LXXXIII. THE ABSORPTION OF PHOSPHATES FROM THE INTESTINE

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THE absorption of Ca and P from the alimentary tract is believed to be influenced by a variety of factors, such as vitamin D, the acidity of the intestinal tract, the Ca/P ratio in the diet, parathormone etc. A discussion of all these is beyond the scope of this work; a summary of published work may be found in a paper by Nicolaysen [1937, 1]. Most of the work on absorption, however, has been carried out till now by estimating the intake of Ca and P and the output of these elements in urine and faeces. The amount of Ca or P found in the faeces is usually the sum of the quantity left unabsorbed and that excreted into the intestines. Hence, although the "balance" method might yield useful information about the total retention of Ca and P, it will not give a true picture of absorption alone. Recently the isolated loop technique of Verzár [1935] has been used for studying the absorption of Ca and P. Nicolaysen [1937, 2] found that the absorption of potassium phosphate and sodium glycerophosphate from isolated loops proceeded equally well in normal and vitamin D-deficient rats. He also found that the absorption of inorganic phosphate increased with increasing concentration. Laskowski [1937], using similar technique, also found that vitamin D did not influence the absorption of P, but parathormone did. A reference to both these papers will be made later on.

No information is available about the route by which Ca and P enter the general circulation after absorption from the intestines. There are two possible routes, (1) the portal system and (2) the lacteals, lymphatics of the mesentery and the thoracic duct. So far as the absorption of P is concerned no work has been reported dealing with this aspect. As Schmidt & Greenberg [1935] suggest, one may speculatively assume that after absorption P is carried by the portal blood and enters the general circulation via the liver. In a study on the absorption of amino-acids into the capillaries and the lacteals of the villi Bolton & Wright [1937] could find no evidence of a selective activity on the part of either. Whether such selective activity is also absent in the absorption of phosphate could only be shown by further investigation.

The main purpose of the present investigation was therefore to find the route of absorption of P from the intestines. Additional light has been thrown on the influence of concentration and *p*H on the absorption of P and on the availability of ester P. These latter aspects are discussed first, and the experiments designed to show the route of absorption are described in the latter part of the paper.

METHODS

The experiments described in the following pages were carried out on normal healthy dogs. The dog selected for any particular experiment was starved for 16–20 hr. before being subjected to ether or chloroform anaesthesia. The

abdomen was opened with a midline incision and the portal vein was exposed by turning over to one side the intestines, including the duodenum. 5–10 ml. blood were then taken from the portal vein by means of a syringe, a known volume of the phosphate solution, previously warmed to 37°, was introduced into the duodenum by means of a hypodermic syringe and the abdomen was closed by adjusting the flaps and holding them in position by means of forceps. At intervals the abdomen was reopened and 5–10 ml. blood were withdrawn from the portal vein. The experiment lasted for 3–4 hr. during which time the dog was kept anaesthetized and warm.

The blood was always delivered into a tube chilled to 0°; it was gently shaken and portions were measured out for analysis. The P analyses were carried out on whole blood as follows.

Total phosphorus. 0·5 ml. whole blood was oxidized with a mixture of HNO_3 and H_2SO_4 (3 : 7). The oxidation was completed with perhydrol and the digested mixture heated long enough to drive out the last traces of HNO_3. About 2 ml. water were added and the contents boiled and left overnight. The digest was then made to 50 ml. with the addition of distilled water and P was determined in an aliquot portion by the Bell & Doisy [1920] modification of Briggs's method.

Acid-soluble phosphorus. 5 ml. whole blood were mixed with 35 ml. water and 10 ml. 20 % trichloroacetic acid. The mixture was well stirred, allowed to stand for 10–15 min. and filtered. Estimations of total and inorganic P were carried out in aliquot portions of the filtrate.

In later experiments where P estimations in lymph were carried out, a weighed quantity of lymph was treated with trichloroacetic acid and water, made up to a known volume and P was estimated as mentioned above.

