INFLUENCE OF HEMATOCRIT AND PLASMA ON THE MECHANISM OF PHOSPHATE UPTAKE BY RABBIT ERYTHROCYTES*

by

HERBERT JONAS

Department of Pharmacology, University of Virginia Medical School, Charlottesville, Virginia (U.S.A.)

INTRODUCTION

A previous study of the mechanism of radioactive orthophosphate uptake by rabbit erythrocytes suggested that a two-stage adsorption process is followed by absorption of phosphate by the cell interior[1]. Phosphate saturation of the cell by these three steps of uptake is influenced by certain hematocrit dependent factors which this study attempts to elucidate by two approaches:

1. Some preliminary experiments indicated fluctuations of phosphate uptake, which parallelled hematocrit readings. This correlation of uptake and cell population is to be expected on the basis of the laws of absorption and permeability as shown by numerous investigators. Further evidence in support of this correlation is presented herein.

2. If there is a direct and simple correlation between cell concentration and phosphate uptake it follows that the same uptake should be observed in different samples of blood in which the cell concentrations have been adjusted to the same level. Such samples can be prepared by diluting aliquots of blood with their own plasma before incubation with orthophosphate. The results of the present experiment, in which this has been done, cast doubt on the concept of a simple and direct proportionality.

The results of this study are described in the same sequence.

METHODS

The methods for these experiments have been described previously[1]. A modification for studies of phosphate (^{32}ph) uptake by blood samples of different hematocrit fractions (H) consisted of incubating in duplicate 11 aliquots each of 7 ml of whole blood plus 1 ml of a solution of $H_3{}^{32}PO_4$ with sufficient NaCl, to preserve isotonicity of 0.90% NaCl of the blood mixture. The final ^{32}ph concentration was $4.170 \cdot 10^{-9}$ moles/ml of incubating mixture (m/ml). Each tube was centrifuged 30 min at 3,000 rpm. after an incubation of 3 hours. Then 0.20 ml of the supernatant fluid (f) of each tube were pipetted on 25 × 25 mm filter paper squares (Eaton Dikeman #613) which had been mounted on 1½″ planchets by 4 marginal drops of Duco Cement. The radioactivity of the planchets was assayed according to the preceding study.

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RESULTS AND DISCUSSION

1. *Uptake of radioactive phosphate by blood samples with different hematocrits.*

Fig. 1 demonstrates uptake-input relationships of the eleven blood samples. A general trend of increased uptake with increased initial phosphate concentration per ml of extracellular fluid is apparent (x/m_f vs. C'_{of}), where the initial phosphate concentration is proportional to the effective hematocrit (H'') (Table I). But the scatter of the data requires further analysis.

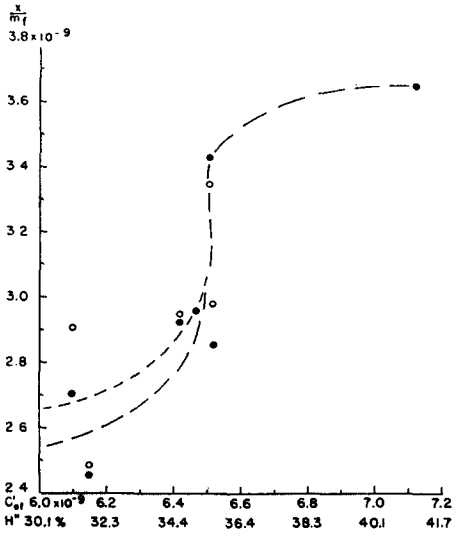

Fig. 1. Uptake of radioactive phosphate by rabbit erythrocytes in blood samples of various hematocrit fractions at pH 7.40, expressed as a function of final hematocrit and of initial ^{32}ph concentration. Abscissas: 1. Initial concentration of ^{32}ph in moles/ml of extracellular fluid (C'_{of}); 2. Reaction hematocrit (H'') in per cent. Ordinate: ^{32}ph uptake as moles/ml fluid (x/m_f).
First series of 6 aliquots = — — ● — —,
Second series of 5 aliquots = – – – O – – –.

