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Biologiske Meddelelser. **XIV**, 5.

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Det Kgl. Danske Videnskabernes Selskabs Publikationer i 8vo:

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Printed in Denmark.
Bianco Lunos Bogtrykkeri A/S, Kbhvn.

List of Symbols.

p = total amount of plasma-phosphate;
c = total amount of acid-soluble phosphorus in corpuscles
s_p = specific activity of plasma phosphate (activity per mgm. phosphorus);
s_i = specific activity of inorganic phosphorus in corpuscles;
s_c = average specific activity of total acid-soluble phosphorus in corpuscles;
a_p = total activity in plasma phosphate;
a_c = total activity of acid-soluble phosphorus in corpuscles;
S_p = value of s_p at the end of an experiment;
S_i = value of s_i at the end of an experiment;
S_e = specific activity of acid-soluble organic phosphorus in corpuscles at the end of the experiment;
S_c = value of s_c at the end of an experiment, etc.;
A_t = total activity of plasma ester at the time;
A_o = total activity of plasma ester at the start of the experiment;
α = coefficient of penetration;
k = rate-constant of the monomolecular decomposition of hexose-phosphate in blood.

The distribution of inorganic phosphate and of organic acid-soluble phosphorus compounds between plasma and corpuscles deviates from equipartition. This difference in the distribution of the phosphate ion could be due to the fact that during the life time of the corpuscles equilibrium between its contents and hose of the surrounding plasma is not yet reached, but it is more probable that we are faced with a case in which the partition coefficient of the ion in question between corpuscles and plasma actually differs from unity.

The distribution coefficient of inorganic phosphate and also that of the acid-soluble organic phosphorus compounds between plasma and corpuscles fluctuates within wide limits. HALPERN[1] investigated the inorganic P content of the plasma and corpuscles of rabbits blood in 33 cases. In 29 cases the inorganic P content of the corpuscles was found to be less than that of the plasma of equal volume, the ratio between the inorganic P content of the corpuscles and that of the same volume of plasma varying between 0.86 and 0.38. In our experiments we find an average content of the plasma inorganic P amounting to 7 mgm. % and of the corpuscles

[1] L. HALPERN, J. Biol. Chem. **114**, 747 (1936). In this paper earlier litterature on this subject is to be found. R. T. BRAIN, H. O. KAY and P. G. MARSHALL (Biochem. J. **22**, 628, 1927) found the inorganic P content of the human blood plasma to be 4.1 mgm. %, that of the corpuscles 2.4 mgm. %.

inorganic P to 4.5 mgm. % making the above mentioned ratio equal to 0.64. When taking into account that the water content of the corpuscles amounts to only 70 % of that of the plasma we obtain for the distribution coefficient of the inorganic P between the corpuscle water and plasma water a value differing not much from unity[1]. While the determination of the inorganic P of the plasma is not difficult, as the acid-soluble plasma P is mostly composed of phosphate ions, the analysis of the corpuscles often gives less reliable results. Some of the organic phosphorus compounds present in the corpuscles may decompose[2] in the course of the separation of the corpuscles, the decomposition giving rise to the formation of additional inorganic phosphate. On the other hand, when the corpuscles have to be obtained quite free of plasma as in our experiments (page 19) it is necessary to wash them with a suitable solution free of phosphate, for example with an isotonic sodium chloride solution; in the course of this operation some phosphate can be lost from the corpuscles by a diffusion process. In view of the great importance the plasma phosphoric esters play in Robison's theory of bone calcification he and his collaborators[3] made a careful study of the amount of phos-

[1] R. T. Brain, H. O. Kay and P. G. Marshall (Biochem. J. **22**, 629, 1928) find for human blood the same distribution coefficient as found by us for canine blood, namely 0.91

[2] Maitland, Hansman, and Robison, Biochem. J. **18**, 1152 (1924), have shown that, if the blood is made acid to $p_H = 7.3$, there is hydrolysis, if made alkaline to $p_H = 7.35$ there is for a short time synthesis of the esters; this, however, soon gives place to hydrolysis and to a corresponding increase of the inorganic phosphate; comp. also H. Lawaczeck, Biochem. Zeitsch. **145**, 351, 1924.

[3] Robison, R., The Significance of Phosphoric Esters in Metabolism, New York 1932, p. 68. Comp. also R. T. Brain, H. O. Kay and P. G. Marshall l. c.

phoric ester present in the plasma from human and rabbit bloods and ascertained an average value of about 0.5 mgm.%.

The problem in which we were interested was the determination of the rate at which phosphate ions and also the phosphoric ester molecules of the plasma penetrate into the corpuscles and vice versa. The usual procedure employed to obtain information on the permeability of the corpuscle membrane to phosphate ions is to introduce sodium phosphate into the plasma and to investigate if and to what extent the phosphate and phosphoric ester content of the corpuscles is increased. By using this line of attack HALPERN found that at 3° inorganic P does not enter or leave the blood cell to an yappreciable extent in the course of 9 hours. Above 23° a very slow, at 37° an appreciable penetration of the additional phosphate into the corpuscles was observed.

A very convenient way of the study of exchange between phosphorus components present in the plasma and the corpuscles is opened by the application of labelled (radioactive) phosphate. By introducing active sodium phosphate of negligible weight into the plasma all the phosphate ions present in the latter get labelled and, if after the lapse of some time radioactive phosphorus compounds are found to be present in the corpuscles, we can conclude that these penetrated during the time in question from the plasma into the corpuscles. We carried out experiments both in vivo and in vitro introducing active sodium phosphate into the plasma and investigating, after the lapse of few hours, the activity and the concentration of the inorganic phosphate and also of the phosphoric esters present in plasma and corpuscles. In other cases active hexosemonophosphate was introduced into the plasma and the activity and concentration of the above mentioned P compounds were measured. In view

of the very slow rate of the formation of labelled "non-acid soluble" phosphorus-compounds present in the blood (phosphatides and phosphorus containing proteins) ascertained in our former work[1] we left those substances out of consideration in this investigation. Some of the acid soluble phosphorus compounds, of which a great variety occurs in the corpuscles, were found to be labelled to a large extent after the lapse of a short time. The problem which first occurs is whether the labelled organic phosphorus compounds (phosphorus esters and others which we will in what follows denote as "phosphorus ester") are formed within the corpuscles from active inorganic phosphate or whether active esters diffuse from the plasma into the corpuscles. As we will see later the labelled esters found in the corpuscles are, at least to a large extent synthesised within the corpuscles.