Calcium. In some experiments Ca also was estimated in deproteinized whole blood filtrates. Ca was precipitated as oxalate and titrated with $N/100$ $KMnO_4$, the procedure adopted being that described by Wang [1935].

I. Factors which influence the absorption of P

The effect of anaesthesia on the P of blood

In order to be certain about the value of the results obtained by the above technique it was necessary to determine the effect of anaesthesia on the P of blood. For this purpose a dog was starved for 16–18 hr. and a sample of blood withdrawn from a peripheral vein in the hind leg. The dog was anaesthetized with chloroform and ether and kept under anaesthesia for 3–4 hr.; at intervals blood was withdrawn from a peripheral vein and analysed. Since the dog was in the post-absorptive stage there would probably be no difference in the P contents of the portal and peripheral blood. Hence one might reasonably assume that the determination of P in blood from a peripheral vein would give results applicable to the whole venous blood of the anaesthetized animal.

Table I. *The effect of ether and chloroform anaesthesia on blood P*

Time	mg./100 ml. whole blood		
	Total P	Acid-soluble P	Inorganic P
Before anaesthesia	47·53	25·15	3·61
0 hr. under anaesthesia	48·03	29·07	4·73
½ ,, ,, ,,	48·21	29·07	6·02
1 ,, ,, ,,	49·21	29·20	6·66
2 ,, ,, ,,	47·71	26·60	5·25
3 ,, ,, ,,	47·18	26·26	5·21

This and similar experiments showed that the inorganic P of the blood increased appreciably under anaesthesia and simultaneously, but to a much less extent, the total P of the whole blood also increased. In both cases the maximum was reached within 1 hr., after which the values tended to diminish. Such increase in the inorganic phosphate of the blood has been observed previously. Marenzi & Gerschman [1934] found the inorganic phosphate increased during ether anaesthesia. They state that this increase was not due to changes in the organic P compounds of the blood; the excess inorganic P came possibly from sources other than blood. That this is partly true is evident from a slight increase in the total P of the blood (Table I), but it is also possible that a fraction of the increase in inorganic P might be due to the changes in some of the organic P compounds of the blood, e.g. changes which cause a large increase in the soluble fraction.

It will be clear from the experiment quoted above that in any study of absorption on anaesthetized animals based on estimations of concentration in blood of the substance under investigation, it is necessary to bear in mind the changes which result from anaesthesia. The alterations in the concentration of substances in blood due to absorption from the intestines will, under such conditions, be superimposed on the changes due to anaesthesia. Since these latter are seldom identical it has not been possible to make quantitative allowances for them. The rate of uptake of the substance in question by tissues introduces a further difficulty in the way of a quantitative study of absorption by this method. Thus the concentration in blood of a substance being absorbed from the intestines will depend upon at least two factors: (1) the rate of absorption from the intestines and (2) the rate of removal by the tissues. As it is difficult to obtain reliable information at present concerning the second factor a strictly quantitative interpretation of the results is not possible. Nevertheless it is possible to derive certain useful information concerning the various factors which can influence absorption from the intestines. The results of the experiments described in the following pages have been interpreted after bearing in mind these difficulties.

It is necessary to explain the reason why only whole blood was selected for analysis. As a result of the absorption of P from the intestines there should be a rise not only in the inorganic P of the blood, but also in the total P of the whole blood. It will appear from Table I that in anaesthesia an increase in the inorganic P of the blood can take place without a corresponding increase in the total P. In such experiments on absorption one might easily be led to conclude, on the strength of an increase in the inorganic P only, that a given substance has been absorbed; for instance, experiments with sodium phytate described in Table III (C and D) show a marked rise in inorganic P, but no rise in the total P. In those experiments it would not have been possible to arrive at any conclusion unless the total and inorganic P had both been determined. That this procedure is justified is shown by the close correspondence between the increase in inorganic P and the total P observed in the early stages of absorption of inorganic phosphate (Table II).

