LXXX. OBSERVATIONS ON PHOSPHATES IN BLOOD AND ON THE URINARY EXCRETION OF PHOSPHATES.

BY REGINALD THOMAS BRAIN,
HERBERT DAVENPORT KAY (*Beit Memorial Research Fellow*)
AND PHILIP GUY MARSHALL.

From the Medical Unit, The London Hospital, E. 1.

(Received February 29th, 1928.)

It has been suggested from time to time (*a*) that "inorganic" phosphate in the blood or plasma is present in some unusual form (*i.e.* not as true ionic phosphate), (*b*) that the excretion of phosphate in the urine is controlled by factors other than the level of "inorganic" phosphate in the blood.

We bring together in this paper a number of observations we have made on these two, and on other related questions. We shall deal, in particular, with the interesting conclusion of Eichholtz, Robison and Brull [1925] that it is not the inorganic phosphate but the acid-soluble organic phosphorus of the plasma that is the normal source of part, if not all, of the inorganic phosphate of the urine, the organic phosphorus undergoing hydrolysis in the cells of the kidney under the action of kidney phosphatase.

FLUID REACHING THE KIDNEY.

Phosphate concentration in the plasma.

Mayrs [1922], Wu [1922] and Cushny [1926] have emphasised that it is the concentration of a substance in the *plasma* and not that in the whole blood which is of major importance in relation to urinary excretion. With regard to inorganic phosphate it has been variously stated that the normal corpuscular content of inorganic phosphate is greater than [Bloor, 1918; McKellips, de Young and Bloor, 1921; Jones and Nye, 1921], equal to [Zucker and Gutman, 1922; Wigglesworth and Woodrow, 1923], or less than [Kerr, 1926; Underhill, 1923] that in the plasma. Richter-Quittner [1921] and Buell [1923] go so far as to conclude that there is no inorganic phosphate in the corpuscles.

We have investigated this question by two methods.

First method. Samples of blood¹ were collected into paraffined centrifuge tubes over ice, and the separation of the plasma and precipitation of the

¹ Human blood has been used throughout our work, unless otherwise stated.

proteins carried out with the utmost celerity. Samples of blood drawn at the same time, but prevented from clotting by heparin and by oxalate were also dealt with similarly. Briggs's method was used for inorganic phosphate determinations. Corpuscle phosphate was calculated from these determinations and the haematocrit reading. Results are in Table I.

Table I. *Inorganic phosphate distribution between plasma and corpuscles, P mg. per 100 cc.*

Exp.* No.	Cooled blood, no anticoagulant				Same blood not cooled							
					Oxalate 0.2 %				Heparin 0.02 %			
	Blood	Plasma	Corps.	Plasma Corps.	Blood	Plasma	Corps.	Plasma Corps.	Blood	Plasma	Corps.	Plasma Corps.
1	3.1	3.3	2.9	1.14	2.7	3.1	2.2	1.41	2.8	2.9	2.7	1.07
2	3.0	3.5	2.4	1.46	2.7	3.2	2.0	1.60	2.9	3.6	2.0	1.80
3	5.0	6.1	3.6	1.69	4.7	5.7	3.3	1.73	4.7	6.1	3.1	1.97
4	3.5	4.3	2.4	1.79	3.5	3.9	2.8	1.39	3.5	4.1	2.5	1.64
5	2.3	2.8	1.5	1.86	2.2	2.6	1.6	1.63	2.2	2.8	1.5	1.87
6	3.6	4.7	1.9	2.47	3.6	4.3	2.5	1.76	3.7	4.5	2.4	1.87
Mean	3.4	4.1	2.4	1.71	3.2	3.8	2.4	1.58	3.3	4.0	2.4	1.67

* Experiments in order of increasing $\frac{\text{plasma}}{\text{corps.}}$ ratio.

It will be seen that for both blood and plasma the results are consistently just a little higher when no anticoagulant is used than in the oxalated specimens, though the difference is not very great. Whatever the method employed to prevent clotting, free phosphate is invariably found within the corpuscles albeit in distinctly lower concentration than in the plasma. If the corpuscles are washed with isotonic sodium chloride solution, appreciable quantities of inorganic phosphate may be found in the washings, at the expense of that in the corpuscles. This probably accounts for the findings of Buell that little or no free phosphate is present in the corpuscles, since she found it necessary to wash the corpuscles in some cases as many as four times before analysis.

We have carried out a large number of determinations during the past two years upon samples of oxalated blood from normal subjects and from patients with nephritis, working without undue delay, but with no special celerity. On the average, the plasma values are somewhat lower, and the corpuscle values slightly higher than in Table I. Average values from 85 determinations on normal blood and 36 on blood from patients with nephritis are:

	Inorg. P mg. per 100 cc.			Plasma Corps.
	Blood	Plasma	Corps.	
Normal subjects or convalescent patients	3.9	4.5	3.3	1.36
Patients with nephritis	6.5	7.3	4.7	1.55

The plasma value for inorganic phosphate in nearly all instances is higher than the corpuscle value, whether the blood phosphate was rising, falling or constant.

The results are shown graphically in Figs. 1 and 2. For normal blood, the points lie on a curve $p = 1.3b - 0.7$, where p is the plasma and b the blood phosphate value. This equation has been found useful for obtaining probable plasma values from the whole blood values of other workers.

Fig. 1. The distribution of inorganic phosphate between corpuscles and plasma in different phases of varying plasma concentration.

Fig. 2. Relation between inorganic phosphate content of plasma and of whole blood, in normal subjects.

Second method. Foetal blood was centrifuged in umbilical cords. By this method the blood remains in endothelium-lined blood-vessels, and as nearly normal in environment as possible, until the plasma has been separated; the red cells therefore are more likely to escape injury during centrifugation.

The cord is obtained as early as possible after the birth of the child; a portion, engorged with blood, is secured between ligatures, the blood being mixed by gently squeezing the cord. A third ligature is then introduced between the other two, and a sample of whole blood withdrawn from one of the two portions of cord and run into a tube containing just sufficient solid oxalate to prevent clotting. The remainder of the cord is suspended in liquid paraffin in a centrifuge tube and spun, the plasma removed by a fine needle and syringe and run on to oxalate. An inorganic phosphate determination is then made on both plasma and whole blood. A small portion of unoxalated blood, prevented from clotting by heparin, is used for the haematocrit determination. The results of four such experiments are shown in Table II.