As to the nature of phosphoric esters present in the corpuscles the presence of various compounds has been recorded, such as adenyltriphosphate, hexosephosphate, triosephosphate, mono- and diphosphoglycerate, glycerophosphate, and phosphopyruvate. The composition of the corpuscles of different animals was found to be markedly different: while the corpuscles of the blood of sheep contain[2], for example, 80% of esters which are hydrolyzed by boiling 1 n. HCl within 3 hours, the corresponding figure amounts, in the case of rats' blood, to only 30. The average ester P content of human blood corpusles is stated to be 20 mgm. per 100 cc. blood, of which about 68% is present as phosphoglycerate, 21% as hexosephosphate, and 11% as adenyltriphosphate[3]. Few data are available as to the

[1] L. Hahn and G. Hevesy, Memoir. Carlsberg Lab. **22**, 188, 1938.
[2] H. v. Euler and K. M. Brandt, Z. f. physiol. Chem. **240**, 215 (1936).
[3] E. Warneg and G. Stearns, J. Biol. Chem. **115**, 567 (1936).
S. E. Kerr and A. Antaki, J. Biol. Chem. **121**, 531 (1927).

phosphorus ester content of the plasma and its composition, the presence of small amounts of hexosemonophosphate being recorded[4]. The phosphoric ester content of the plasma varies within wide limits, the average value being about 0.5 mgm. %. In the normal human plasma values varying between 0.0 and 0.9 mgm. % and an average valwe of 0.33 mgm. % were recorded[5].

Diffusion of Phosphate Ions into the Corpuscles.

Radioactive sodium phosphate containing a negligible amount of phosphorus is added to 10 cc. of heparinised rabbit blood. The sample is shaken in a thermostat at 37° under a mixture of oxygen and carbon dioxid, after the lapse of few hours plasma and corpuscles are separated by centrifuging, the corpuscles washed 2—3 times with a physiological sodium chloride solution. The acid-soluble components of the plasma and also those of the corpuscles are isolated in the usual way (extraction with ice-cold trichloro-acetic acid). While the acid-soluble fraction of the plasma is practically (90% or more) composed of inorganic P, the corpuscles contain mostly organic phosphorus compounds and, in addition, an appreciable amount of inorganic P. The latter can be isolated by precipitation as ammonium magnesium phosphate. The organic phosphorus compounds present in the filtrate are then converted into inorganic salts and precipitated as such.

When carrying out such experiments we find that in the course of few hours an appreciable part of the labelled plasma inorganic phosphate diffused into the corpuscles. At the same time we find a formation of labelled organic

[4] Comp. R. Robison, The signficance of phosphoric esters in metabolism, New York 1932, p. 69.

[5] R. T. Brain, H. D. Kay and P. G. Marshal, Biochem. I. **22**, 635, 1928.

Table 1.

Activation in vitro of acid-soluble phosphorus present in the corpuscles.

Relative specific activities of corpuscles' phosphate and of organic acid-soluble phosphorus present in the corpuscles, taking the specific activity of the plasma-phosphate at the end of the experiment to be = 1.

	Time	Rel. spec. activity of corp. phosphate $\left(\frac{S_i}{S_p}\right)$	Rel. spec. activity of corp. org. acid-soluble P. $\left(\frac{S_e}{S_p}\right)$
Rabbit G	90 min.	0.25	0.14
	90 min.	0.38	0.21
Rabbit H	30 min.	0.27	0.11
	90 min.	0.46	0.23
	175 min.	0.69	0.36

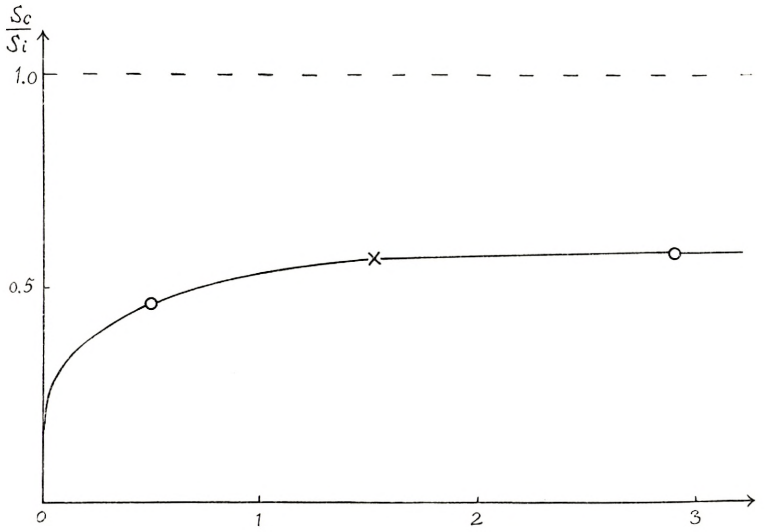
phosphorus compounds in the corpuscles. What actually happens is that the individual iorganic phosphate ions of the plasma diffuse into the corpuscles and are converted in the latter into phosphorus esters. The question, which now occurs, is which is the faster process, the diffusion of HPO_4'' into the corpuscles or the ester formation. This can be decided by comparing the specific activities of the different phosphorus fractions isolated from the corpuscles. Such a comparison is seen in table 1. After the lapse of only half an hour about half the ester was in exchange equilibrium with the inorganic P present in the corpuscles. The resynthesis of the acid soluble organic P compounds must thus be a fast process and, from the fact that with increasing time the ratio between the specific ativity of the ester P and inorganic P of the corpuscles only slightly increases and yet strongly differs from 1, we must conclude

that only one part of the diverse organic phosphorus compounds present in the corpuscles is renewed and thus activated in the course of the experiment while the other part, composed of diphosphoglycerate, hexosephosphate and other compounds remains, at least practically, inactive. This conclusion is supported by the results of the following experiments. Instead of destroying the total esters we hydrolysed[1] them with 1 n. HCl or H_2SO_4 for 100 min. at 100° and determined the specific activity of the hydrolysed P. While the average ester P secured from the corpuscles of a rabbit (G, comp fig. 1) was found to have a specific activity amounting to 55 % of the corpuscle inorganic P the corresponding figure for the hydrolysable ester was 80 %. In the case of another rabbit (H), the figures were 53 % and 100 % respectively[2]. From the facts mentioned above it follows that the exchange reaction between inorganic phosphate ions and the hydrolysable esters is a very fast process. That the inorganic P present in the corpuscles does not reach exchange equilibrium with the plasma phosphate in the course of a few hours is due to the fact that a large part of the active phosphate ions are incorporated into the organic compounds of the corpuscles, while simultaneously non-active phosphate ions are freed to take the place of the active ones and "dilute" the active inorganic phosphate which penetrated into the corpuscles, diminishing thus the specific activity of the corpuscle inorganic P.