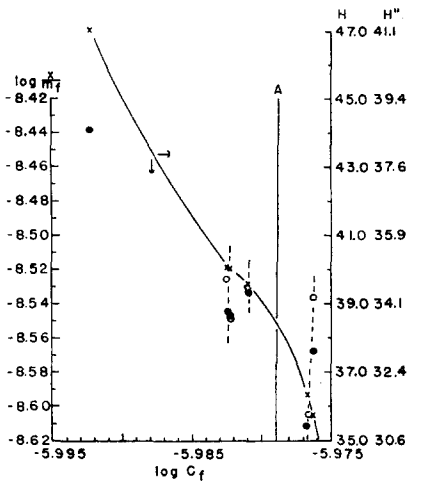

Fig. 2. a. Uptake of ^{32}ph by rabbit erythrocytes of blood samples of various hematocrit fractions at pH 7.40, expressed as a series of FREUNDLICH adsorption isotherms of the form $\log x/m_f = n \log C_f + \log b$, — — —. Abscissa: Log of final $^{31+32}$ph concentration (C_f) in m/ml of extracellular fluid. Left ordinate: Log of uptake of ^{32}ph (x/m_f) in m/ml fluid. Symbols as in Fig. 1. A = section of complete adsorption isotherm[1], drawn, but not placed to scale. Dashed lines indicate slopes within log x/m_f values of groups of neighboring hematocrits. b. Log of final concentration of $^{31+32}$ph (Cf) as a function of hematocrits, —×—×—. Abscissa coincident with (a). Right ordinate: Original and reaction hematocrit percentages (H, H'').

A previous analysis of similar data[1] had indicated strong adsorption of ^{32}ph on and in the cell membrane when the cells had been incubated with several ^{32}ph concentrations. They produced a FREUNDLICH adsorption isotherm, which could also be expected in this investigation in which the final concentrations of total phosphate in the fluid (C_f) is influenced by the cell concentration. As it rises, adsorption by the cells should rise logarithmically. To test this expectation the uptake data have been inserted into a FREUNDLICH isotherm (Table I), in which the influences of both the originally present ^{31}ph and the added ^{32}ph are combined. It turns out in Fig. 2 that the isotherm is discontinuous over the whole range of log C_f. Rather, adsorption manifests itself only within narrow limits of log C_f, in which accidentally many samples were grouped.

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TABLE I

ERYTHROCYTE PROPORTIONS AND UPTAKE OF RADIOACTIVE PHOSPHATE BY RABBIT BLOOD

Function	*Units*	*Symbols*	*Values*						*Mean Value*
Original blood									
hematocrit	vol. %	H	0.357	0.363	0.396	0.406	0.408	0.470	0.400
plasma fraction	vol. %	$1-H$	0.643	0.637	0.604	0.594	0.593	0.530	
Blood after dilution with ^{32}ph									
hematocrit	vol. %	H''	0.312	0.318	0.346	0.355	0.357	0.412	
plasma fraction	vol. %	$1-H''$	0.688	0.683	0.654	0.645	0.644	0.589	
Initial ^{32}ph concentration									
moles/sample of 7 ml	m/s$\cdot 10^{-8}$	C'_o	3.357	3.357	3.357	3.357	3.357	3.357	
moles/ml of mixture	m/ml $m\cdot 10^{-9}$	C'_{om}	4.196	4.196	4.196	4.196	4.196	4.196	
moles/ml of fluid	m/ml $f\cdot 10^{-9}$	C'_{of}	6.099	6.148	6.412	6.506	6.520	7.130	
Uptake of ^{32}ph moles of fluid									
Series 1	m/ml $f\cdot 10^{-9}$	x_1/m_f	2.707	2.454	2.922	3.430	2.854	3.648	3.002
Series 2	m/ml $f\cdot 10^{-9}$	x_2/m_f	2.909	2.486	2.949	3.349	2.980	—	2.935
Final concentration of ^{32}ph moles/ml fluid									
Series 1	m/ml $f\cdot 10^{-9}$	C'_{1f}	3.383	3.694	3.494	3.007	3.666	3.482	
Series 2	m/ml $f\cdot 10^{-9}$	C'_{2f}	3.190	3.661	3.467	3.157	3.541	—	
^{31}ph moles/ml plasma	m/ml $p\cdot 10^{-6}$	C''_p	1.127	1.127	1.127	1.127	1.127	1.127	
/ml fluid	m/ml $f\cdot 10^{-6}$	C''_f	1.053	1.051	1.042	1.039	1.038	1.014	
$^{31+32}$ph moles/ml fluid									
Series 1	m/ml $f\cdot 10^{-6}$	C_{1f}	1.056	1.055	1.045	1.042	1.041	1.018	1.043
Series 2	m/ml $f\cdot 10^{-6}$	C_{2f}	1.056	1.055	1.045	1.042	1.041	—	1.048

Legends: c = erythrocytes; C = phosphate concentration; f = extracellular fluid; H = hematocrit; m = moles; p = plasma; s = saturation; x = moles, removed from extracellular phase per indicated mass of material.