Table II. *Blood centrifuged in the vein. P mg. per 100 cc.*

Exp. No.	Inorg. P concentration in			Plasma
	Blood	Plasma	Corps.	Corps.
1	4.4	5.8	2.2	2.64
2	3.1	4.7	1.7	2.76
(2 a*)	3.1	4.4	2.1	2.10
3	4.6	6.1	2.7	2.26
4	5.1	6.7	3.2	2.09
(4 a*)	5.1	5.8	4.0	1.45
Average for 1, 2, 3 and 4				2.44

* Specimen of whole blood from same cord centrifuged *after* removing from vein and oxalating.

The distribution of inorganic phosphate between the plasma and corpuscles in foetal blood centrifuged in the vein differs, therefore, from that in adult blood centrifuged, however speedily, *in vitro*. There is relatively much more phosphate in the plasma in the former case than in the latter.

The foregoing results are not altogether surprising in view of two inter-related factors which will clearly influence the distribution of phosphate: (a) the much greater protein concentration (and therefore smaller quantity of available water) in the corpuscles than in the plasma, (b) the Donnan equilibrium¹ between the cells and plasma. Thus, (a) plasma contains about 91.5 cc. of water per 100 cc., corpuscles some 65 cc., so that a substance present in simple aqueous solution would be distributed between 100 cc. plasma and 100 cc. of corpuscles in the ratio 140 : 100; (b) taking the Donnan ratio, r , for the distribution of univalent anions between the water of plasma and corpuscles of normal venous blood at p_H 7.4, to be 0.76 [Henderson, Bock, Field and Stoddard, 1924] and remembering that at this p_H phosphate ions are about four-fifths HPO_4'' and one-fifth H_2PO_4' , calculation indicates a theoretical ratio of 165 : 100 for the distribution of inorganic phosphorus.

¹ The red cell appears to be freely permeable to the *inorganic* phosphate of the blood.

between 100 cc. water of plasma and 100 cc. water of corpuscles. This value, however, does not take into consideration the large difference in the amount of water in the two phases (see (a)). If we now correct for this we find

$$\frac{\text{concentration of inorganic P per 100 cc. plasma}}{\text{concentration of inorganic P per 100 cc. cells}} = 2.32 : 1.$$

The average of the results obtained with foetal blood (plasma concentration : corpuscle concentration = 2.44 : 1) is in much closer agreement with this theoretical value of 2.32 : 1 than is the mean of the experimental findings for the blood of adults, given in Table I, in samples centrifuged in absence of anticoagulant (plasma concentration : corpuscle concentration = 1.71 : 1) or in presence of heparin (plasma concentration : corpuscle concentration = 1.67 : 1). In fact the latter values are not far removed from the theoretical value of 1.85 : 1 which is obtained if the assumption is made that the inorganic phosphate ions in blood are all univalent. There is, however, no reason to believe that this is the case. Does then the mere act of removing blood from a vein bring about (possibly as a result of changes occurring in the first stages of clotting or its prevention) a breakdown of the true *in vivo* equilibrium to a factitious equilibrium with a considerably lower plasma value than that which exists in the blood when it is circulating? The figures shown in Tables I and II support this hypothesis to some extent, though we cannot lay too much stress on a comparison between foetal blood and adult blood taken directly from active circulation. Foetal blood has inevitably been stagnant for some minutes in the vein, and its Donnan ratio, r , has not been directly determined and may be slightly different from that of adult venous blood.

Effect of temperature on r . Marrack and Thacker [1926, 2] found that when plasma was separated from whole blood at 38° or at 20° and its p_H then determined at 38°, there was, in normal subjects, an average increase of only 0.02 in the p_H of the plasma separated at 20°. Martin and Lepper [1926] found that the effect of temperature (with the concentration of total CO₂ constant) was the same upon the c_H of corpuscles and corresponding plasma. It would therefore appear that there is little interchange of ions between the corpuscles and the plasma in cooling from 38° to 20°, and thus the Donnan ratio r is probably not markedly affected by cooling the blood over this range.

Apart from the use of umbilical cords, we are not acquainted with any practicable means, applicable to the human subject, for obtaining plasma direct from the vein and thus being able to determine the true *in vivo* concentration of inorganic phosphate in the plasma. For working purposes we have taken the inorganic phosphate concentration in the plasma of venous blood centrifuged *in vitro* as a measure of the true concentration of inorganic phosphate in the plasma of blood entering the kidney, although in reality the latter value may be as much as 20 % higher than the former.

State of inorganic phosphorus in the plasma.

Is the inorganic phosphate of blood present in a diffusible form, or is it, like calcium, present partly in the form of ions, and partly in the form of an unionised, indiffusible compound?

Holt, La Mer and Chown [1925] brought forward evidence to show that normal blood-serum was apparently supersaturated with tertiary calcium phosphate to the extent of more than 200 %. They found, however, that artificial solutions containing $\text{Ca}_3(\text{PO}_4)_2$ remained supersaturated for days, as was also shown by Marrack and Thacker [1926, 1] for artificially supersaturated solutions of CaCO_3 . A possible explanation of this apparent supersaturation is that the calcium phosphate is present in serum or plasma in a colloidal form, and, indeed, Eichholtz and Starling [1925], from work on the excretion of phosphate by the heart-lung-kidney preparation, came to the conclusion that a great part of the inorganic phosphate present in normal serum is present as a non-ionised, colloidal calcium compound, and not in the form of sodium orthophosphate.

The balance of evidence up to the present is, nevertheless, against the view of these authors, at least in so far as mammalian blood is concerned. Marrack and Thacker [1926, 1] find no evidence of the presence of colloidal phosphate or carbonate in artificially supersaturated solutions, whilst Cushny [1919] and Pincus and his collaborators [1923, 1926] consider that the whole of the inorganic phosphorus of serum is present in the form of ions or ultrafilterable electrolytes.