[1] Under these conditions, diphospho-l-glycerate and also hexosediphosphate are only hydrolysed to a negligible resp. small extent. (G. Warweg and E. Stearns, J. Biol. Chem. **115**, 567 (1936)).

[2] The difference between the rate of activation of the "hydrolysable" and "nonhydrolysable" fractions is still better brought out when comparing the specific activity of the pyrophosphate, with of from adenosintriphosphate after 7 min. hydrolysis, to that the residnal P, as found in a recent investigation the result of which will be published shortly.

If the whole ester-phosphorus could exchange with the inorganic P, the activity-ratio should finally reach a value 1.0 (indicated by the dotted line). It is clear that the limiting value of $\frac{S_c}{S_i}$ is much lower, which is due to the presence of an organic phosphorus fraction in the corpuscles exchanging at a slow rate.



Ratio in the corpuscles of the specific activities of the total acid-soluble phosphate and the inorganic phosphorus. x = Rabbit G. o = Rabbit H.

Diffusion of Plasma HPO_4'' into Corpuscles.

We may obtain information as to the rate of penetration of the HPO_4'' from the plasma into the corpuscles by comparing, after a lapse of time, the specific activities of the plasma inorganic P and that of the total acid-soluble P present in the corpuscles. An alternative method would be to compare the specific activities of the plasma inorganic P and the corpuscle inorganic P, but in this way the rate of penetration would be underestimated for the following

reason: While active esters are forming in the corpuscle a corresponding amount of non-active P ester decomposes producing non-active inorganic P which dilutes the active inorganic P present. (We denote as specific activity the activity per mgm. P). Therefore to arrive to a proper figure for the rate of penetration into the corpuscles we have to consider the total acid-soluble P present in the corpuscles. As already mentioned, one part of the organic P of the corpuscles reaches exchange equilibrium very rapidly while the other becomes activated at a slow rate; and therefore the ratio of the specific activities of the corpuscle inorganic P and total acid-soluble P does not become = 1, but 1.8. This figure is only valid for the blood of the rabbit, while a different figure will presumably be obtained for blood containing appreciably more or less hydrolysable phosphorus. In the following discussion the calculation of the penetra-penetratition of HPO_4'', and of a magnitude which we will call a penetration coefficient and denote as α will be demonstrated. (Comp. the list of symbols).

Calculation of the Coefficient of Penetration.

The total activity of the plasma (a_p) is equal to the product of the specific activity (s_p) and the amount of phosphate (p) present in the plasma. (Definition of the specific activity.)

$$a_p = p \cdot s_p$$

and similarly for the corpuscles

$$a_c = c \cdot s_c$$

c denoting the total acid-soluble P of the corpuscles. During the exchange process $a_p + a_c$ remains constant, and therefore

$$-\Delta s_p = \frac{c}{p} \Delta s_c .$$

At the beginning of the experiment the specific activity of the corpuscles P was = O and, accordingly, when denoting the specific activities at the end of the experiment as S_p and S_c, we arrive at a value of the specific activity of the plasma phosphate at the beginning of the experiment

$$s_p = S_p + \frac{c}{p} S_c .$$

The rate of activation of the corpuscle P being proportional to $s_p - s_i$ we can write

$$\frac{ds_c}{dt} = \alpha (s_p - s_i)$$

were, as already mentioned, α is the coefficient of penetration. In an early stage of the experiment the change of s_p and s_i with time is linear and therefore, as far as we choose such experimental conditions that most of the activity is still to be found in the plasma, we arrive at the average value of s_p during the experiment

$$S_p + \tfrac{1}{2} \frac{c}{p} S_c$$

and in analogous way on the average

$$s_i = \tfrac{1}{2} S_i .$$

From which follows, considering that s_c increases linearly with time,

$$S_c = \alpha (S_p + \tfrac{1}{2} \frac{c}{p} S_c - \tfrac{1}{2} S_i) t .$$

We found in our best experiment $\frac{c}{p} = 4$ and, as we saw that $S_i = 1.8\ S_c$, we conclude that the end value of the specificativity of the corpuscles total acid-soluble P when the experiment, as was in our case, is of restricted duration,

$$S_c = \alpha (S_p + 1.1\ S_c) t .$$

The value of the penetration coefficient of the phosphate ions into the corpuscles, a magnitude we will make use of in our later calculations, is given in the table 2.

Table 2.

Calculation of the Penetration-Coefficient of Phosphate-Ions (α) into Corpuscles.

S_c denotes the specific activity of the total acid-soluble P in the corpuscles; S_p that of the plasma at the end of the experiment.

	Time in min.	$\frac{S_c}{S_p}$	α
Rabbit C	170	0.19	0.0009
	170	0.20	0.0010
Rabbit G	90	0.14	0.0014
	90	0.21	0.0019
Average value			0.0013
Average value (Rabbit C alone)			0.0010

In what follows we desire to compare the rate of interpenetration of labelled phosphate ions into the corpuscles with that of labelled hexose-monophosphate molecules. As the latter are easily decomposed in the plasma, we have first to discuss the behaviour of the hexose-monophosphate in this medium.

Rate of Decomposition of Hexose-monophosphate.

The usual method applied to the study of the decomposition of hexose-monophosphate under the action of enzymes is the determination of the amount of phosphate ions split off in the course of the experiment. This is a highly satisfactory method if, at the start of the experiment, no appreciable amount of inorganic phosphate is present. It is not very satisfactory, however, if the decomposition of a slight amount of phosphorus ester is to be determined in the

presence of large amounts of inorganic phosphate. In such a case the use of labelled hexose-monophosphate and the determination of the amount of radioactive phosphate split off is much to be prefered to the first mentioned method. Even large amounts of inorganic phosphate present at the start of the experiment will in no way influence the results as these are, in contrary to the phosphate split off from the labelled phosphorus ester, not radioactive, and thus not recorded by activity measurements.

In our experiments we have shaken 10 cc of rabbits' blood for a few hours in a thermostat, after the addition of labelled hexosemonophosphate containing about $^1/_{15}$ mg. P. The hexosemonophosphate (Embden ester) was prepared by Dr. Ostern and kindly presented us by Professor Parnas. At the end of the experiment the activity of the total acid-soluble P of the corpuscles, that of the organic acid-soluble P of the plasma, and also that of the inorganic phosphate present in the plasma was determined. The results obtained are seen in table 3.

Table 3.

Hydrolysis of Labelled Hexosemonophosphate Added to Blood in vitro at 37°.