It would appear that three isotherms with almost identical slopes exist. Hardly any deviation can be detected graphically—even on the vastly extended scale of log C_f—between these slopes and the slope of the complete isotherm (A) of previous experiments[1]. The apparent equality of these sectional adsorption isotherms supports the hypothesis that uptake proceeds partly via a common adsorption system.

The sequence of the three sectional adsorption isotherms of Fig. 2 follows a trend that is determined by the magnitude of their intercepts b on the log x/m_f ordinate in the equation $x/m = bC^n$, if log x/m_f is extrapolated to log $C_f = 0$. Since the relation of log C_f (a function of log x/m_f) to H and H'' is to be investigated, a plot of both hematocrits against log C_f may explain how cell concentration influences final phosphate concentration and ^{32}ph uptake, which in turn determine the ordinate shift of the FREUNDLICH isotherms. Fig. 2 illustrates this plot by the full curve which falls approximately linearly with log Cf. It indicates a negative exponential dependence of C_f on H. Now both plots of Fig. 2 can be summarized by this sequence of events:

$$\text{Increasing } H \longrightarrow \text{decreasing log } C_f \begin{cases} \nearrow \text{increasing log } x/m_f \\ \searrow \text{increasing log } b. \end{cases}$$

This means that cell concentration affects adsorption and also absorption, which is at least partly independent of phosphate diffusion into the cell because diffusion would exhibit a linear relation of b with H. This fact may be taken as indirect evidence for metabolic accumulation in contrast to passive diffusion, as might be expected from

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the formation of phosphoesters by the cell. Metabolic accumulation may mask any diffusion, which may occur at a relatively lower rate, as discussed by KAMEN AND SPIEGELMAN in the case of unicellular plants[2].

2. ^{32}ph uptake by blood with equalized hematocrits

Taking up the introductory statement that blood samples with equalized cell concentrations can be expected to exhibit a phosphate uptake independent of the original hematocrit this hypothesis will be examined in the light of the following data[1]: A disagreement with this idea would mean the existence of a plasma-bound factor which can modify ^{32}ph uptake.

TABLE II

COMPARISON OF BLOOD VOLUMES AND DILUTIONS OF TWO SAMPLES OF RABBIT BLOOD

Function	*Values*		*Fraction of lower to higher value*	*Fractional difference*	*Derivation of Function*
	A	B	$F_e:F_h$	$\Delta F:F_h$	
H	0.436	0.401	0.918		hematocrit of whole blood
H'	0.350	0.350	1.000		hematocrit of blood diluted with plasma
V_b	3.500	3.500	1.000		ml of original blood sample
V'_b	4.354	3.977	0.913		$V_b+\Delta V_p$; $\Delta V_p=V_b\{[(H\times(1-H')/H']-(1-H)\}$; ΔV_p = addition of plasma
V''_b	4.854	4.477	0.922		V'_b + 0.5 ml ($H_3{}^{32}PO_4$ + NaCl).
V_p	1.974	2.100	0.940		ml of plasma in original blood sample
V'_p	2.828	2.577	0.911		$V_p+\Delta V_p$
V''_f	3.328	3.077	0.925		V'_p + 0.5 ml ($H_3{}^{32}PO_4$ + NaCl).
D'_b	0.244	0.136	0.599	0.441	$(V'_b-V_b)/V_b$
D''_b	0.387	0.279	0.721	0.279	$(V''_b-V_b)/V_b$
$D_b{}'^{-''}$	0.115	0.125	0.915	0.085	$(V''_b-V'_b)/V'_b$
D'_p	0.433	0.227	0.524	0.476	$(V'_p-V_p)/V_p$
D''_b	0.687	0.466	0.678	0.322	$(V''_f-V_p)/V_p$
$D_f{}'^{-''}$	0.177	0.194	0.909	0.091	$(V''_f-V'_p)/V'_p$