We have carried out a number of ultrafiltrations of both plasma (heparinised or oxalated) and serum, and have found in all cases that a large proportion of the inorganic phosphate of the plasma is ultrafilterable through cellophane (which is, on the whole, a more resistant ultrafiltering medium than collodion and would be more likely to effect the complete retention of substances in the colloidal state). The ultrafilter used is that described by Aitken and Kay [1927]¹, and a micro-modification of the Bell and Doisy or of the Briggs method was used for inorganic phosphorus. In all except 3 (out of 32 determinations) with rabbit's or human plasma or horse serum (either heparinised or oxalated) there was slightly less inorganic phosphorus in the ultrafiltrate

Table III. *Ultrafiltration experiments. P mg. per 100 cc.*

Kind of plasma	Ultra-filtration membrane	No. of exps.	Inorg. P in original plasma	Inorg. P in residual plasma	Inorg. P in ultra-filtrate	Percentage of ultra-filterable inorg. P*
Rabbit heparinised	Cellophane	8	3.8	—	3.1	75
Rabbit oxalated	"	4	3.4	—	3.5	95
Human heparinised	"	5	3.8	4.0	3.3	80
Human oxalated	"	4	4.0	4.1	3.8	90
"	Collodion	4	4.0	4.2	3.5	80

* To nearest 5 %. Corrected for protein concentration.

¹ For work with phosphate it is essential that this ultrafilter should be nickel plated. It is advantageous to use a double thickness of cellophane.

than in either the original or the residual plasma. Usually about one-third of the volume of the plasma in the ultrafilter came through during the experimental period. Results are summarised in Table III.

Plasma was also dialysed at 0° in collodion sacs against from 1 to 3 volumes of distilled water or 0.9 % NaCl, and the inorganic phosphate concentration within and without the membrane determined after equilibrium had been reached. In no case was protein found in the dialysate.

Table IV. *Dialysis experiments.*

Fluid dialysed	Dialysed against	Time hours	P mg. per 100 cc.	
			Inorg. P in sac	Inorg. P in dialysate
Rabbit's heparinised plasma	0.9 % NaCl	18	1.4	1.3
" " "	"	60	2.1	1.8
" " "	"	18	1.2	1.25
" " "	Distilled water	18	0.7	1.0
Rabbit's oxalated plasma	0.9 % NaCl	18	1.1	1.4
" " "	Distilled water	18	0.8	0.7
Human " "	"	18	0.8	0.9
" " "	"	18	1.4*	1.7*
			1.3*	1.5*
Mean			1.17	1.27

* Duplicate dialyses.

It is perhaps permissible to take a mean of these findings, when it will be seen that on the whole, allowing for the protein content within the sac, the inorganic phosphate of normal human or rabbit plasma may be considered to be fairly completely dialysable.

Since this work was completed a paper by Grollman [1927] has appeared in which he states that the inorganic phosphate in the serum of blood from normal dogs or pigs is completely ultrafilterable. He has found, however, that the addition of CaCl_2 to plasma, or the hypercalcaemia produced by parathyroid injections renders some of the inorganic phosphorus no longer filterable.

Many of our phosphate determinations have been done by Briggs's method, after precipitating the proteins with trichloroacetic acid. Briggs's method entails the use of fairly strong acid (about 0.5 *N*) and the question arises as to whether some or all of the apparent "inorganic" phosphate of blood may not, in fact, result from the breakdown of an organic phosphorus compound unstable in acid solution, such as phosphagen [Eggleton and Eggleton, 1927], a compound of creatine and phosphoric acid.

Using precautions to avoid contact with acids for more than very short periods and that at low temperatures, we have been unable to detect any evidence of such a substance being present in blood, and Eggleton [1927] states that phosphagen is in all probability absent from blood.

The foregoing experiments have of necessity been carried out upon oxalated or heparinised samples of blood or plasma *in vitro*. It may be objected therefore that the results do not truly indicate the state of inorganic phosphate in

the normal circulating blood. Nevertheless, until definite evidence in favour of this objection is produced, it seems to us justifiable to accept the data furnished by the above experiments as showing that the "inorganic" phosphate of normal plasma is present mainly as a simple, ultrafilterable and dialysable salt of orthophosphoric acid.

The presence and quantity of phosphoric esters in the plasma.

Feigl [1917] showed that, in addition to the inorganic phosphate of the serum, organic acid-soluble phosphorus compounds, of unknown constitution, were also present in relatively small amount. This has been confirmed by other workers. Since, however, there is a large amount of organic phosphorus in the corpuscles, it is possible that the finding of very small and varying amounts of it in the serum or plasma might simply be an indication of slight haemolysis¹ or of incomplete removal of the corpuscles on centrifuging. Martland and Robison [1926] and Kay [1926, 1], avoiding these errors as far as possible, have shown that varying amounts of acid-soluble phosphoric esters are present in plasma freed from formed elements, and in which there was no sensible haemolysis, and that these esters are hydrolysable by body phosphatases. In particular it has been shown that the plasma-esters are probably completely hydrolysed by an extract of kidney.

Using normal human plasma, or the plasma of nephritic patients, obtained by centrifuging the blood at 7000 revs. per min. for 15 minutes, and free from visible haemolysis, we have obtained results completely confirmatory of the findings of the authors just quoted. These are summarised in Table V.

Table V. *Concentration of ester-P in plasma and urine.*

		Plasma organic P mg. per 100 cc.	Urinary organic P	
			mg. P per 100 cc.	mg. P per hour
Normals (17)	{ highest	0.9	2.8	3.0
	{ lowest	0.0	0.0	0.0
	{ mean*	0.33	0.8	1.2
Nephritics (31)	{ highest	2.2	2.1	3.4
	{ lowest	0.0	0.0	0.0
	{ mean*	0.52	0.6	0.7

* The majority of the determinations are grouped around the mean.

