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tatively. These constants of the law of mass action reduce to ratios of internal to external phosphate concentrations because the proportion of solvents and of adsorbants are constant within each experimental series.

TABLE VI

APPARENT CONCENTRATION EQUILIBRIA OF TOTAL PHOSPHATE ADSORPTION, ABSORPTION, AND UPTAKE BY RABBIT ERYTHROCYTES

Function	*Symbol*	*Unit*	*Values*		*Mean*
			A	B	
Absolute concentration of $^{31+32}$ph in fluid at complete adsorption	C_{fs}'	$M \cdot 10^{-3}$	1.100	1.100	1.100
^{32}ph in antisphering film at complete adsorption		$M \cdot 10^{-2}$	0.598	2.641	1.619
$^{31+32}$ph in cell membrane		$M \cdot 10^{-2}$	1.348	5.525	3.437
$^{31+32}$ph in cell volume		$M \cdot 10^{-3}$	2.98	7.70	4.00
Apparent equilibrium constants $K_e = \frac{\text{(P) ads. or abs.}}{\text{(P) in next outer phase}}$					
for adsorption by antisphering film	K_e'		5.43	23.93	14.68
for adsorption by cell membrane	K_e''		2.25	2.09	2.17
for absorption by cell interior	K_e'''		0.022	0.147	0.085
for uptake	K_e		0.270	7.34	3.81

Transport of inorganic phosphate from the phosphate complex into the cell lumen appears to be partly by diffusion because of the comparatively low value for K_e''' of 0.0845. It actually represents only a partial equilibrium due to the formation of phosphate esters from inorganic phosphate. The esters are located principally in the cell interior as described by GOURLEY[1], SACKS[15], and by SACKS AND ALTSCHULER[16]. They suggest as the site of ester formation some phase of the cell membrane, which is conceivably in the phosphate-lecithin complex. Its inorganic phosphate concentration surpasses that of the other external and internal phosphate phases.

Labile P of ATP, which was found by GOURLEY to be a precursor for phosphoesters, consequently appears to be utilized for the formation of ATP from ADP in the intermediate P–complex. Similarly, SACKS AND ALTSCHULER demonstrated the formation of organic phosphate compounds on muscle cell membranes. SACKS[17] showed subsequently, that glucose-6-phosphate can be formed at muscle surfaces. Further evidence by CLARKSON AND MAIZELS[18] and by ROTHSTEIN, MEIER AND SCHARFF[19] may be interpreted in favour of the existence of phosphate splitting enzymes on external cell surfaces.

ROSENBERG[20] discussed the necessity of an energy potential gradient between outside and inside reaction sites of single cells to activate the transport of any particular metabolite. Since a membrane or interface must exist to maintain an energy gradient, a phosphate carrier is prerequisite for active phosphate transport. The question to be answered is: Does the carrier, in this case apparently an adenosine polypyrophosphate, obtain its potential activity inside or outside the cell interior? If it were formed internally, it would posses an outwardly directed diffusion tendency, and could not act as a carrier in the opposite direction. Therefore it must be assumed to be transformed outside of the cell interior into an active stage from a precursory form. LE FEVRE AND LE FEVRE[21] expressed a similar thought for glucose transfer into human cells and GIBSON[22] indicated an activation reaction in the interface itself.

The present observations suggest that the highest inorganic phosphate concentration exists in the cell membrane itself and not in the antisphering layer, which must

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be interpreted to mean that inorganic phosphate is metabolized from the precursor ($Ca_{2.1} \cdot Le_{2.1} \cdot Pr_{4.5} \cdot ph$). Furthermore, differences of the degree of blood dilution of samples *A* and *B* have apparently no significant effect on K_e'' and on the shape of the FREUNDLICH adsorption isotherm (Table VI). The absence of such an effect means that the summation of the reaction rates of $^{31}+^{32}ph$ adsorption on lecithin and of the simultaneous partial incorporation of $^{31}+^{32}ph$ into organic substrates is constant.

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SUMMARY

1. Rabbit erythrocytes were incubated for 3 hours with $1 \cdot 10^{-9}$ to $5 \cdot 10^{-5}$ moles of saline radioactive orthophosphate per ml of cells.

2. Phosphate uptake was postulated to proceed by a) adsorption on the cell according to a Freundlich adsorption isotherm, b) absorption by the cell interior.

3. The cell surface became saturated with phosphate when its ^{32}P supply exceeded $2.5 \cdot 10^{-7}$ moles/ml cells and at a total phosphate equilibrium concentration of $1.1 \cdot 10^{-6}$ moles/ml extracellular fluid. The cells achieved uptake saturation at a mean ^{32}P supply of $5 \cdot 10^{-6}$ moles/ml cells and at a total phosphate equilibrium concentration of $1.2 \cdot 10^{-5}$ moles/ml of fluid.

4. ^{32}P adsorption could possibly proceed on the lipoid antisphering film. Its concentration of effective surface electrons approximated the number of adsorbed ^{32}P ions, and the area occupied by both was almost equal.

5. Total phosphate appeared to be adsorbed by a complex which is composed of 2.1 Ca ions + 4.5 protein chains + 2.1 lecithin molecules with one phosphate ion. Its estimated molecular weight is 80,745. The surface area of a spherical layer of this complex can accommodate approximately twice as many phosphate ions as were found experimentally subject to hematocrit irregularities. The fraction of cell volume occupied by the complex approaches the fraction enclosed by a subsurface proteinaceous membrane.