Results of equalizing the cell concentrations of samples of rabbit blood (Table II) demonstrate the fact that the relative increase of blood and plasma volumes due solely to the addition of ^{32}ph (i.e. $D_b{}'^{-''}$ & $D_f{}'^{-''}$) are inverse functions of hematocrit because the successive contributions of ^{32}ph to increasing blood and plasma volumes (V_b' & V_p') decrease in dense blood. In order to obtain a quantitative measure of the comparative dilutions by additions of plasma and ^{32}ph to both blood and extra cellular fluid of both blood samples, two expressions are tabulated: a. the fraction of the lower value of a function to its higher counterpart, F_l/F_h, and b. the complementary ratio of the increment of a function to its higher limit, $\Delta F/F_h$, the sum of which equals 1. A comparison of these ratios with the differences of ^{32}ph uptake by both blood samples in its several stages may be used to elucidate any intrinsic blood factors which influence ^{32}ph uptake after hematocrit equalization. The data on ^{32}ph uptake, taken from previous experiments[1], are summarized in Table III and compared with F_l/F_h and its complement in Table IV. Certain deductions can be made about functional relations between specific expressions of blood dilution (Table II) and of ^{32}ph uptake (Table III) by approximating all expressions of similar magnitudes. These deductions are discussed and simplified in the text accompanying Table IV. Its conclusion is that a factor exists in plasma which

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TABLE III

DIFFERENTIAL DISTRIBUTION OF ORIGINAL PHOSPHATE (^{31}ph) AND ADDED PHOSPHATE (^{32}ph) ON AND WITHIN RABBIT ERYTHROCYTES

Function	*Symbols*	*Units*	*Values*		*Difference*	
			A	*B*	*Absolute*	*% of max.*
Hematocrit of whole blood	H		0.436	0.401		8.18
Equilibrium ^{32}ph at complete adsorption	C'_f	m/ml $f \cdot 10^{-6}$	1.100	1.100		<1
^{32}ph adsorption at saturation						
in moles/ml fluid	x/m_{fs}'	m/ml $f \cdot 10^{-8}$	1.628	7.427		78.1
in moles/ml cells	x/m_{cs}'	m/ml $c \cdot 10^{-8}$	3.583	16.26		78.0
^{32}ph ions adsorbed/cell		ions $\cdot 10^{6}$	1.32	5.97		77.9
$^{31+32}$ph ions adsorbed/cell		ions $\cdot 10^{7}$	1.204	4.790		74.9
Stromatin-protein sites/$^{32+31}$ph ion			11.24	2.82		74.8
Ca/$^{31+32}$ph ion			5.33	1.33		75.1
Lecithin molecules/$^{31+32}$ph ion			5.33	1.33		75.1
Volume of $^{31+32}$ph adsorption complex with lecithin		ml $\cdot 10^{-12}$	1.571	6.255		74.8
Fraction of complex of cell volume		%	2.32	9.21	6.89	74.8
Equilibrium $^{31+32}$ph at complete uptake	C_{fs}	m/ml $f \cdot 10^{-6}$	1.532	22.59		93.2
Total $^{31+32}$ph at complete uptake						
moles/ml fluid	x/m_{fs}	m/ml $f \cdot 10^{-7}$	3.379	40.43		91.6
moles/ml cells	x/m_{cs}	m/ml $c \cdot 10^{-7}$	6.197	88.28		93.0
Equilibrium $^{31+32}$ph at complete adsorption	C_{fs}''	m/ml $f \cdot 10^{-6}$	0.432	21.49		98.0
Complete $^{31+32}$ph absorption, moles/ml cells	x/m_{cs}''	m/ml $c \cdot 10^{-7}$	2.914	75.21		96.1
$^{31+32}$ph ions/cell interior		ions $\cdot 10^{7}$	1.057	27.63		96.2
Fraction of adsorption of uptake		% by cells	52.9	14.7	38.2	72.2
Fraction of absorption of uptake		% by cells	47.1	85.3	38.2	44.8
Absolute concentration of $^{31+32}$ph in fluid at complete adsorption	C_{fs}'	$M \cdot 10^{-3}$	1.100	1.100		<1.0
^{32}ph in antisphering layer		$M \cdot 10^{-3}$	5.975	26.41		77.4
$^{31+32}$ph in lecithin complex		$M \cdot 10^{-2}$	1.348	5.525		75.6
$^{31+32}$ph in cell volume		$M \cdot 10^{-4}$	2.98	77.0		96.1
Apparent equilibrium constants for						
adsorption by antisphering film	K_e'		5.43	23.93		77.3
adsorption by cell membrane	K_e''		2.25	2.09		7.14
adsorption by cell interior	K_e'''		0.022	0.147		84.9
uptake	K_e		0.270	7.340		96.3

inhibits the uptake of ^{32}ph under the conditions of this experiment[1]. This inhibition is perhaps similar to the observation by REID AND RYAN[3] that human blood serum may contain a dialyzable and ionically inactive principle which inhibits phosphate metabolism. The presently observed inhibition is most apparent in absorption and less so in adsorption of ^{32}ph on the antisphering layer of the cell. But there is no marked effect on the adsorption of originally present ^{31}ph and newly added ^{32}ph on the calcium-lipoprotein complex internal to the antisphering layer[4]. The phosphate which was present in this complex before the experiment apparently exists in a relatively stable form.