In normal human plasma we find an average of 0.33 mg. phosphorus as phosphoric esters in 100 cc., in nephritic plasma there is slightly more. Patients with nephritis appear to show some retention of organic phosphorus, as well as of inorganic phosphate, nitrogen, etc., in the plasma. There also appears to be some difficulty in the excretion of organic phosphorus by the

¹ Mâcheboeuf [1927] claims to have isolated phosphoglyceric acid from serum. This substance is known to be present in red cells, and since Mâcheboeuf used the serum from defibrinated blood, in which there is notoriously a good deal of haemolysis, his results cannot be completely accepted until they are repeated on serum or plasma obtained with more adequate precautions.

nephritic kidney, despite the greater "pressure-head" in the plasma. (All the urine figures in Table V are for urine collected in the period 8.30–10.30 a.m. after a very light breakfast at 6 a.m.)

We have, then, considerable experimental foundation for the conclusion that in normal plasma the phosphorus is present in two forms: (a) the major portion, inorganic, mainly if not completely dialysable, (b) a relatively small amount of organically combined phosphorus, probably about 10 % of the total acid-soluble phosphorus of the plasma.

The importance of this definite information with regard to the presence and approximate quantity of ester phosphorus in the plasma will be evident when the hypothesis of Eichholtz, Robison and Brull is under consideration.

Uptake of phosphorus from the intestine.

In the food phosphorus occurs entirely as the salts or esters of phosphoric acid. In no case, as far as we are aware, has phosphorus been demonstrated to occur naturally, either in plant or animal tissues, in any other state of oxidation¹.

There is no evidence at present that organic phosphorus compounds (with the possible exception of lipins) are taken up as such into the blood through the wall of the intestine. Hydrolysis, at least in the adult, appears to occur invariably, the powerful phosphatase in the succus entericus and in the intestinal mucosa being the active agent.

We have made several attempts to raise the organic phosphorus content of the plasma by the ingestion of one of the most stable esters of phosphoric acid. Fairly large doses of sodium glycerophosphate have been taken by mouth by several individuals, without any detectable increase in the organic phosphorus of the plasma or the whole blood, though increases in the inorganic phosphate followed invariably.

The protocols of a typical experiment are given below: 8 a.m., 300 cc. water taken; 8.30 a.m., light breakfast; 9.0 a.m., first urine sample; 25 g. sodium

Table VI. *Effect of ingestion of glycerophosphate on plasma and urinary phosphates.*

Time of taking blood	Plasma mg. P per 100 cc.		Time of collection	Urine	
	Inorg. P	Organic P		Inorg. P mg. per hr.	Mean plasma-P
10.15 a.m.	2.8	0.34	9.0–10.30	16.3	2.8
11.20	3.6	0.31	10.30–11.30	13.5	3.2
12.20 p.m.	4.40	0.31	11.30–12.30	59.5	4.1
1.20	4.36	0.28	12.30–1.30	69.1	4.38
2.20	4.30	0.34	1.30–2.30	70.2	4.33
3.20	4.26	0.40	2.30–3.30	70.2	4.28
4.20	4.23	0.32	3.30–4.30	80.3	4.24
5.20	3.98	0.25	4.30–5.30	75.6	4.1

¹ The statement occasionally seen in text-books that the phosphates of the urine are derived in part by the oxidation of the phosphorus of the phosphoproteins is fallacious, since the phosphorus in these proteins is already fully oxidised, and requires hydrolysis only to become free phosphate.

glycerophosphate in 500 cc. water. From this hour till 5.30 p.m., samples of blood and urine were taken and 250 cc. water was drunk hourly. Results are shown in Table VI.

Altogether nine similar experiments were performed, in some of which additional determinations were made on the total acid-soluble phosphorus in whole blood, and on the lipin phosphorus. The results seem to show quite definitely that when the relatively stable phosphoric ester, sodium glycerophosphate, is present in the gut (*a*) there is no demonstrable rise in the organic phosphorus of plasma or of whole blood, (*b*) there is a definite rise in the inorganic phosphate of the plasma followed by a rise in the rate of urinary excretion of inorganic phosphate. Moreover, a physiological increase in the inorganic phosphate of the plasma does not affect the level of the plasma phosphoric esters, though the rate of urinary excretion may rise fivefold. There is therefore, at least under these experimental conditions, no correlation whatever between the amount of phosphoric esters in the plasma and the rate of inorganic phosphate excretion by the kidney.

PHOSPHATE EXCRETION BY THE KIDNEY.

We now proceed to examine the suggestion of Eichholtz, Robison and Brull mentioned earlier in this paper that the inorganic phosphorus of normal urine has its immediate origin in the acid-soluble organic phosphorus compounds of the plasma, by hydrolysis of these in the kidney, a hypothesis which at the same time provides a logical primary function for the very active phosphatase known to be present in kidney tissue. Briefly stated, the experimental basis for this suggestion is as follows: these authors added sodium glycerophosphate to the fluid perfusing the heart-lung-kidney preparation, and found that whereas the kidney was previously unable to concentrate inorganic phosphorus, it now began (after an interval) to excrete both organic and inorganic phosphorus at definitely higher concentrations than those in the circulating (defibrinated) blood.

On examining this hypothesis in the light of these and other experimental findings the following conclusions may be drawn: (1) there is quantitatively more than enough phosphatase present in the human kidney to bring about the necessary hydrolysis [Kay, 1926, 2]; (2) putting the organic phosphorus of the plasma at 0.3 mg. P per 100 cc. (see later section) there is sufficient substrate present to provide, on hydrolysis, the whole of the inorganic phosphate of the urine; (3) there is no doubt that phosphoric esters, added to the perfusing blood, are to a marked extent hydrolysed in the heart-lung-kidney preparation. The above mentioned authors, however, make one or two assumptions that should not pass without comment. They assume that the kidney is the only tissue in their preparation which is capable of hydrolysing phosphoric esters. This is not the case, since blood itself, heart muscle to a slight degree, and lung tissue to a much larger degree also possess this hydrolytic power. Nevertheless, it is probably true that in their preparation the