	Time in min.	Fraction decomposed	Velocity constant of decomposition in min^{-1}.
Rabbit A	150	0.26	0.0021
	150	0.37	0.0031
Rabbit C	80	0.15	0.0021
	80	0.15	0.0020
	170	0.29	0.0020
	170	0.32	0.0023

The first two samples were shaken in air after the addition of oxalate, the four latter samples in a mixture

of CO_2 and O_2 after addition of heparin. The velocity constant was calculated by making use of the equation valid for mono-molecular reactions

$$\ln \frac{A_o}{A_t} = kt,$$

A_t being the total activity of the ester present in the blood at the time t, A_o at the beginning of the experiment. It follows from the fair constancy shown by the velocity constant recorded in table 2 that in the hydrolysis of the hexosemonophosphate equilibrium is far from being reached in three hours. It is of interest to compare the velocity constant of the hydrolysis of the labelled hexosemonophosphate which we obtained under the action of enzymes present in the blood with the value ROBISON[1] found when hydrolysing the ester by 0.1 n H_2SO_4. The acid was found to be much less effective in hydrolysing hexosemonophosphate than the enzymes, the value of $k = 2.2 \times 10^{-6}$ min^{-1} being found by him.

In one case labelled hexosemonophosphate was added to plasma and we found, after the lapse of 175 min., 23 % of the ester to be decomposed, thus approximately the same amount which decomposes during the same time in the presence of blood corpuscles (31 %).

When labelled ester disappears we have to distinguish between two possibilities, in one case the number of ester molecules actually diminishes, in the other case the active phosphorus atom present in the ester molecules is replaced through an enzymatic exchange process by a non radioactive one and no change in the number of ester molecules occurs. That in the above discussed cases we have to deal with

[1] R. ROBISON, Biochem. J. **27**, 2191 (1932).

the first mentioned possibility (decomposition of the ester) follows from the experiment to be described. To 10 cc. plasma non active hexosemonophosphate containing 0.15 mgm. P and radioactive sodium phosphate of negligible weight was added and after the lapse of 170 min. the activity of the plasma esters determined. If the loss of activity by the active ester in the experiments recorded in table 3 should be due to an exchange process we would have to expect about 5% of the activity of the sodium posphate to be incorporated in the originally non active hexosemonophosphate added to the plasma. The result of our experiment, however, has shown that the activity of the added hexosemonophosphate isolated together with the other minute amounts of esters present in the plasma amounted to less than $^1/_{10}$ of the calculated activity. We have therefore to conclude that at least 90% of the activity loss of the labelled hexosemonophosphate recorded in table 3 is actually due to decomposition and not to an exchange process.

General Remarks on the Resynthesis of the Molecules of the Acid Soluble Organic Phosphorus Compounds Present in the Corpuscles.

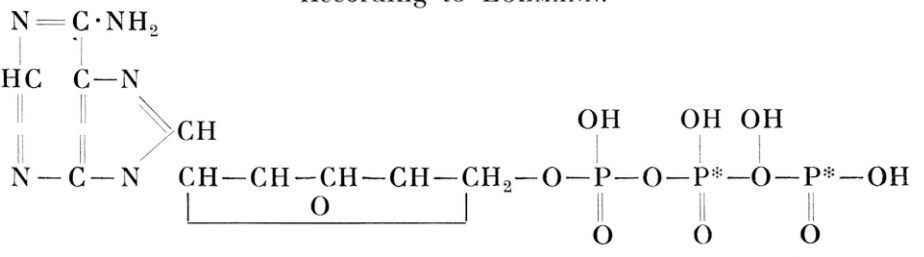
The process of the incessant new formation and thus activation of, for example, the adenosine triphosphate molecule present in the corpuscles shows a formal analogy to the incessant new formation of acetic acid radicals in aqueons solution. The acetic acid molecule is only dissociated to a small degree in turn; however, within the shortest time all molecules will pass alternatively through the dissociete and undissociated state. This can be best demonstrated by dissolving acetic acid in heavy water, in which case within

the shortest time an equipartition of the heavy hydrogen atoms between the labile H atom of the acetic acid molecule and the water will be noted. This is made possible by the incessant dissociation and rebuilding of the acetic acid molecule. In this process, however, only the acid H atom denoted by an asteric in the formula below

$$CH_3COOH^*$$

is involved, the CH_3 radical being held together by strong forces of attraction does not take part in such a new-formation process and is therefore not converted to CD_3 when acetic acid is placed in heavy water. Out of three P atoms of of the adenyltriphosphoric molecule only two, those denoted by an asteric, are moveable and involved in the alternative decomposition and new formation process going on in the corpuscles and made possible by the presence of a suitable phosphatase. The loosening of the third P atom of the adenosintriphosphoric molecule requires deeper going processes just as does the loosening of the H atoms of the CH_3 radical. The relative rate of replacement of the labile and stabile P atoms present in adenosintriphosphate extracted from the muscles of rabbits was recently found by PARNAS to be about 10 : 1.

Formula of Adenosintriphosphate.
According to LOHMANN.



Uptake of Hexosemonophosphate by the Blood Corpuscles.

We interpreted the formation of active phosphorus esters found in the corpuscles as due to an enzymatic exchange process inside the corpuscles, (the number of ester molecules decomposed being presumably replaced by an equal number of newly formed molecules) into which some of the active inorganic phosphate added to the plasma penetrated. An alternative explanation would be that active phosphorus ester molecules are formed in the plasma, diffuse into the corpuscles and are replaced, in experiments in vitro, by an equal number of non active molecules leaving the corpuscles. We can test the correctness of this explanation for cach compound by adding to the plasma the active phosphorus ester and determining, after the lapse of few hours, the activity of the phosphorus ester molecules and the inorganic phosphate of the corpuscles. So far we only carried out such experiments with active hexosemonophosphate, prepared by Dr. Ostern and presented to us most kindly by Professor Parnas. Several of the other labelled phosphorus ester compounds were also synthesized in the laboratory of the latter. The result obtained by us is that, if hexosemonophosphate molecules diffuse at all into the corpuscles, the rate of their penetration must be much slower than that of the phosphate ions. We arrived at this result by supposing: The amount of active P to be expected in corpuscles after the lapse of a certain time is wholly due to the penetration of active phosphate ions from the plasma into the corpuscles and independent of the presence of active hexosemonophosphate in the plasma. The next step is to compare the calculated values for the activity of the corpuscles P with those found by the experiment