6. Inorganic phosphate absorption approached cell hemoglobin content at incipient hemolysis. Apparent equilibrium constants for both phases of adsorption and for absorption suggested formation of adenosine polypyrophosphates as phosphate carriers on the lecithin complex, from where inorganic and organic phosphates are transferred into the cell.

RÉSUMÉ

1. Des erythrocytes de lapin ont été incubés pendant 3 heures en présence de quantités d'orthophosphate radioactif comprises entre $1 \cdot 10^{-9}$ et $5 \cdot 10^{-5}$ moles par ml de cellules.

2. L'auteur suppose que la consommation de phosphate est due: a) à une adsorption sur la cellule selon un isotherme d'adsorption de Freundlich, b) à une absorption à l'intérieur de la cellule.

3. La surface de la cellule est saturée en phosphate quand la concentration initiale est plus de $2.5 \cdot 10^{-7}$ moles de ^{32}P par ml de cellule, la concentration totale à l'équilibre du phosphate dans le liquide extracellulaire étant de $1.1 \cdot 10^{-6}$ moles/ml. Le maximum de consommation par les cellules est atteint, en moyenne, à une concentration initiale de $5 \cdot 10^{-6}$ moles de ^{32}P/ml de cellules, la concentration totale à l'équilibre en phosphate étant de $1.2 \cdot 10^{-5}$ moles/ml de liquide.

4. L'adsorption de ^{32}P a peut être lieu à la surface du film lipoïdique antisphère. La concentration de ce film en électrons effectifs de surface est à peu près égale au nombre des ions ^{32}P adsorbés, et les superficies occupées par les électrons et par les ions ^{32}P sont presque identiques.

5. Il semble que le phosphate total soit adsorbé par un complexe constitué de 2.1 ions Ca + 4.5 chaînes protéiques + 2.1 molécules de lécithine pour 1 ion phosphate. Le poids moléculaire estimé serait de 80,745. L'aire de la surface d'une couche sphérique de ce complexe peut fixer environ deux fois plus d'ions phosphate qu'il a été déterminé expérimentalement avec considération d'irrégularités hematocrites. La fraction du volume cellulaire occupée par le complexe est approximativement égale à la fraction limitée par une membrane protéique sous la surface.

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6. L'absorption du phosphate minéral correspond sensiblement à la teneur de la cellule en hémoglobine au début de l'hémolyse. Les constantes d'équilibre apparentes pour les 2 phases de l'adsorption et pour l'absorption suggèrent que des adénosine polypyrophosphates transportent les phosphates sur le complexe lécithinique, à partir duquel les phosphates minéraux et organiques sont absorbés par la cellule.

ZUSAMMENFASSUNG

1. Erythrocyten von Kaninchen wurden 3 Stunden lang mit $1 \cdot 10^{-9}$ bis $5 \cdot 10^{-5}$ Mol isotonischen radioaktiven Orthophosphat pro ml Zellen inkubiert.

2. Es wurde postuliert, dass die Phosphataufnahme über a) die Adsorption an den Zellen nach einer Freundlich'schen Adsorptionsisotherme und b) über die Absorption vom Zellinneren verläuft.

3. Die Zellenoberfläche wurde mit Phosphat gesättigt, wenn der ^{32}P-Vorrat $2.5 \cdot 10^{-7}$ Mol/ml Zellen überschritt und bei einer Gesamtphosphatgleichgewichtskonzentration von $1.1 \cdot 10^{-6}$ Mol/ml Extrazellularflüssigkeit. Die Sättigungsaufnahme wurde von den Zellen bei einem mittleren ^{32}P-Vorrat von $5 \cdot 10^{-6}$ Mol/ml Zellen und bei einer Gesamtphosphatgleichgewichtskonzentration von $1.2 \cdot 10^{-5}$ Mol/ml Flüssigkeit erreicht.

4. Die ^{32}P-Adsorption könnte möglicherweise an dem Lipoidantisphären-Film vorsichgehen. Seine Konzentration wirksamer Oberflächenelektronen war annähernd gleich der Anzahl der adsorbierten ^{32}P-Ionen, und die von beiden besetzte Zone war beinahe gleich.

5. Es scheint, dass das gesamte Phosphat von einem aus 2.1 Ca-Ionen + 4.5 Proteinketten + 2.1 Lezithinmolekülen mit einem Phosphation zusammengesetzten Komplex adsorbiert wird. Das geschätzte Molekulargewicht beträgt 80,745. Das Oberflächengebiet einer kugligen Schicht dieses Komplexes kann ungefähr zweimal soviel Phosphationen unterbringen als experimentell gefunden wurde unter Berücksichtigung der Hämatokritunregelmässigkeiten. Der von dem Komplex eingenommene Bruchteil des Zellvolumens kommt dem von einer unter der Oberfläche befindlichen aus Proteinen bestehenden Membran gleich.

6. Die Absorption anorganischen Phosphats kommt dem Hämoglobingehalt der Zellen gleich bei beginnender Hämolyse. Anscheinende Gleichgewichtskonstanten für beide Phasen der Adsorption und für die Absorption lassen die Bildung von Adenosinpolypyrophosphaten als Phosphatträger auf dem Lezithinkomplex vermuten, von dem aus anorganische und organische Phosphate in die Zelle übergebracht werden.

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