kidney is quantitatively the most important tissue as far as phosphatase action is concerned. They also assume that a relatively small rise in the inorganic phosphate of the blood would hardly affect the rate of excretion of phosphate by the kidney. The findings of many other workers, and even their own figures¹ bear witness that this is a very doubtful assumption. If all the serum inorganic phosphate figures given by these authors are entered on their charts, it will be seen that there is a closer correlation between the level of inorganic phosphorus in the serum and the rate of excretion of inorganic phosphorus in the urine, than between the latter and the level of organic phosphorus in the serum. Our interpretation of the results of Eichholtz, Robison and Brull is that the phosphatases in the various tissues—the chief hydrolysing tissue being, in their experiments, the kidney—have slowly decomposed the circulating glycerophosphate, thereby raising the inorganic phosphate concentration in the serum above a threshold level, and, by continuous hydrolysis, have kept it at a high but slowly sinking level as long as any glycerophosphate has been available. In glucose tolerance tests it is frequently found that once the kidney threshold is overstepped, glucose continues to be excreted at plasma values which are normally below the threshold, and this seems to have occurred in one or two of these authors' experiments with phosphate. If the urinary inorganic phosphate is derived from the plasma organic phosphate it would be expected that the higher the plasma organic phosphate, the greater the rate of phosphate excretion. This relation is not seen either in their results, or in our own with the intact animal, in which after the injection of organic phosphorus we have repeatedly found that the excretion of inorganic phosphate may be greater with a low value for plasma organic phosphorus than with a high one.

We are definitely of opinion that in the normal human subject or in the normal rabbit the source of inorganic phosphate in the urine is not the organic but the inorganic phosphorus of the plasma, and that kidney phosphatase is not primarily concerned in the appearance of urinary phosphates. We will first summarise our reasons for these conclusions and then give the experimental evidence on which they are based.

Our reasons are as follows.

(1) Although the inorganic phosphate of the urine may be markedly increased by increasing the intake of either inorganic or of organic phosphate by the mouth, it does not appear possible to raise the organic phosphorus of the plasma by this means; *i.e.* the rate of inorganic phosphorus excretion may be increased whilst the level of organic phosphorus in the plasma remains normal.

¹ Thus, in their Table IV, after the injection of 50 mg. P as sodium phosphate the excretion of inorganic phosphate rises from 2 units to 30 units in 10 minutes, with a rise of inorganic P in the serum from 4.0 to 7.8 mg. P per 100 cc. With the serum inorganic P still at a high level, 50 mg. *organic* phosphorus causes a rise in phosphate excretion in the urine from 17.9 to 32 units in 10 minutes, but the maximum rate of excretion of 42 units takes 1 hour to attain, and is reached with the organic phosphorus in the serum actually falling.

(2) On increasing the organic phosphorus content of the plasma of an intact animal by intravenous injection of a phosphoric ester readily hydrolysable by kidney phosphatase (and, therefore, by other body phosphatases), the kidney excretes organic phosphate in a concentrated form. At the same time the excretion of inorganic phosphate increases, but only *pari passu* with the increase in inorganic phosphate in the plasma.

(3) Conditions have frequently been observed in which, while the inorganic phosphate in the urine was increasing, the organic phosphate in the plasma was definitely decreasing. In all these cases the rate of excretion of organic phosphate by the kidney was related to the level of organic phosphorus in the plasma, while the rate of excretion of inorganic phosphate clearly depended on the level of inorganic phosphate in the plasma.

(4) Organic phosphate injected into the circulation of a nephrectomised animal is hydrolysed at about the same rate as if it had been introduced into the circulation of an intact animal; *i.e.* hydrolysis of phosphoric esters in the circulating plasma goes on quite well without the intervention of the phosphatase of the kidney at all.

(5) There is an approximately linear relationship between the inorganic phosphate level in plasma and the rate of its excretion in the urine, whether the amount of organic phosphorus in the plasma is normal or is artificially increased.

The experiments on which these conclusions rest will now be briefly described.

A. Large male rabbits were given intravenous injections of (a) inorganic phosphate at p_H 7.4, (b) neutral sodium glycerophosphate, (c) neutral sodium hexosephosphate. Samples of blood were taken, in each case, before, and at intervals after the injection, and specimens of urine were obtained by catheterisation (bladder washed out each time with warm water in order to secure the whole of the urine secreted).

(a) The results of a typical experiment with inorganic phosphate are shown in Fig. 3.

Doubling the plasma concentration of inorganic phosphate results in an increase of rate of excretion of about tenfold. This immediate response of the kidney to a rise in plasma content supports very strongly a direct relationship between the plasma and the urinary values. In any case no appreciable amount of organic phosphorus was present in the plasma either at the beginning or during the course of these experiments.

(b), (c). Sodium glycerophosphate and fermentation sodium hexosediphosphate behave very similarly. The rapid rise in the plasma level of organic phosphorus after injection is followed by a fairly rapid fall. At the same time the inorganic phosphate in the plasma increases, undoubtedly as the result of hydrolysis of the injected ester by the tissue phosphatases. If the inorganic phosphate of the urine results from the hydrolysis of the organic phosphate of the plasma by kidney phosphatase, there should be a maximum urinary

Fig. 3. Rabbit. Intravenous injection of sodium phosphate (at p_H 7.4).

Fig. 4. Rabbit. Intravenous injection of sodium glycerophosphate.

Fig. 5. Rabbit. Intravenous injection of sodium hexosediphosphate (10 mg. inorganic P, 98 mg. organic P).

excretion of inorganic phosphate during the first hour, when the organic phosphate of the plasma is at its maximum. Actually the excretion of inorganic phosphate by the kidney in the first hour is nearly doubled in the second hour (Figs. 4 and 5) although the concentration of organic phosphorus in the plasma is then approaching its pre-injection level. It will be noted that organic phosphate appears in the urine in considerable amounts when its concentration in the plasma is increased. In its excretion it appears to behave as a substance with very low threshold.

B. Normal men drank a solution of 25 g. of sodium glycerophosphate in 400 cc. water. Samples of blood and urine were collected before, and at 2-hourly intervals afterwards. A moderate water intake was allowed, but no attempt was made to produce a diuresis. A typical experiment has been given in Table VI. In none of nine experiments was there any appreciable

Fig. 6. Normal man. Intravenous injection of sodium glycerophosphate.

rise in the plasma content of organic phosphorus. It may be concluded that ingested sodium glycerophosphate is completely hydrolysed during absorption. It is virtually equal, in its effect on blood and urinary phosphates, to an equivalent amount of ingested inorganic phosphate, the rates of change of concentration of plasma and urinary phosphates after ingestion of sodium glycerophosphate agreeing fairly well with those reported by Wigglesworth and Woodrow [1923] to follow the ingestion of sodium phosphate.