No attempt has been made to study the partition of the absorbed P between the plasma and the corpuscles. It is possible that the sudden and large influx of inorganic P in the plasma might upset the equilibrium existing between the several P compounds of the red blood cells and the plasma. The conclusion of Buell [1923] that the red blood cells contain no inorganic P has not been confirmed by Halpern [1936], who has shown that at 37° the membrane of the red blood cell is permeable to phosphate ions, the extent and direction of the

exchange of these ions depending upon the metabolic activity of the R.B.C. It is proposed to investigate shortly the effect which the absorption of P has on the distribution of P compounds in blood.

The absorption of inorganic phosphate

Aqueous solutions of Na_2HPO_4, adjusted to definite *p*H by titrating with solutions of NaH_2PO_4 and containing known amounts of P, were introduced into the duodenum and blood from the portal vein was analysed at intervals. The details and results of the experiments are given in Table II.

Table II. *The absorption of sodium orthophosphate*

Particulars	Time, hr.	mg./100 ml. whole blood: Total P	Acid-soluble P	Inorganic P	Calcium
A. Wt. of dog, 11·0 kg. Phosphate solution injected, 40 ml. containing 933 mg. P at *p*H 9·4 (23·3 mg. P/ml.)	0	59·27	41·21	7·19	6·90
	½	70·06	51·63	17·64	5·60
	1	66·74	44·80	14·78	3·60
	2	63·10	43·14	14·61	3·50
	3	63·73	43·73	14·80	4·00
B. Wt. of dog, 8·13 kg. Phosphate solution injected, 40 ml. containing 870 mg. P at *p*H 7·0 (21·75 mg. P/ml.)	0	46·46	28·61	8·61	6·79
	½	49·02	31·56	12·01	6·43
	1	51·12	37·83	14·28	4·64
	2	53·99	36·86	—	2·58
	3	—	41·16	18·17	—
C. Wt. of dog, 6·07 kg. Phosphate solution injected, 40 ml. containing 492 mg. P at *p*H 7·0 (12·3 mg. P/ml.)	0	46·49	24·40	6·14	7·38
	½	49·62	28·47	9·48	5·58
	1	51·86	27·97	10·79	5·40
	2	52·07	25·10	11·36	—
	3	52·94	26·03	12·33	—
D. Wt. of dog, 8·88 kg. Phosphate solution injected, 35 ml. containing 764 mg. P at *p*H 4·9 (21·8 mg. P/ml.)	0	48·09	27·96	5·00	—
	½	54·76	36·94	12·03	—
	1	55·0	42·41	14·65	—
	2	56·9	42·71	16·49	—
	3	63·8	46·59	19·76	—

It appears that the rate of absorption of P is influenced by the *p*H of the solution introduced. In experiments at *p*H 9·4, 7·0 and 4·9 the concentration of P per ml. of solution injected was very nearly the same. It was found that *p*H influenced mainly the rate of absorption and not so much its extent. A rise in inorganic P of more than 10 mg./100 ml. blood was obtained in all these experiments; at *p*H 9·4, however, the peak was obtained within half an hour of the intraduodenal injection while at lower *p*H it was reached in about 3 hr. A comparison of experiments B and C showed that at the higher concentration of P the extent of absorption was more than at the lower concentration. Another interesting observation was that in the early stages of absorption there was a close correspondence between the increase in the inorganic P and the total P of the whole blood, but in later stages the increase in inorganic P over the original level was more than the corresponding increase in the total P.