Assuming that adsorption is proportional to the concentration of exchangeable negative surface charges[1,5], the inhibitor must either compete with them or retard their formation. The observation of simultaneous inhibitions of adsorption of added ^{32}ph and of its absorption points to the second possibility, because reduced absorption can be a sequel to reduced formation of phosphoesters and release of organic anions.

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TABLE IV

ADSORPTION AND ABSORPTION OF RADIOACTIVE PHOSPHATE BY RABBIT ERYTHROCYTES IN RELATION TO DILUTION OF BLOOD BY PLASMA AND PHOSPHATE

Hematocrits $H = 0.436$ and $H = 0.401$ designated by A and B, respectively. Expressions explained in Tables II and III.

Groups of expressions of similar magnitudes	*Interpretation*
I.	
$\frac{H_B}{H_A} \approx \frac{V'_{bB}}{V'_{bA}} \approx \frac{V'_{pB}}{V'_{pA}} \approx \frac{D'^{-''}{}_{bA}}{D'^{-''}{}_{bB}} \approx \frac{D'^{-''}{}_{fA}}{D'^{-''}{}_{fB}} \approx \frac{\Delta x/m_{fs}}{x/m_{fsB}} = 0.911$ to 0.918	The ratio of the hematocrit of B to that of A corresponds to an increase of the same proportion of the total uptake of phosphate ($^{31+32}$ph) of sample B over A. The ratios of the percentages of volume increase of the blood, and also of the extracellular fluid, of sample A to that of B, produced by the addition of phosphate, correspond to an increase of the same proportion of ^{32}ph uptake of B to that of A at complete uptake.
Conclusion: Separated plasma contains a factor which inhibits the uptake of phosphate if added to its own blood.	
II.	
$\frac{V''_{bB}}{V''_{bA}} \approx \frac{V''_{fB}}{V''_{fA}} \approx \frac{\Delta x/m_{cs}}{x/m_{csB}} \approx \frac{\Delta C_f}{C_{fsB}} = 0.922$ to 0.932	The ratios of the final volume of blood, and also of fluid, of B to that of A correspond to an increase of the same proportion of the uptake of ^{32}ph of B to that of A. The relevant equilibrium concentrations of $^{31+32}$ph at complete uptake change proportionally.
Conclusion: The addition of ^{32}ph to a sample of blood containing added plasma does not remove the inhibiting factor. Perhaps it is even intensified. And, because blood which has received more inhibitor takes up less phosphate, the related final equilibrium phosphate concentration in the fluid at complete uptake is reduced proportionally.	
III.	
$\frac{V_{pA}}{V_{pB}} \approx \frac{\Delta x/m_{cs}}{x/m_{csB}} \approx \frac{\Delta x/m_{cs}''}{x/m_{csB}''} \approx \frac{\Delta C_{fs}}{C_{fsB}} = 0.930$ to $0.961 \longrightarrow \frac{\Delta C_{fs}''}{C_{fsB}''}$	The ratio of the original plasma volume of A to that of B corresponds to an increase of the same proportion of phosphate uptake from blood A to B, caused particularly by adsorption. The equilibrium concentrations of phosphate at complete uptake and absorption change accordingly.
Conclusion: The inhibiting factor may possibly be most influential on the absorption process. Unchanged equilibrium concentrations at complete adsorption of $^{31+32}$ph and only a slight change of the equilibrium constant for adsorption by the lipoprotein layer of the cell support this argument.	
IV.	
$\frac{D''_{bB}}{D''_{cA}} \approx \frac{D''_{fB}}{D''_{fA}} \approx \frac{\Delta\ ^{31+32}\text{ph ions adsorbed/cell}}{^{31+32}\text{ph ions adsorbed/cell}} \approx \frac{\Delta\ (\%\ ^{31+32}\text{ph adsorption complex of cell volume})}{\%\ ^{31+32}\text{ph adsorption complex of cell volume of } B}$	The ratios of the final percentages of volume increase of blood B to that of A and of fluid B to that of A correspond to an increase of the same proportion of the number of $^{31+32}$ph ions adsorbed per cell.

