CXLVIII. CHANGES IN THE PHOSPHORUS PARTITION IN HUMAN BLOOD DURING AMMONIUM CHLORIDE ACIDOSIS.

By HERBERT DAVENPORT KAY (*Beit Memorial Research Fellow*).

From the Biochemical Laboratory, Cambridge.

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Phosphorus occurs in at least four forms in human blood: inorganic phosphate, two forms of organic acid-soluble phosphoric esters, one of which is hydrolysed by an enzyme occurring in bone [Kay and Robison, 1924] and in other tissues, and "lecithin." Bloor [1918] showed in 1918 that the acid-soluble portion of protein-free blood filtrates and the portion soluble in ether-alcohol contained between them all the phosphorus, i.e. that no phospho-protein is present. In determining, therefore, the distribution of phosphorus at any given time between these four groups, although there may be more than one substance present in any one group, no phosphorus-containing compound of importance is neglected.

The acid-soluble portion, in which there are at least two, and possibly four, different phosphorus-containing substances, contains among these only one compound—adenine nucleotide—which has up to the present been identified with any degree of certainty [Jackson, 1924]. It is nevertheless feasible, even with the meagre knowledge already acquired, to give some answer to the question as to the possible functions of these unidentified phosphorus compounds, and there seems to be no doubt, for example, that bone formation is as intimately concerned with one of the organic phosphoric esters present in blood [Kay and Robison, 1924; Robison and Soames, 1924] as is fat metabolism with the ether-soluble phosphorus compounds [Bloor, 1916].

From the experiments of Haldane, Wigglesworth and Woodrow [1924] on J. B. S. H. it is known that ammonium chloride acidosis leads to a marked disturbance of the phosphorus partition in blood, and it was with a view to obtaining further evidence as to the possible functions of these different phosphorus compounds that advantage was taken of a recent experiment[1] in this laboratory in which the same subject ingested large quantities of ammonium chloride on each of three successive days, to examine this disturbance in more detail.

For the analyses, blood was taken from an arm vein each morning about half an hour after rising, while the subject was still fasting. Beginning in the

[1] Other results of this experiment will shortly be published.

morning of the day during which the first 25 g. of ammonium chloride were taken, these analyses were continued for 6 days after the last ammonium chloride day, until the inorganic phosphorus had returned to its normal concentration. When the daily blood analyses were discontinued, the amounts of the other phosphorus compounds in the blood had almost, but not quite, reached their normal level, and when a final blood sample was taken 4 days later the correspondence between the first (normal) day sample and this last sample was complete, i.e. the phosphorus distribution had returned to the normal.

The inorganic phosphorus was determined by the Briggs method; the total acid-soluble phosphorus by ignition with a sulphuric-nitric acid mixture followed by the same colorimetric procedure; the "lecithin" phosphorus by what was practically the method of Randles and Knudson [1922] and the "hydrolysable" phosphorus by the method of Kay and Robison using enzyme freshly prepared from rat bone. The blood filtrates for the hydrolysable phosphorus determination were neutralised, a little chloroform added, and then placed in the cold chest at 0°. There they remained until the end of the experiment, when all were treated together under strictly comparable conditions with the bone enzyme. It is known from previous work that keeping the blood filtrate at 0° for a much longer period has no effect on the hydrolysable phosphoric ester. Since all the organic acid-soluble phosphorus of blood is present in the corpuscles, it was necessary, in order to follow the important changes taking place within them, to determine the percentage of corpuscles in each blood sample.

The results are shown in Table I.

Table I. *Changes in phosphorus distribution in blood during ammonium chloride acidosis.*

		In milligrams P per 100 cc. *blood*						In mg. P per 100 cc. *corpuscles*		In mg. P per 100 cc. *blood*	
Day of month	Haematocrit percentage corpuscles	(*a*) Inorganic phosphorus	(*b*) Total acid soluble phosphorus	(*c*) Organic phosphorus (*b* − *a*)	(*d*) Hydrolysable phosphorus	(*e*) Non-hydrolysable phosphorus (*c* − *d*)	(*f*) Percentage hydrolysable phosphorus in whole blood	(*g*) Hydrolysable phosphorus to nearest mg.	(*h*) Non-hydrolysable phosphorus to nearest mg.	(*i*) Lecithin phosphorus	(*j*) phosphorus (*b* + *i*)
29th[1]	46	3·2	27·5	24·3	6·0	18·3	25	13	39	11·2	38·7
30th[2]	48	3·4	24·9	21·5	6·7	14·8	31	14	31	12·1	37·0
31st[3]	51·5	3·9	24·0	20·1	6·6	13·5	33	13	26	12·4	36·4
1st	52·5	3·7	24·4	20·7	7·0	13·7	34	13	26	12·6	37·0
2nd	47·5	2·7	23·1	20·4	6·7	13·7	33	14	29	10·9	34·0
3rd	45·0	2·6	23·3	20·7	5·8	14·9	29	13	33	10·0	33·3
4th	39·5	2·5	22·0	19·5	5·5	14·0	28	14	35	10·1	32·1
5th	39·0	3·0	23·3	20·3	4·9	15·4	24	13	39	10·3	33·6
6th	40·0	3·4	24·9	21·5	4·6	16·9	21	12	42	10·4	35·3
10th[4]	44·0	3·4	27·1	23·7	5·4	18·3	23	12	42	11·3	38·4