The absorption of phosphoric esters

Sodium glycerophosphate. Aqueous solutions of sodium glycerophosphate adjusted to definite *p*H by dilute HCl were introduced into the duodenum and the experiments were carried out as in the case of inorganic phosphate. The absorption of P took place both at *p*H 7·0 (Table III, Exp. A) and 4·9 (Table III, Exp. B). When these experiments were compared with those on the absorption

of sodium phosphate (Table II, Exps. C and D) at the corresponding *p*H values, it was evident that at *p*H 7·0 the absorptions of sodium phosphate and glycerophosphate proceeded at practically the same rate, but at *p*H 4·9 the rate of absorption of the latter was slower than that of the orthophosphate. An explanation of this curious behaviour which suggests itself to the authors is that possibly the rate of absorption of glycerophosphate is limited by the rate of liberation of inorganic P from it by enzymic hydrolysis of the ester. Nicolaysen [1937, 2] found that when glycerophosphate was injected into isolated loops of rat's intestines at *p*H 6·0 only a very small fraction of total P remaining in the loop at the end of 1 hr. was present as inorganic P, while in a similar experiment at *p*H 8·5 one to two thirds of the remaining P were found as inorganic P. Nicolaysen therefore concluded that glycerophosphate could be absorbed without previous hydrolysis. How far Nicolaysen's experiments lend themselves to this interpretation is not quite clear. It is possible that at *p*H 8·5, which is near the optimum *p*H for the activity of phosphatase, the rate of hydrolysis of glycerophosphate might exceed that of the absorption of the liberated inorganic P and at *p*H 6·0 this relation might be reversed. This could provide an alternative explanation of Nicolaysen's observations. Laskowski [1937], also using the loop technique, found that at *p*H 7·2 the absorptions of inorganic phosphate and of glycerophosphate proceeded practically at the same rate. He further found that those esters which were hydrolysed very slowly were also absorbed very slowly, an observation which suggests that liberation of phosphate in inorganic form is necessary before it can be absorbed. Similar conclusions could be drawn from the experiments described in the present communication (Table III). At *p*H 7·0, at which the phosphatase might be active, hydrolysis and also absorption might have proceeded at a rapid rate but at *p*H 4·9 the enzyme,

Table III. *The absorption of phosphoric esters*

Particulars	Time, hr.	mg./100 ml. whole blood: Total P	Acid-soluble P	Inorganic P	Calcium
A. Sodium glycerophosphate.	0	48·58	28·04	2·77	6·41
Wt. of dog, 7·63 kg. 40 ml.	½	—	30·15	4·35	5·49
injected contained 520 mg.	1	52·64	35·72	7·14	4·59
P at *p*H 7·0 (13·0 mg. P/ml.)	2	51·25	35·09	7·87	3·60
	3	54·59	38·84	8·70	3·51
	4	53·31	33·34	7·29	3·15
B. Sodium glycerophosphate.	0	50·40	35·68	4·70	—
Wt. of dog, 7·57 kg. 30 ml.	½	56·30	41·35	7·89	—
injected contained 687 mg.	1	57·20	40·82	10·81	—
P at *p*H 4·9 (22·6 mg. P/ml.)	2	60·67	39·14	10·46	—
	3	58·68	43·60	10·59	—
C. Sodium phytate. Wt. of	0	60·24	30·15	2·63	—
dog, 10·75 kg. 55 ml. in-	½	60·81	30·46	3·61	—
jected contained 372 mg. P	1	61·55	31·11	4·11	—
at *p*H 3·8 (6·76 mg. P/ml.)					
D. Sodium phytate. Wt. of	0	59·46	—	5·15	—
dog, 10·83 kg. 35 ml. in-	½	59·74	38·98	7·94	—
jected contained 334 mg. P	1	58·88	37·82	7·96	—
at *p*H 5·2 (9·54 mg. P/ml.)	2	58·46	—	9·30	—
	3	59·17	38·05	8·08	—
E. Calcium glycerophosphate.	0	50·69	27·46	3·67	6·30
Wt. of dog, 9·20 kg. 40 ml.	1	51·98	29·12	5·38	6·86
injected contained 340 mg.	2	54·26	30·98	6·74	7·60
P at *p*H 4·9 (8·5 mg. P/ml.)	3	—	33·63	7·16	7·23
	4	57·11	35·58	7·77	7·23

being considerably less active on account of the unfavourable H ion concentration, might not bring about the hydrolysis rapidly enough to permit the maximum rate of absorption, and hence the concentrations of inorganic and total P in the blood of the dog absorbing glycerophosphate at *p*H 4·9 would be considerably lower than in a corresponding experiment with inorganic phosphate.