and to ascertain if any difference is shown by the two values. Should such not be the case, then we must conclude that the rate of penetration of the hexosemonophosphate molecules into the plasma is negligible compared with that of the phosphate ions. The amount of labelled P in the corpuscles is zero at the start, i. e. after addition of active hexosemonophosphate to the plasma, and increases with time as discussed on p. 9. As in the course of the experiment only a small part of the active hexosemonophosphate is hydrolysed, we are entitled to make the simplifying assumption that the increase of the specific activity of plasma inorganic P takes place in a linear fashion. We also assume that the decrease of the difference in the specific activities of plasma phosphate P and corpuscle phosphate P with increasing time will also take place according to a linear function.[1] The average value (comp. p. 13) of the last mentioned difference will be

$$\tfrac{1}{2}\,(S_p - S_i) = 0.5\,S_p - 0.9\,S_c$$

and the specific activity of the corpuscles P at the end of the experiment

$$S_c = \alpha\,(0.5\,S_p - 0.9\,S_c)\;t.$$

The value of α being known ($\alpha = 0.0010$), the quantity $\frac{S_c}{S_p}$ can be evaluated. The figures thus obtained and also those supplied by the experiment are recorded in table 4.

Table 4.

	Time of experiment in min.	Values of $\frac{S_c}{S_p}$ calculated	found
Rabbit C	80	0.04	0.03
	80	0.04	0.04
	170	0.07	0.07
	170	0.07	0.09

[1] This assumption though incorrect does not cause an appreciable error.

Uptake of Phosphate Ions by the Blood Corpuscles in Experiments in vivo.

The interpretation of the results of experiments in vivo is much complicated by the fact that active phosphate introduced into the blood stream is rapidly exchanged with phosphate of bone tissue. Such an interaction leads to a very rapid decrease in the activity of the blood after intravenous administration of active phosphate as the specific activity of the plasma inorganic P at the end of the experiment differs very strongly from the value prevailing on the average during the experiment. To facilitate the interpretation of the result the labelled sodium phosphate was injected into the ear vein of a rabbit drop by drop in the course of the experiment which took 3 hours. We have to thank Professor Lundsgaard for injecting the rabbit in this and many other cases. During the experiment small blood samples (0.1 — 0.3 mg.) were taken from the vein of the other ear of the rabbit and their activity determined. The results of the experiment are seen in tables 5 and 6.

Table 5.

Percentage of the total labelled P injected during the course of the experiment present in the circulation.

(Labelled sodium phosphate administered drop by drop in the course of three hours).

Time in minutes	Percentage of the total P administered in the course of 3 hours present in circulation
18	1.16
61	2.64
117	4.00
168	5.36

Table 6.

Specific Activities Obtained.

Labelled P injected drop by drop during the course of the experiment (3 hours).

Fraction	Relative specific activity
Plasma inorganic P	100.0
Corpuscle inorganic P	11.0
Corpuscle acid-soluble organic P	7.7
Marrow inorganic P	7.5
Muscle inorganic P	6.9
Muscle creatine P	0.95
Muscle acid-soluble after removal of inorganic creatine P	0.78
Tibia diaphysis P average	0.27

As seen from the figures of table 5 the specific activity of the plasma inorganic P increases during the experiment somewhat slower than proportional to time. If the increase of the specific activity would be proportional to the time of the experiment the average specific activity of the plasma inorganic P, which we wish to know, should be $= \frac{1}{2} S_p$. This can be considered to be a lower limit of the average specific activity. To arrive at an upper limit we could assume the average specific activity to be equal to the maximum value observed, which is S_p. Making the assumption $s_p = \text{prop.} \sqrt{t}$, we arrive at the average value of the specific activity of the plasma inorganic P $= \frac{2}{3} S_p$. The value for the specific activity of the total acid-soluble corpuscle P (comp. p. 13) is approximately given by the formula

$$S_c = \tfrac{2}{3}\, \alpha t\, (S_p - 1.8\, S_c).$$

For the ratio $\frac{S_c}{S_p}$, after the lapse of 175 min., we arrive at the figure 0.12 (using the value of α found in our

experiments in vitro), while the experimental value of the specific activity of the acid-soluble P of the corpuscles (comp. table 6), after the lapse of 175 min., was found to be 8 % of that of the plasma inorganic P at the same time, or $\frac{S_c}{S_p} = 0.08$. The calculated and experimentally found values of the rate of labelling of the corpuscles acid-soluble P are thus in good agreement. We arrived at the calculated figure by making use of the α value obtained with rabbits' blood in experiments in vitro (taking $\alpha = 0.0013$ for rabbit I).[1]

Administration of Labelled Hexosemonophosphate.

Labelled hexosemonophosphate containing 6 mgm P was administered to a rabbit weighing 2.7 kg. by intravanous injection. After the lapse of $1\frac{1}{2}$ hour the animal was killed and the specific activity of the fractions recorded in table 5 determined.

When interpreting the above figures we must bear in mind that the greatest part of labelled inorganic P formed through the decomposition of active hexosemonophosphate enters the tissues. That the specific activity of the plasma inorganic P is, in spite of this fact, higher than that of the plasma ester P clearly indicates that a very large part of the active hexosemonophosphate injected into the blood must have been decomposed in the course of the experiment. We must also take in consideration that besides the 4 mgm. % hexosemonophosphate P added the plasma contained also its normal ester P content of about 0.5 mgm. % which became partly labelled, this being made possibly

[1] When isolating the inorganic P and the different fractions of the ester P present in the corpuscles and comparing their specific activity in experiments in vitro and in vivo some differences were found. The results of these experiments will be shortly published.

through the presence of labelled inorganic P formed through the decomposition of active hexosemonophosphate. But even disregarding the presence of esters other than hexosemonophosphate, by comparing the activity of the hexosemonophosphate introduced with that of the total plasma ester present, after the lapse of $1^1/_2$ hours, we arrived at the result that more than 99.9 % of the labelled hexosemonophosphate administered left the circulation within $1\frac{1}{2}$ hours. In Making the above calculation we assumed the blood content of the rabbit to amount to 160 cc. In our in vitro experiments $^1/_{15}$ mgm. of hexosemonophosphate was added to 10 cc. blood, thus the concentration of the latter was about $^1/_6$ of that in the experiment in vivo[1]. While in the in vitro experiment in the course of $2^1/_2$ hours $\frac{1}{5}$ of the labelled hexosemonophosphate was decomposed, in the in vivo experiment in the course of $1^1/_2$ hours more than 99.9 % was removed.[2] A powerful agency producing hydrolysis of hexosemonophosphate is ROBISON's bone enzyme. The presence of small amounts of this enzyme in the plasma was found by MARTLAND and ROBISON[3] which according to them is possibly derived from bone by slow diffusion. These small amounts of bone enzymes were presumably responsible for the hydrolysis of hexosemonophosphate in our experiments in

[1] When comparing the results of the in vitro and in vivo experiments we have to consider that the rate of decomposition depends on the concentration of the substrate as well, through a change of the concentration in a ratio 1:6 should not strongly influence the rate of decomposition.