[1] Blood sample 10 a.m. 25 g. NH_4Cl eaten between noon and midnight.
[2] Blood sample 9.30 a.m. 25 g. NH_4Cl eaten between noon and midnight.
[3] Blood sample 9.30 a.m. 20 g. NH_4Cl eaten between noon and midnight.
[1, 4] Normal days.

The change in the inorganic phosphate of the blood confirms in general that found in a previous experiment of Haldane, Wigglesworth and Woodrow

[1924]. In the experiment described in the present paper the rise in inorganic phosphorus was more marked and the subsequent fall not quite so severe. In Haldane, Wigglesworth and Woodrow's experiment, sodium phosphate was taken on the fourth day after the last ammonium chloride day and brought the inorganic phosphorus rapidly back to the normal, whereas here the return to the normal level was more leisurely, being first accomplished on the sixth day after the last ammonium chloride day. On this day, although the total acid-soluble phosphorus per 100 cc. blood was not yet back to the normal, the total acid-soluble phosphorus *per* 100 *cc. corpuscles* had already reached its normal value. The discrepancy is due to the fact that the normal percentage of corpuscles had not yet been reached.

The "lecithin" or lipin phosphorus changed in a similar way to the inorganic phosphorus, but it was more clearly connected with changes in the haematocrit value, which is to be expected, since corpuscles contain two and a half times as much lipin phosphorus as the plasma [Bloor, 1916]. The lecithin phosphorus *per* 100 *cc. corpuscles*, calculated from the haematocrit value and the ratio found by Bloor (assuming that this ratio still holds during acidosis) shows only a small variation during the period of the experiment, an initial rise in the first three days being followed by a corresponding fall in the next three days, and a rise again to normal at the end of the period of experiment. The organic acid-soluble phosphorus per 100 cc. blood and per 100 cc. corpuscles shows an immediate fall at the outset, followed by a slow return to normal after several days.

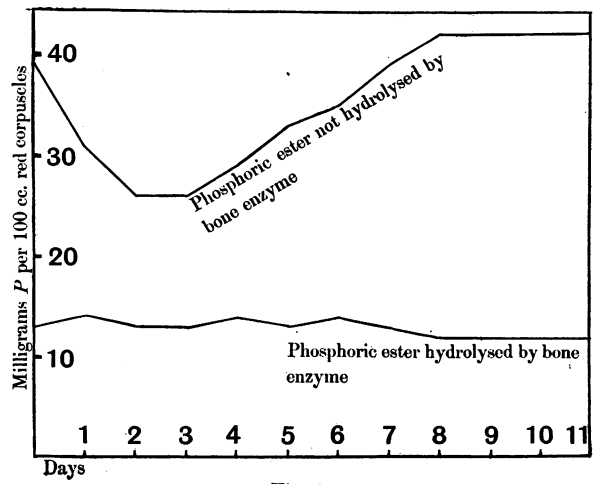

Fig. 1.

It is in the partition of this organic acid-soluble phosphorus into enzyme-hydrolysable and non-hydrolysable portions that the most interesting development occurs. Fig. 1 shows the amounts in 100 cc. corpuscles of the enzyme-hydrolysable and non-hydrolysable phosphorus.

It seems clear that the quantity of hydrolysable phosphoric ester in the

corpuscles remains constant during acidosis, and that the total loss of acid-soluble organic phosphorus from the corpuscles is borne by the portion resistant to hydrolysis by the bone enzyme, which portion is evidently one of the reserves to be called upon to make up the loss of phosphorus from the body.

(The urinary excretion of phosphate during this particular experiment is not yet available, but there is no reason to suppose that it will be found markedly different from that in a previous experiment with the same subject, when about the same amount of ammonium chloride was eaten and the maximum excretion of urinary phosphorus was found to occur on the last two days on which this salt was taken. This peak in the excretion curve would thus be associated with the big fall in the non-hydrolysable phosphoric ester in the corpuscles.)