C. Since it was not possible to raise the level of plasma organic phosphorus by ingestion, normal men were given sodium glycerophosphate intravenously, the plasma inorganic and organic phosphate and the urinary excretion being followed. The findings with rabbits described in A above have been abundantly confirmed for normal human adults. A typical experiment (one of ten) is shown in Fig. 6¹.

¹ In two experiments the lipin-P in the blood was also followed. In neither case was there a rise in the amount present.

In these experiments, as before, it was repeatedly observed that the rate of urinary excretion of inorganic phosphate could never be correlated with the concentration of the organic phosphate, but always with that of the inorganic phosphate in the plasma.

In view of our experimental findings, a short comment on two other theories of the origin of urinary phosphates may be made here. The first is that of György [1925] who believes that the urinary phosphates have their origin, for the major part, in the organic phosphoric esters of the kidney tissue. This author has studied the autolysis of kidney tissue *in vitro*, and finds that the free phosphate increases, but gives no evidence, other than this, for his views. We think it is just as legitimate to conclude that the carbon dioxide of the expired air is derived from the combustion of the carbon compounds of lung tissue. While it is possible, and indeed probable, that a small portion of the urinary phosphate is derived from the breakdown of kidney tissue, it seems to us in the highest degree unlikely that upwards of a kilogram of kidney tissue is autolysed in the body per day to provide the 1.2 g. of urinary phosphorus excreted in man: this result follows if György's theory is worked out arithmetically.

The second theory, which belongs to a different category of probability, is that of Jost [1927]. In an able investigation of the biological significance of phosphoglyceric acid in the red corpuscle, this author comes to the cautious conclusion that if any part of the urinary phosphate is derived from the blood phosphoric esters, this compound is its most important mother substance. We agree. In normal blood, however, it appears to be entirely within the corpuscle, and nothing we have tried, short of laking the cells, is able to bring it, in the unhydrolysed form, into the plasma. Until, therefore, we have some definite evidence that it can emerge, under physiological conditions (apart of course from the normal breakdown of red cells which is continually proceeding), from the cell and become hydrolysed by the kidney enzyme we find it difficult to accept the suggestion that it is an important source of the urinary phosphate.

Is there a renal threshold for phosphate?

In the experiments described under B above, the inorganic phosphate level in plasma and the corresponding rate of urinary excretion of inorganic phosphate have been determined for six normal individuals (9 experiments, 23 values). If the plasma values are plotted against the rate of excretion (Fig. 7), the points are found to lie fairly close to a straight line which, if produced, cuts the abscissa at a plasma phosphate value of 2.8 mg. P per 100 cc.

If the results obtained following the intravenous injection of sodium glycerophosphate in man are similarly plotted, the line cuts the axis at a plasma value of about 3.5 (Fig. 8). The more scattered appearance of the points in this group of experiments is due, at least in part, to the more rapid changes which are occurring in the level of inorganic phosphate in the plasma,

which render the mean plasma value during any given period of urinary excretion a somewhat uncertain figure¹. As, however, the 34 points recorded in Fig. 8 are from as many as 9 persons of different ages and sex, the concordance is satisfactory. It may be concluded that when the inorganic phosphate content of the plasma rises above a certain level, the excretion of phosphate by the kidney either begins, or changes fairly suddenly from a very low rate to a much higher one. We use the word "threshold" to describe the level of a urinary constituent in the blood at which this *sudden increase* in the excretory rate takes place. Above this level, as is demonstrated by Fig. 7, the kidney excretes phosphate at a rate approximately proportional

Fig. 7. Rate of excretion of inorganic phosphate and plasma concentration, after ingestion of sodium glycerophosphate. (Six normal adults.)

Fig. 8. Rate of excretion of inorganic phosphate and plasma concentration, after intravenous injection of sodium glycerophosphate. (Nine subjects.)

to the extent of the increase of plasma phosphate above the threshold level. The graph in Fig. 7 is represented by the equation $U = r(P - a)$ where U is the rate of excretion of phosphorus in mg. per hour, P is the concentration of inorganic phosphorus in the plasma in mg. per 100 cc., a is the threshold in the same units, and r is the excretory ratio—a constant. In both Figs. 7 and 8 the slope of the line is the same, thus r is the same in both and works

¹ Also the subjects varied in weight, and therefore presumably in kidney size and excretory capacity, so that the excretion per hour uncorrected by some function of the weight is a somewhat unsatisfactory ordinate. Nevertheless, we consider that there are fewer objections to its use than to the use of the "excretion per kilogram per hour" as the ordinate. The metabolism in any case is not a linear function of the body weight, nor is the weight more than a very dubious estimate of the amount of kidney tissue.

out at about 60. This is approximately the value found for r for urea excretion in man, under similar conditions, by Hewlett, Gilbert and Wackett [1916] (see Adolph [1925]).

Wigglesworth and Woodrow [1923] ingested inorganic (sodium) phosphate and investigated the subsequent changes in the blood phosphate levels and rates of urinary excretion. We have recalculated their data by applying the formula for converting blood phosphate figures to plasma values. Their threshold ("a") value is about 3.4 mg., and their r works out at about 50.

The results of these workers, and our own, suggest a fairly high threshold for inorganic phosphate, to wit, one only slightly below the normal plasma level. A threshold, in the stricter sense of the word, implies that below that level inorganic phosphate is not excreted. Addis, Meyers and Bayer [1925] stated that there was no reference in the literature to the complete absence of phosphate from the urine, which with a threshold of the above value one might expect to have been observed. But under normal and under the majority of abnormal conditions, the inorganic phosphate never goes below the threshold value. This occurs however in insulin hypoglycaemia, in overbreathing and occasionally in rickets. Sokhey and Allen [1924] showed that in both normal and diabetic dogs insulin hypoglycaemia was accompanied by a greatly diminished rate of excretion of phosphate in the urine, traces only being found for some hours after insulin administration. It is well known that insulin produces a definite, though not a very severe, hypophosphataemia, so that it would appear that the phosphate threshold in dogs is also not very far below the normal plasma level. In man, Haldane, Wigglesworth and Woodrow [1924] found that after a period of overbreathing the inorganic phosphate of the blood fell from the normal value of 3 to 0.9 mg. P per 100 cc. or even lower. During one experiment, whilst the blood inorganic phosphate fell from 3.2 to 0.95 mg., the rate of excretion in the urine fell from over 20 mg. per hour to about 2 mg. With shorter periods for urinary collection, even lower excretory values might have been attained. It is likely, however, that even with a very low blood phosphate, *i.e.* one far below the threshold level, small quantities of inorganic phosphate, produced during the functional wear and tear of the phosphorus-rich kidney tissue itself, will continue to appear in the urine. This may account for the fact that urine completely free from phosphate has not yet been reported.