[2] A large part of the hexosemonophosphate removed may have been taken up by the tissues. R. T. BRAIN, H. O. KAY and P. G. MARSHALL (l. c.) found namely that in the course of 5 min. more than three-quarters of the injected glycerophosphate left the human circulation and as in this time it had not been excreted in the urine, nor hydrolysed into inorganic P in the blood, it must have been taken up by the tissue.

[3] MARTLAND and ROBISON, Biochem. J. **20**. 847, 1926.

vitro, while the much more rapid disappearance of the labelled hexosemonophosphate from the blood in vivo is probably due to the much larger amounts of bone enzyme present in the bones and other organs, especially the kidneys[1]. As seen in table 7 the specific activity of the corpuscle inorganic P is appreciably lower than that of the plasma inorganic P, while that of the corpuscle ester P is only slightly lower than that of the corpuscle inorganic P.

Table 7.

Specific Activity of P Fractions $1^1/_2$ Hours After Administration of Labelled Hexosemonophosphate.

Fraction	Specific activity
Plasma inorganic P	1.
Plasma ester P	0.9
Corpuscles inorganic P	0.3
Corpuscles ester P	0.23
Liver inorganic P[2]	0.62
Liver ester P	0.22

On the Origin of Phosphatides of Plasma, and Corpuscles.

We have already mentioned that the amount of labelled phosphatides formed in the blood within a few hours is

[1] Bodansky (I. Biol. Chem. **118**, 391, 1937) concludes that the phosphatase in question comes from the bone, kidney ov some other tissue, but not from the intestinal mucose.

[2] We were prevented in extracting at once the inorganic and acid soluble organic P of the liver and therefore some of the latter may have been decomposed (comp. E. Jacobsen, Biochem. Z. **242**, 292, 1931); supplying inorganic P of low activity. The specific activity of the inorganic P present as such in the intact liver may therefore have been higher than stated above. In this connection it is of interest to know that in the case of a rat, which had been injected with labelled hexosephosphate and killed 2 hours later, no such difference was found. Relative specific activities: Plasma inorganic phosphate 1.0; liver inorganic phosphate 0.97; liver ester 0.58).

entirely negligible compared to that of labelled acid-soluble organic compounds formed. The phosphatides present in the plasma are released by the organs in which phosphatides are synthesised, primarily by the liver, but also by the intestinal mucosa, and possibly by other organs. The synthesis of phosphatides in the different organs was investigated in recent years using fatty acids, which could be traced by chemical analysis[1], and also by the use of radioactive P as an indicator[2]. In several cases the change in the degree of saturation of the fatty acid component of the phosphatides extracted from the intestinal mucosa, liver, etc., was studied after feeding cod liver oil which contains a large amount of unsaturated fats. Within a short time an increase in the iodine number of the fatty acids was found. For example, within 2 days after the change of diet the iodine number of phosphatides of the intestinal mucose increased from 93 to 160. From this result it follows that within 2 days an appreciable amount of the phosphatide molecules present in the intestinal mucose were renewed. Instead of feeding a mixture of fatty acids showing a different degree of saturation and having a high average iodine number, SINCLAIR fed, in his later experiments, fats containing pure (85 %) elaidic acid, a geometric isomer of oleic acid, an easily traceable substance, since it forms a lead salt which is insoluble in ether, differing in this regard

[1] Comp. R. G. SINCLAIR's report in Physiological Reviews **14**, 351 (1934). The papers of the same author and collaborators, J. of Biochem. **115**, 211 (1936); **118**, 122 (1937); **121**, 361 (1937). C. ARTOM, Archiv intern. Physol. **36**, 101 (1933).

[2] C. ARTOM, C. PERRIER, M. SANTANGELO, G. SARZANA, and E. SEGRE, Archiv Internat. de Physiol. **45**, 32 (1937); **47**, 245, 1938. L. HAHN and G. HEVESY, Skandinav. Archiv f. Physiol. **77**, 148 (1937). G. HEVESY and E. LUNDSGAARD, Nature **140**, 275 (1937). B. A. FRIES, S. RUBEN, I. PERLMAN and I. C. CHAIKOFF, J. Biol. Chem. **122**, 169 (1937); **123**, 587 (1938).

from all other unsaturated acids. 8 hours after feeding elaidic acid to cats, 15 % of the fatty acids extracted from the plasma phosphatides were found to contain elaidic acid, while hardly any was found in the phosphatides obtained from the corpuscles. This result may be interpreted as a proof of lack of a phosphatide turnover in the corpuscles. Another chemical indicator used to follow up the turnover of phosphatides is iodised fat which was fed by Artom to animals. Also in this case the presence of iodised fatty acid in the phosphatide molecule is a proof of their formation after the administration of iodised fat. By using this method Artom found the presence of iodised fatty acids in the phosphatides extracted not only from the plasma but also from the corpuscles. In the latter the concentration of the iodised fat was even higher (3.3 % of the total fatty acids) than in the former (2.0 %), a still lower content being found in the phosphatide fatty acids secured from the liver. The result obtained as to the turnover of phosphatides in the corpuscles, when using the elaidic acid method, is thus just the opposite of that arrived at when applying iodised fat as an indicator. While these indicators proved to be very useful to show that a rapid turnover of phosphatides actually takes place in some of the organs they are less adapted to permit conclusions of a quantitative nature to be drawn.