It is unlikely that such a large proportion of the blood phosphorus (18 mg. P per 100 cc. whole blood or six times the amount of inorganic phosphorus) would be carried about by such a highly specialised cell as the red blood corpuscle unless it played some useful part, and the result of this experiment indicates its possible function as a readily available reserve supply of phosphorus, possibly the first reserve called upon, to enable the kidney to excrete acid within the limits of hydrogen ion concentration compatible with the health of this organ.

A further analysis of the figures in Table I reveals the fact that the total loss of phosphorus from the whole of the blood at any stage in the acidosis is very nearly the same as the loss of phosphorus from the enzyme-stable ester fraction of the organic phosphorus in the corpuscles (Table II).

Assuming that the blood volume of the subject was six litres to begin with, and that the corpuscular volume was therefore 46 % of this, i.e. 2·76 litres, and that this corpuscular volume was constant throughout the acidosis, we get the following figures:

Table II.

(After correcting for changes in blood volume.)

	Total P in whole of blood		Non-hydrolysable P in whole of blood		Lecithin P in whole of blood	
Day of month	Total in grams	Excess or deficiency	Total in grams	Excess or deficiency	Total in grams	Excess or deficiency
29th[1]	2·32	+0·0	1·08	+0·0	0·67	+0·0
30th	2·13	−0·19	0·86	−0·22	0·70	+0·03
31st	2·05	−0·27	0·72	−0·36	0·66	−0·01
1st	2·06	−0·26	0·72	−0·36	0·66	−0·01
2nd	1·99	−0·33	0·80	−0·28	0·63	−0·04
3rd	2·04	−0·28	0·91	−0·17	0·61	−0·06
4th	2·05	−0·27	0·96	−0·12	0·71	+0·04
5th	2·32	+0·0	1·08	+0·0	0·73	+0·06
6th	2·37	+0·05	1·16	+0·08	0·72	+0·05
10th[1]	2·39	+0·07	1·16	+0·08	0·71	+0·04

[1] Normal day.

The "lecithin" figures similarly corrected for blood volume are given in the last two columns of Table II. It will be seen that the lecithin rose above

normal during the rapid breaking up of the enzyme-stable phosphoric ester at the beginning of acidosis, and is again above normal during the recovery period. This may possibly mean that this ester both in hydrolysis and synthesis passes through the "lecithin" or ether-soluble stage. Inorganic phosphate may be the next stage in the hydrolysis, as it remains high whilst the ester is being hydrolysed, then falls through normal and reaches its lowest point on the two days when the re-synthesis of the ester, and therefore the demand for phosphorus to make it, is most marked. Perhaps this may be putting more strain on the figures than they can bear, but it is at least a suggestive possibility and connects the sequence of many of the observed changes.

The presence of adenine nucleotide in blood corpuscles has already been mentioned. Quantitatively (taking Jackson's [1924] figures of 15–25 mg. nucleotide per cent.) it accounts for some 2 mg. P per 100 cc. whole blood, or 5 mg. P per 100 cc. corpuscles. The maximum decrease in the non-hydrolysable phosphorus in corpuscles during the acidosis period is 13 mg. P %, so that even were the adenine nucleotide to disappear completely it would only account for the lesser portion of the missing phosphorus. The only known organic phosphorus compound in the corpuscles, therefore, gives no clue to the problem.

That these unknown phosphorus compounds in the corpuscles, which are chemically fairly stable substances, not hydrolysed at room temperatures or at 37° on standing in neutral, alkaline or acid solution, and requiring to be boiled with $N/2$ alkali or acid for many hours before complete hydrolysis is obtained, should be isolated and identified is a matter of some urgency[1]. It is possible that the responsibility for changes in the reducing power of blood filtrates after acid hydrolysis remarked by Cooper and Walker [1921, 1922] and certain anomalies in the relationship of the reducing power to the polarimetric value of blood filtrates such as those observed by Winter and Smith [1922] may find an explanation in the nature of the organic phosphorus compounds in the corpuscles.

Summary.

Marked changes occur in the distribution of phosphorus compounds in human blood during the acidosis which follows the ingestion of ammonium chloride. The significance of these changes is discussed.

The author desires to express his thanks to Mr J. B. S. Haldane for his patience in trying circumstances and for suggestions and criticism.

[1] Dr Robison informs me that he is at present engaged on this work and will shortly publish a paper on the subject.

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