If a phosphate threshold actually exists, as appears probable, its level even for the same individual will differ at different times. Havard and Reay [1925] have found that the inorganic phosphate of the blood shows a distinct seasonal variation. A change in the kidney threshold for phosphate must clearly accompany, or possibly be the cause of, this variation, otherwise there would be a very serious loss of phosphate from the body in June, July and August when the blood inorganic phosphate was at its seasonal maximum of from 3.6 to 4.0 mg. P per 100 cc. blood (= plasma 4.0 to 4.5 mg. per 100 cc., cf. Fig. 2).

The fact that a large proportion of the phosphate of the plasma is dialysable and ultrafilterable seems to dispose of the possibility that the threshold is due to the presence of most of the "inorganic" phosphate of plasma in a form which, owing to its colloidal nature, is held back at the glomeruli.

Rate of disappearance of injected glycerophosphate from the circulation.

In a number of cases blood was taken just before, and at intervals after, the intravenous injection of sodium glycerophosphate in normals and in nephritics. The rise in organic phosphorus took place entirely in the plasma, in no case was there a measurable increase in the organic phosphorus of the corpuscles. There was a rapid disappearance of injected glycerophosphate from the blood; usually within 5 minutes more than three-quarters of the injected salt was no longer circulating. In this time it had not been excreted in the urine, nor had it been hydrolysed into inorganic phosphate in the blood. It must therefore have been taken up by the tissues [see Underhill, 1923].

Typical figures illustrating this point are given in Table VII.

Table VII. *Rate of disappearance of injected glycerophosphate from the plasma.*

No. of exp.	Type of case	Time after injection (mins.)	mg. P per 100 cc.		Percentage disappeared
			Rise in organic P of plasma	Theoretical* rise in plasma	
40	Normal man	8	5.0	23	80
42	"	5	4.7	26	80
45	"	5	5.4	22	75
41	Nephritic	6	3.6	17	80
80	"	3	5.0	19	75
81	"	5	4.9	19	75
38 {	"	5	4.5	19	75
	"	10	3.1	19	85
	"	60	1.2	19	95
58 {	Normal rabbit	3	47	170	70
	"	40	19	170	90
	"	75	12	170	95

* Blood volume taken as 1/20th body weight.

Phosphate excretion and urinary volume.

Cushny [1926] states that in diuresis the rate of excretion of urinary phosphate rises only slightly, if at all. We have found that, following the same stimulus, the excretion of both organic and inorganic phosphate in the urine of normal individuals is independent of the amount of water excreted. In Table VIII is found a series of consecutive observations on ten normal individuals.

The independence of inorganic phosphate excretion on urinary volume is also strikingly shown in certain results recorded by Havard and Reay [1926] in which the diuresis reached 1200 cc. per hour, and the actual concentration of inorganic phosphate in 100 cc. urine was less than that in blood.

Table VIII.

Inorganic P excreted in 1 hour following intravenous injection of 5 g. sodium glycerophosphate in a volume of from 100 cc. to 10 cc. of aqueous solution.

Exp. No.	Urine volume in cc.	Inorganic P excreted mg.	Organic P excreted mg.
25	500	105	60
26	229	129	47
28	212	118	93
31	43	118	78
33	70	98	62
35	93	110	70
40	600	107	83
42	221	93	88
45	246	115	137
95	199	109	86
Mean		110	80

Relation between plasma concentration and rate of urinary excretion.

In our discussion of the possible threshold for phosphate excretion, and indeed throughout most of the present paper, we have assumed that the plasma and not the whole blood concentration of a urinary constituent gives a truer measure of the pressure head with which the kidney has to deal. When, however, the details of transference of a urinary constituent out of the blood to or through the kidney cells are considered, the real value of this measure becomes somewhat doubtful. We mention two only of the questions that arise.

(1) Do the conditions of changing concentration in the blood circulating through the capillaries supplying the excretory elements permit of equilibration of diffusible substances between plasma and corpuscles? Even assuming that there is no such movement across the corpuscle boundary, the pressure head of the effective concentration of any one substance in the plasma must, owing to changes in the protein concentration of the latter, be continually altering in a complicated way during the progress of the blood through the renal vessels. We may know the initial plasma concentration of any given urinary constituent in the renal artery (approximately equal, for most substances, to that in venous plasma), but we do not know its final concentration in the renal vein, which may or may not be considerably lower than that in the artery. Even if we did know the final concentration, owing to such changes the mean value could not be taken with certainty as representing the effective plasma concentration. To be taken into account also is the probable continuous back-diffusion of certain of the urinary constituents from the concentrated urine in the tubules back again into the plasma, owing to partial permeability of the tubule walls [Rehberg, 1926]. This back-diffusion would be expected to depend on the rate of excretion of water, so that urinary volume may enter as another factor into our conception of the threshold level.

(2) Where a corpuscle is squeezing through a narrow capillary, may there not be direct interchange of substances between corpuscular contents and the cells of the capillary wall, particularly if the capillary is part of an excretory

or secretory system? Krogh [1922] has shown that in certain cases there is considerable mechanical squeezing of the corpuscles in passing through the capillaries. It is just conceivable that the phosphoric esters within the red cells may in certain conditions leave the cells to appear in the urine either as unhydrolysed ester or as inorganic phosphate.