By introducing elaidic acid or iodised fat into the phosphatide molecule the properties of the latter are appreciably changed and not only will the different organs utilise the above mentioned substances in the formation of phosphatides only to a restricted extent, but the rate of uptake of these compounds may differ for different organs. In rats provided with large amounts of elaidic acid through-

out the entire period of prenatal and postnatal development the elaidic acid content of the fatty acids in the phosphatides of the brain was found to be only $^1/_4$ of that of the liver and muscles. If one finds a slower turnover of elaidic acid in the brain phosphatide than in other organs, this result can be partly due to a slower phosphatide turnover in the brain, and partly to a greater degree of selection in the building up of phosphatides in the brain than in the liver. The rate of the incorporation of elaidic acid into the phosphatide molecules will therefore fail to be a quantitative measure of the rate of phosphatide rejuvenation in the brain tissue, though this method revealed much important information as, for example, that the phosphatide turnover in the muscles is much slower than that of the liver and the intestinal mucose. In rats the incorporation of elaidic acid into the liver phosphatides was found to be essentially completed within one day but in the muscle transformation had occured only after a period of many days. One of the great advantages of the application of isotopic indicators is that the replacement of ^{31}P by ^{32}P for example in the phosphatide molecule does not change the chemical character of the substance to any noticeable extent and therefore any possible preference of an organ for the ^{32}P phosphatide can be disregarded. A quantitative comparison of the phosphatide turnover in different organs by using ^{32}P as an indicator was carried out by different experimentors, a slow turnover being found in the brain and muscles, a fast one in the milk gland, the liver, the kidneys and the intestinal mucose, while we find a fairly fast rate in tumor tissue. Taking the specific activity of the phosphatide P extracted from the liver of a mouse to be 100, we find for the specific activity of that extracted from the muscles

and the graft of the brest tumor 18 and 9 % respectively. These figures indicate the relative rate of resynthesis of the phosphatide molecules. They were obtained by extracting and analysing the phosphatides from the organs, 4 hours after the administration of labelled sodium phosphate. These figures, however, fail to inform us as to the percentage of the phosphatide molecules which were renewed within the last 4 hours. Most of the active phosphate ions will exchange with bone and other tissue phosphate and will thus be prevented from taking part in the synthesis of phosphatide molecules. Knowledge relative to the percentage of, for example the liver phosphatides, renewed within 4 hours can be obtained, as already discussed on p. 21, by administrering the sodium phosphate solution drop by drop and thus keeping the active phosphate concentration of the blood at an approximately constant level, or in perfusion experiments carried out on the isolated liver. In the latter case active sodium phosphate is added to the blood circulating through an isolated liver and, after the lapse of a few hours, the specific activity of the plasma inorganic P and that of the phophatide P extracted from the liver are compared. Through the kindness of Prof. Lundsgaard we were able to carry out such a determination from which it was concluded that, in the course of $2^1/_2$ hours, about 2 % of the phosphatides in the cat liver are renewed[1]. In the same time, when blood is shaken with active sodium phosphate, only a very slight amount of active phosphatide was formed. From these experiments it may be concluded that less than 0.1 % of the phosphatide molecules present in the isolated blood was renewed in the course of $2^1/_2$ hours. In experiments on living goats, 4 hours after injecting labelled sodium phosphate, less and probably

[1] L. A. Hahn and G. C. Hevesy, Biochem. J. **32**, 342, 1938.

much less than 1 % of the blood phosphatide molecules were labelled. We have therefore to conclude that the phosphatide molecules present in the blood can only get rejuvenated by an influx of molecules from the organs like the liver in which they were synthesised. As a possible source of formation of the blood phosphatides the liver is first to be considered in view of the fast phosphatide turnover found in the liver and the large amounts of phosphatides stored in it. In this connection the results obtained by NEDSWEDSKY[1] should be recalled according to which blood leaving the liver contains 23 % more phosphatide than that entering the liver from the portal vein. Laying hens are especially well suited to the study of phosphatide metabolism. A hen laying daily incorporates into the yolk 1—2 gms. of phosphatides corresponding to about 60 mgms. of P. We found that these phosphatides were not produced in the ovary but were carried by the plasma in the main from the liver. To what extent phosphatides are carried into the circulation through the lymph from the intestinal mucosa is not yet settled. In the above mentioned experiment the specific activity of the phosphatide P extracted from the hens' intestinal mucosa was found to be only 1/4 of that of the plasma phosphatide P. The greatest part of the active phosphatide molecules present in the plasma of the hen could therefore not originate from the intestinal mucosa but must have been formed in the liver and, possibly to minor extent, in other organs. As the plasma of the hen contains only about 20 mgm. % phosphatide P, thus only 1/3 of that incorporated in the yolks daily, over 9/10 of the plasma phosphatides of the blood is removed from

[1] S. W. NEDSWEDSKY and K. ALEXANDRY, Z. physiol. Chem. **219**, 619 (1928).

the latter, in the course of a day, and replaced by newly formed molecules. If the plasma phosphatides originate from the liver, after the lapse of a day, the specific activity of the plasma phosphatide P should no longer differ materially from that of the liver phosphatide P. In an experiment in which the hen was killed 28 hours after injecting the labelled phosphate, the plasma phosphatie P showed a specific activity amounting to 82 % of that of the liver phosphatide P. When interpreting the low figure found for the phosphatide turnover in the intestinal wall of the hen compared with that found in the liver we have however to bear into mind that the labelled inorganic phosphate reaches the digestive tract at a later and thus more "diluted" (with inactive phosphate) state than the liver. The active phosphate injected will be promptly carried to the liver while it enters the intestine only in the form of saliva, gastric juice, bile and pancreatic juice and, possibly to some extent, through the intestinal wall[1] in the digestive tract. Therefore, when comparing the specific activities of the liver phosphatide P with that of the intestinal mucosa phosphatide P after injection of active phosphate we are apt to overestimate the phosphatide turnover of the liver while, when feeding the active sodium phosphate, we must expect the opposite to be the case. A comparison of the specific activity of the liver inorganic P with that of the liver phosphatide P leads to the result that, after the lapse of 28 hours, the former was about two times greater than the latter. Thus only less than half of the phosphatide molecules present in the hen's liver was newly formed within that time, the rate of regeneration of the tissue phosphatides being thus a comparatively slow process, since the removal of the tissue phos-

[1] G. F. Youngburg, Proc. Exp. Physiol. Mediz. **36**, 230, 1932.

phatides into the plasma also takes time the low rate of rejuvenation of the plasma phosphatides is just what we would expect. In experiments we carried out on human subjects, in the course of a day less than 30 % of the plasma phosphatide was regenerated and a rough estimate, taking into account the change of the spec. activity of the phosphate, indicates that after the lapse of a week this fraction is still less than one-half. (comp. table 8).