Sodium phytate. A solution of the salt of inositolhexaphosphoric acid prepared according to the method described by Patwardhan [1937] was used in concentrations of 6·76 and 9·54 mg. P/ml. In higher concentrations than these the salt would not remain in solution unless acid were added to depress the *p*H below 3·0. Actually the two experiments were carried out at *p*H 3·8 and 5·2. There was a rise in the inorganic phosphate of the portal blood, but the elevation of total P in blood was such as could be obtained merely by anaesthesia. Hence it was considered that phytate was not absorbed. This experiment can be compared with that on calcium glycerophosphate (Table III, Exp. E). The solutions of the respective salts contained practically the same concentrations of P. While in the case of calcium glycerophosphate there was a rise of 6·5 mg. in the total P, in the case of phytate the rise was less than 0·5 mg. From these experiments on organic P compounds the conclusion can reasonably be drawn that P must be released from inorganic combination before absorption and that when it is not liberated by hydrolysis for want of suitable conditions it is not absorbed from the intestines, thus confirming Laskowski's conclusions reached by different methods.

The effect of the absorption of phosphate on blood Ca

Simultaneously with the rise of inorganic P of the blood as a result of anaesthesia there is observed a slight but definite fall of blood Ca. That there is a fall in the blood Ca under the influence of narcotics had been observed by Cloetta *et al.* [1934] in dogs to which were administered paraldehyde, chloral hydrate or "numal", singly or in mixtures. On the other hand Emerson [1928] reported a rise in serum Ca under ether anaesthesia, but he also found that under ether anaesthesia accompanied by a certain amount of asphyxia serum Ca showed a slight fall. Patwardhan & Chitre (unpublished experiments) observed a slight depression of serum Ca level after the administration of urethane; similar results were obtained after giving a mixture of ether and chloroform. Any change in Ca level which results from the influx of phosphates into the blood of an anaesthetized animal will therefore be superimposed on the changes caused by anaesthesia as in the case of phosphates. Fortunately, however, the fall in blood Ca due to anaesthesia is small, never exceeding 1·0 mg./100 ml. serum.

In the experiments in which P was being absorbed from the intestines the amount of blood Ca was found to fall as the concentration of inorganic phosphate in the blood increased (Tables II and III), this fall being larger than that which would be caused by anaesthesia alone. Binger [1917] noticed that intravenous injection of a large dose of sodium phosphate into a dog lowered the blood Ca. That this was not merely due to the dilution of blood involved in the injection of large volumes of sodium phosphate solution was shown by Tisdall [1922], who injected stronger solutions and obtained similar results. That the amount of Ca in blood is lowered also during the absorption of phosphate from the intestines is evident from the experiments quoted in Tables II and III. But when Ca and P were being absorbed simultaneously the effect on blood Ca was found to be different. For such an experiment it was necessary to ensure that Ca and P could be administered together without the formation of insoluble calcium phosphate.

Such a condition could be fulfilled by using calcium glycerophosphate in acid solutions. Hence an aqueous solution of the calcium salt at *p*H 4·9 was injected into the duodenum and the Ca and P were estimated in the portal blood at intervals. It was observed that Ca was absorbed more rapidly than P. Although the rise in Ca is, at its maximum value, only 1·4 mg. it becomes significant when it is remembered that ordinarily the level would have fallen much below the original. The absorption of P in this experiment proceeded slowly, more slowly than that of Ca; it was probably limited by the extent of hydrolysis. Higher concentrations of calcium glycerophosphate could not be used as even at *p*H 4·9 the salt precipitated out of solution.