The following conclusions with regard to the excretion of phosphate are, we believe, in agreement with the experimental facts found by ourselves and by others. The inorganic phosphate in normal urine arises from two immediate sources: (a) a small, basal quantity excreted at a fairly constant rate no matter what the level of inorganic or organic phosphate in the plasma may be, produced during the normal wear and tear of the renal cells, being in a real sense endogenous and derived from the inorganic and organic acid-soluble phosphorus compounds of kidney tissue; (b) the main quantity, under normal conditions much larger than (a), derived immediately from the inorganic phosphate of the plasma, and appearing so long as the level in the plasma is maintained above a threshold value. This fraction (b) is actively excreted by the kidney. The amount excreted increases in linear proportion to the rise in the plasma level of inorganic phosphate above the threshold value, and has nothing to do directly with the plasma level of acid-soluble phosphoric esters. If by some means this latter value is artificially raised, the excess of ester is partly excreted unchanged, and partly slowly hydrolysed by all the active tissues of the body (including the kidney itself) and the resulting inorganic phosphate produced is excreted by the kidney in the usual way. The possibility is not denied that some of the ester circulating under abnormal conditions in the blood may, by being hydrolysed in the kidney, provide a small portion of the extra excretion of inorganic phosphate. Whereas, however, in the heart-lung-kidney preparation, in which the kidney is a relatively large fraction of the phosphatase-containing tissue, the quantity of inorganic phosphate so produced may be an appreciable fraction of the total inorganic phosphate excretion, these results cannot be applied to the intact animal where quantitative relationships of an entirely different order have to be taken into account.

SUMMARY.

1. Acid-soluble phosphorus in the plasma is present in two forms: (1) as a simple salt of orthophosphoric acid, of which at least 80 % is ultrafilterable and dialysable and therefore neither colloidal nor bound to the plasma colloids, (2) as organically combined (ester) phosphoric acid. The amount of (2) is usually about 10 % of the amount of (1) both in normal plasma and in that from patients with nephritis.

2. The amount of inorganic phosphate in the plasma, as determined by the usual methods, is less than the amount to be expected from the Donnan equilibrium between corpuscles and plasma.

3. The very low concentration of ester phosphorus in the plasma cannot be raised by taking phosphoric esters by mouth. It can be raised temporarily by intravenous injection of phosphoric esters.

4. In the intact rabbit or in man, there is no evidence in favour of the view that the ester phosphorus of the plasma controls the rate of excretion of inorganic phosphate. On the contrary, the evidence is all in favour of the control of such excretion by the plasma level of inorganic phosphate. There appears to be a renal threshold for phosphate excretion.

5. Within wide limits there is no relationship between the amount of urinary phosphate and the urinary volume.

The expenses of this work have been defrayed in part by grants from the Research Fund of the London Hospital Medical College.

REFERENCES.

- Addis, Meyers and Bayer (1925). *Amer. J. Physiol.* **72**, 125.
 Adolph (1925). *Amer. J. Physiol.* **74**, 93.
 Aitken and Kay (1927). *J. Physiol.* **62**; *Proc.* xxix.
 Bloor (1918). *J. Biol. Chem.* **36**, 49.
 Buell (1923). *J. Biol. Chem.* **56**, 97.
 Cushny (1919). *J. Physiol.* **53**, 391.
 — (1926). Secretion of urine. (Longmans, London.)
 Eggleton (1927). Private communication.
 Eggleton and Eggleton (1927). *Nature*, **119**, 194.
 Eichholtz, Robison and Brull (1925). *Proc. Roy. Soc. Lond. B*, **99**, 91.
 Eichholtz and Starling (1925). *Proc. Roy. Soc. Lond. B*, **98**, 93.
 Feigl (1917). *Biochem. Z.* **83**, 81.
 Grollman (1927). *J. Biol. Chem.* **72**, 565.
 György (1925). *Biochem. Z.* **161**, 157.
 Haldane, Wigglesworth and Woodrow (1924). *Proc. Roy. Soc. Lond. B*, **96**, 1.
 Havard and Reay (1925). *Biochem. J.* **19**, 882.
 — (1926). *Biochem. J.* **20**, 99.
 Henderson, Bock, Field and Stoddard (1924). *J. Biol. Chem.* **59**, 403.
 Hewlett, Gilbert and Wackett (1916). *Arch. Int. Med.* **18**, 636.
 Holt, La Mer and Chown (1925). *J. Biol. Chem.* **64**, 566.
 Jones and Nye (1921). *J. Biol. Chem.* **47**, 321.
 Jost (1927). *Z. physiol. Chem.* **165**, 171.
 Kay (1926, 1). *Brit. J. Exp. Path.* **8**, 240.
 — (1926, 2). *Biochem. J.* **20**, 791.
 Kerr (1926). *J. Biol. Chem.* **67**, 689.
 Krogh (1922). Anatomy and physiology of the capillaries. (Yale Press.)
 Macheboeuf (1927). *Ann. Inst. Pasteur*, **41**, 1036.
 Marrack and Thacker (1926, 1). *Biochem. J.* **20**, 580.
 — (1926, 2). *Brit. J. Exp. Path.* **7**, 265.
 Martin and Lepper (1926). *Biochem. J.* **20**, 1071.
 Martland and Robison (1926). *Biochem. J.* **20**, 847.
 Mayrs (1922). *J. Physiol.* **56**, 58.
 McKellips, de Young and Bloor (1921). *J. Biol. Chem.* **47**, 53.
 Pincus and Neuhausen (1923). *J. Biol. Chem.* **57**, 99.
 Pincus, Peterson and Kramer (1926). *J. Biol. Chem.* **68**, 601.
 Rehberg (1926). *Biochem. J.* **20**, 447.
 Richter-Quittner (1921). *Biochem. Z.* **124**, 106.
 Sokhey and Allen (1924). *Biochem. J.* **18**, 1170.
 Underhill (1923). *Brit. J. Exp. Path.* **4**, 117.
 Wigglesworth and Woodrow (1923). *Proc. Roy. Soc. Lond. B*, **95**, 559.
 Wu (1922). *J. Biol. Chem.* **51**, 21.
 Zucker and Gutman (1922). *Proc. Soc. Exp. Biol. Med.* **19**, 169.