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TABLE IV (continued)

Groups of expressions of similar magnitudes	*Interpretation*
$\approx \frac{\Delta \text{ (\% adsorption of uptake)}}{\text{\% adsorption of uptake}}$ = 0.678 to 0.749	Both related data of cell volume utilization and the proportion of uptake taken care of by adsorption behave accordingly.
$\rightarrow \frac{\Delta\ ^{31}\text{ph ions adsorbed/cell}}{^{31}\text{ph ions adsorbed/cell}}$	

Conclusion: Reducing the addition of plasma stimulates the adsorption of $^{31+32}$ph. But the facts that the adsorption solely of ^{32}ph at the saturated antisphering layer varies more with changes of hematocrit than the adsorption of $^{31+32}$ph, *i.e.* 77% to 78% versus 68% to 75% (Table III), and that the equilibrium constant K'_e of ^{32}ph adsorption is influenced about 10 times more than the constant K''_e for $^{31+32}$ph adsorption on the internal lipoprotein complex, indicate a predominant inhibitor action on the adsorption process on the antisphering film.

V.

$\frac{\Delta D'^{-''}_b}{D'^{-''}_{bB}} \approx \frac{\Delta D'^{-''}_f}{D'^{-''}_{fB}} \approx \frac{K''_{eB}}{K''_{eA}}$ = 0.0714 to 0.0905	The ratio of the increment between the percentages of volume increase of sample A and B by addition of ^{32}ph to the percentage of volume increase of B corresponds to a similar ratio of the equilibrium constants of $^{31+32}$ph adsorption by the cell membrane of sample B to that of A.

Conclusion: The relative dilution effect on blood and on plasma of the ^{32}ph solution is greater on low hematocrit blood and causes a proportionate reduction of the equilibrium constant for the formation of the phosphate-lipoprotein adsorption complex.

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SUMMARY

1. Samples of rabbit blood with various hematocrits were incubated with radioactive phosphate to determine the effect of cell concentration on phosphate adsorption and absorption.

2. Phosphate uptake follows a FREUNDLICH adsorption isotherm if the final phosphate concentration of the extracellular fluid is varied by small changes of cell concentration. Changes of hematocrit over a relatively wide range affect absorption more than adsorption, absorption apparently being caused more by active accumulation than by diffusion.

3. Evidence is presented for the existence of an inhibitor of phosphate uptake. It seems to be in centrifuged plasma and retards absorption of radioactive phosphate more than adsorption.

RÉSUMÉ

1. Des échantillons de sang de lapin, avec divers hématocrites, ont été incubés en présence de phosphates radioactifs, dans le but de déterminer l'influence de la concentration en cellules sur l'absorption et l'adsorption des phosphates.

2. La fixation des phosphates obéit à un isotherme d'adsorption de FREUNDLICH lorsque l'on fait varier la concentration finale en phosphates par de petites modifications de la concentration en cellules. Des modifications de l'hématocrite dans un domaine relativement étendu ont plus

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d'influence sur l'absorption que sur l'adsorption, l'absorption étant plutôt la conséquence d'une accumulation active que d'une diffusion.

3. L'existence d'un inhibiteur de la fixation des phosphates a été indiquée par les auteurs. Cet inhibiteur serait présent dans le plasma centrifugé et retarderait l'absorption des phosphates radioactifs plus que leur adsorption.

ZUSAMMENFASSUNG

1. Kaninchenblutproben mit verschiedenen Hämatokriten wurden mit radioaktiven Phosphat inkubiert um die Wirkung der Zellkonzentration auf die Phosphatadsorption und -adsorption zu bestimmen.

2. Wenn die Endphosphatkonzentration der Extracellularflüssigkeit durch geringe Änderungen der Zellkonzentration variiert wird, folgt die Phosphataufnahme einer FREUNDLICH'schen Adsorptionsisotherme. Die Hämatokritänderungen über einen relativ grossen Bereich haben eine grössere Wirkung auf die Absorption als die Adsorption. Die Absorption wird augenscheinlich mehr durch eine aktive Ansammlung als durch Diffusion verursacht.

3. Es ist augenscheinlich ein Inhibitor der Phosphataufnahme vorhanden. Er scheint im zentrifugiertem Plasma gegenwärtig zu sein und verzögert die Absorption radioaktiven Phosphats mehr als die Adsorption.

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