II. The route of absorption

The experiments described in the first section of this paper had shown that the absorption of phosphates proceeded by way of the portal circulation. An alternative route lies via the lacteals, lymphatics of the mesentery and the thoracic duct, for the investigation of which the following addition to the technique already described was made. Lymph can be obtained from the thoracic duct or the *cysterna chyli*. The easiest way of obtaining it, however, is that described by Verzár [1936] for use with rabbits. The lymphatics of the intestines of the dog meet in the root of the mesentery and form into large lymphatics which lead to the *cysterna chyli*. When these lymphatics were cut the lymph oozed out fairly rapidly and could be sucked up by a pipette. It was delivered into weighed tubes which were weighed again to find out the quantity of lymph taken for analysis. This was essential since lymph clotted very rapidly, making it difficult to measure the volume accurately. Trichloroacetic acid was added to deproteinize the lymph and P was estimated as already described. Total P estimations were not carried out on lymph. Blood samples taken at intervals from the femoral or carotid artery were also analysed as before. Two typical experiments are given below, one with sodium phosphate and another with sodium glycerophosphate.

Table IV

Particulars	Time, hr.	Total P	Acid-soluble P	Inorganic P
			Lymph*	
A. Orthophosphate. Wt. of dog, 10·0 kg. 28 ml. at *p*H 4·9 injected, containing 862 mg. P (30·78 mg. P/ml.)	0		7·49	5·71
	0·5		10·42	8·46
	1		12·62	11·99
	2		14·52	13·51
	3		13·27	12·30
			Blood†	
	0	45·4	22·65	5·63
	2	52·6	29·27	12·63
	3	52·5	28·41	12·63
			Lymph*	
B. Glycerophosphate. Wt. of dog, 8·83 kg. 38 ml. at *p*H 4·9 injected, containing 744 mg. P (19·58 mg. P/ml.)	0		6·26	4·20
	0·5		11·93	8·20
	1		12·31	11·49
	2		12·43	10·44
	3		11·62	10·05
			Blood†	
	0	46·5	29·77	3·17
	2	54·2	36·98	10·59
	3	54·0	35·92	9·38

* mg. P/100 g. lymph. † mg. P/100 ml. whole blood.

From Table IV it will appear that there is a gradual rise in the inorganic and the acid-soluble P of the lymph which runs almost parallel with that in the arterial blood. The experiments described before showed that P is absorbed via the portal circulation; those described now show that it is also absorbed by the lymphatic route. Patwardhan & Chitre (unpublished experiments) have found that Ca is also absorbed in a similar manner.

This conclusion opens up a new field for work. The problem arises as to whether under certain conditions the absorption of P into the capillaries or the lacteals of the villi could be altered in favour of either of these two routes; it remains also to be seen whether those conditions which affect beneficially or adversely the absorption of P from the intestines have any influence on the route of absorption. Further work is in progress to elucidate these and other connected problems.

Summary

The absorption of sodium phosphate, glycerophosphate and phytate has been studied by estimations of P in blood and lymph after the intraduodenal injection of the phosphate solutions into anaesthetized dogs. Orthophosphate was absorbed very rapidly at *p*H 9·4. The rate of absorption diminished at *p*H 7·0 and *p*H 4·9. At *p*H 7·0 the glycerophosphate was absorbed as rapidly as the orthophosphate, but at *p*H 4·9 the former was absorbed much more slowly than the latter. Sodium phytate was not absorbed at *p*H 3·8 or 5·2. Blood Ca fell during the absorption of P, but when calcium glycerophosphate was given the blood Ca rose with the blood P.

When sodium orthophosphate or glycerophosphate was injected into the duodenum the concentrations of inorganic and acid-soluble P in blood and lymph increased considerably, showing that P was being absorbed by the capillaries as well as the lacteals of the villi.

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