We have already mentioned that in vitro experiments have shown that in the blood only a very minimal new formation of phosphatide molecules takes place; the phosphatides present in the corpuscles must therefore have been incorporated in the latter during their formation, or alternatively diffused from the plasma into the corpuscles.[1] As already mentioned, within a day, the plasma phosphatides of a daily laying hen were replaced up to 82 %, by phosphatide molecules carried into the circulation from the liver and other organs. In spite of this thorough-going replacement of the plasma phosphatides, the phosphatide molecules present in the corpuscles are renewed only to an extent equal to $^1/_3$ of that of the plasma phosphatides in the course of 28 hours. In human subjects, after the lapse of one day, the corpuscle phosphatide shows a specific activity amounting to only $^1/_5$ or less of that of the plasma phosphatide P and even after the lapse of 8 days the ratio is still $^1/_2$. When the corpuscles are formed from plasma containing labelled phosphatides they are bound to contain such. As seen above, even after the lapse of a week the

[1] An investigation carried out recently incollaboration with L. HAHN lead to the result that a fairly slow exchange between a part of the phosphatide molecules present in the corpuscles and those present in the plasma takes places. In the course of 4 hours about 5 % of the phosphatides present in the corpuscles were exchanged.

labelled fraction of the plasma phosphatides is still twice as high as that of the phosphatides present in the corpuscles. From this result we can conclude that less than half of the corpuscles are produced in a week. One could object to the above conclusions on the ground that the phosphatides are composed of different constituents, lecithin, cephalin and so on, each of which may contain very different fatty acids and, if those components which are mainly represented in the corpuscles are renewed at a slower rate than those chiefly found in the plasma, this difference may also tend towards a low value for the specific activity of the corpuscle phosphatide P. In human blood, the composition of the corpuscle phosphatides was found to be not very different from that of the plasma phosphatides[1] and as we have found that, after a lapse of a day, the specific activity of the corpuscle phosphatides is only 8—19 % of that of the plasma phosphatides (comp. table 8) we are justified in concluding that at least the most active component of the phosphatide mixture of the corpuscles must show a lower specific activity than its conterpart in the plasma.

This fact definitely excludes the possibility that the active phosphatides are formed in the corpuscles and diffuse into the plasma. The result discussed on page 27, according to which the corpuscle phosphatides contain more iodised fat than the plasma phosphatides, is presumably to be explained by a greater preference of the corpuscles for phosphatides containing an iodised fatty acid component.

[1] E. Kirk, J. Biol. Chem. **123**, 637 (1938). According to this experimentor plasma phosphatide contains as an average 13 % lecithin, 47 % cephalin, and 40 % sphingomeyelin, while the corresponding figures for the corpuscles are 16, 60, and 24.

Table 8.

Parts in Million of the Total Activity Injected Subcutaniously Present in 1 mgm. P extracted from the Plasma and Corpuscles of Human Subjects.

a) Boy	
Sample taken after 120 min.	
Plasma phosphate	201
Corpuscle total acid soluble P	57
Sample taken after 7 days	
Plasma phosphate	10
Plasma phosphatides[1]	14
Corpuscle phosphatides	6
b) Young female	
Sample taken after 130 min.	
Plasma phosphate	480
Corpuscles inorganic phosphate + hydrolysable ester P (hydrolysed for 7 min. at 100° in 1 n HCl)	87
Corpuscles non hydrolysable ester P	62
Sample taken after 1 day	
Plasma phosphate	105
Corpuscles total acid soluble	173
Plasma phosphatide	31
Corpuscles phosphatide	2.3
c) Old female	
Sample taken after 140 min.	
Plasma phosphate	224
Corpuscles inorganic phosphate + hydrolysable ester P (hydrolysed for 7 min. at 100° in 1 n HCl)	61
Corpuscles non hydrolysable ester	34
Sample taken after 1 day	
Plasma phosphatide	7.5
Corpuscles phosphatide	1.3
Sample taken after 8 days	
Plasma phosphate	10
Plasma phosphatide[1]	16
Corpuscles phosphatide	7

[1] We witness here an example of the case discussed in detail in former papers (for example HAHN and HEVESY, Skandin. Archiv f. Phys. **77**, 148 (1937)) in which, due to the rapid exchange between bone phosphate and plasma phosphate the active inorganic P is renewed from the plasma at a rapid rate while the removal of the labelled phosphatide P is a much slower one. Some of the phosphatide molecules were formed

Summary.

The rate at which labelled phosphate ions added to the plasma of rabbits' blood penetrate into the corpuscles was determined. A comparison of the specific activity (activity per mgm. P) of the inorganic P and of the ester P extracted from the corpuscles suggests that the penetration of the labelled inorganic phosphate into the corpuscles is a comparatively slow process while the process in which the labelled inorganic phosphate is incorporated into the easily hydrolysable organic compounds inside the corpuscles is a fast one.

The rate of increase of the specific activity of the total acid soluble phosphorus (s_c) present in the corpuscles with time (t) is proportional to the difference in the specific activity of the inorganic P of the plasma (s_p) and that of the corpuscles (s_i).

$$\frac{ds_c}{dt} = \alpha (s_p - s_i).$$

The proportionality factor of the above equation (α), denoted as coefficient of penetration, was calculated; the latter was found to be about the same in experiments in vitro and in vivo.

By making use of labelled sodium hexosemonophosphate it was found that, when shaking the latter with rabbits' blood, $^1/_5$ hydrolyses within $2^1/_2$ hours. From sodium hexosemonophosphate administered, by intravenous injection, to a rabbit after the lapse of $1^1/_2$ hours less than $^1/_{10}$ % was left in the circulation.

from a highly active plasma and were still present after a lapse of 7 days, while the greater part of the inorganic P dissappeared at this time from the plasma. Similar cases can also be found when investigating the acid soluble P.

By comparing the rate of formation of labelled ester P in the corpuscles after introducing in one case labelled hexosemonophosphate in the other labelled inorganic phosphate we arrive at the result that the labelled phosphate ions which penetrate into the corpuscles are utilised for the formation of labelled ester molecules. If any labelled hexosemonophosphate penetrates into the corpuscle at all, the rate of its penetration is much lower than that of the phosphate ions.

The analogy between the formation of labelled adenyltriphosphate and other acid-soluble P compounds present in the corpuscles and the formation of "heavy" acetic acid when dissolving acetic acid in "heavy" water is emphasized. The role played by the electrolytic dissociation in the latter case is taken over by the enzymatic action of the phosphatases present in the corpuscles.

The rate of regeneration of the phosphatide molecules present in the blood was found to be very much slower than that of the acid-soluble compounds. The regeneration of the former was found to take place in the organs from which they are carried into the circulation. After the lapse of a week, about one half of the phosphatide molecules present in human plasma was replaced by within that time newly formed molecules. The replacement of the corpuscle phosphatides is a much slower process than that of the phosphatides present in the plasma, the ratio in human blood after one day being 6 : 1, after a week 2 : 1.

We want to extend our thanks to Prof. Niels Bohr and Prof. J. N. Brønsted for putting many facilities at our disposal, to Prof. E. Lundsgaard for many valuble sugge-

stions and the biological material used, and to Dr. Hilde Levi for carrying out the counting experiments.

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Indleveret til Selskabet den 4. November 1938.
Færdig fra Trykkeriet den 21. Marts 1939.

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