CXLII. STUDIES UPON THE MODE OF ACTION OF VITAMIN D

V. THE ABSORPTION OF PHOSPHATES FROM ISOLATED LOOPS OF THE SMALL INTESTINE IN THE RAT

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IN a preceding paper [Nicolaysen, 1937, 1] metabolism experiments on rats were described, from the results of which it was inferred that vitamin D in the gut exerts its action primarily on the absorption of Ca, its influence on the absorption of P being a secondary effect. The direct action of vitamin D on the absorption of Ca was confirmed in experiments in which the rates of absorption from isolated loops were studied [Nicolaysen, 1937, 2]. It must be borne in mind, however, that in the metabolism experiments only restricted amounts of phosphates could be given, which the vitamin D-deficient rats were able to absorb completely. It appeared still possible, therefore, that vitamin D might influence the rate of absorption of P in a way which could not be detected when feeding limited amounts. In the experiments described below the "isolated loop technique" which permits a direct measurement of the rate of absorption, as well as testing with large amounts was employed. The present paper also deals with several outstanding points which remained to be decided with regard to the mode of absorption of phosphates in normal animals.

EXPERIMENTAL

The rats for experiments with vitamin D were given the Steenbock-Black rachitogenic ration 2965 at least 6 weeks before experiments were performed on them. The controls received 50 I.U. vitamin D (as calciferol) daily, at least 1 week before experiment. The rats used for the other experiments here presented were kept on the stock diet the whole time. The rats weighed 80–130 g. The experimental technique was essentially the same as previously reported [Nicolaysen, 1937, 2].

Absorption of inorganic phosphate from isolated loops

Solutions of KH_2PO_4 ranging from 0.4 to $1.6 \times$ isotonic were used for these experiments. The rate of absorption of phosphate was measured in 4 normal and 4 vitamin D-deficient rats for each strength.

Fig. 1 gives the results. From this it appears that the rates of absorption of inorganic phosphate are identical in normal and vitamin D-deficient rats. From the $1.2 \times$ isotonic solution about 5 mg. P are absorbed in 1 hr. from the 30 cm. loop. This is a rate about five times that of Ca absorption in normal rats and about ten times that in vitamin D-deficient rats.

The shape of the smoothed curve indicates a direct relationship between the speed of absorption and the concentration of phosphate in the lumen. It is established [Verzar, 1935] that glucose is absorbed at a rate which is independent of concentration, and this is thought to be due to a phosphorylation of glucose in the gut wall. The difference in the type of the absorption curves for

inorganic phosphate and for glucose indicates that the absorption of phosphate is not essentially dependent on esterification with glucose.

Fig. 1. The absorption of inorganic P from isolated loops of the jejunum 3 ml. KH_2PO_4 solutions injected into 30 cm. loops.
 • Normal rats. × Vitamin D-deficient rats.

The absorption of phosphates from isolated loops of the intestines of rats poisoned with iodoacetic acid

It is known that inhibition of phosphorylation with iodoacetic acid reduces the rate of absorption of glucose. In order to confirm the difference in the mechanisms of the absorption of phosphates and glucose, experiments were performed to decide if iodoacetic acid reduced the rate of absorption of phosphates as well. 1.6 and 0.8 × isotonic solutions of KH_2PO_4 were used, and each solution tested on 4 normal rats and 4 rats poisoned with iodoacetic acid (0.15 mg. per g. body wt.). No difference was observed in the rates of absorption between the two groups of animals. The rate of absorption was the same as given for these solutions in Fig. 1. It appeared still possible that esterification could take place in the bowel. Direct P estimation and P estimation after ashing, however, gave identical results. The absorption of inorganic phosphate is therefore independent of esterification with glucose.

The absorption of organic phosphate from isolated loops

Mathieu [1935] studied the absorption of different hexosephosphate esters and found that they were readily absorbed as esters. No hydrolysis occurred in his experiments. At the end of the experiments all phosphate remaining in the lumen was still present in organic combination. In the metabolism experiments the author found that organic phosphate was absorbed completely in normal as well as in vitamin D-deficient rats. Since, however, the rats would eat limited amounts only, it was of interest to follow up the question by a direct measurement of the rate of absorption. Isotonic solutions of alkaline sodium glycerophosphate were used for this purpose.

From Table I it will be seen that glycerophosphate is absorbed as quickly in vitamin D-deficient as in normal rats.

Table I. *The absorption of sodium glycerophosphate from isolated loops of the small intestine*

14.5 mg. P as glycerophosphate injected into 30 cm. loops

mg. P absorbed during 1 hr.	
Normal rats	Vitamin D-deficient rats
3.1	3.4
3.1	3.6
3.2	3.8
3.4	4.2

The hydrolysis of organic phosphate in the intestine

To decide whether any hydrolysis of the ester was taking place direct P estimations were performed in the content remaining in the lumen at the end of the experiments. The values are given in Table II. Inorganic phosphate constitutes 1/3–2/3 of the remaining P (determined after wet ashing). The phosphatases which perform this hydrolysis have a *pH* optimum about 9, and they

Table II. *The hydrolysis of sodium glycerophosphate in the small intestine at pH 8.5*

14.5 mg. P as glycerophosphate injected. 1 hr. experiment

	Unabsorbed P mg.	Organic P mg.	Inorganic P mg.
Vitamin D-deficient rats	11.1	8.1	3.0
	10.9	7.5	3.4
	10.7	6.2	4.5
	10.3	3.6	6.7
Normal rats	11.4	8.0	3.4
	11.4	7.6	3.8
	11.3	5.8	5.5
	11.2	3.8	7.4

are very slightly active at about *pH* 6. Mathieu does not give the *pH* of the solutions used in his experiments. As hexosephosphates are hydrolysed as readily as glycerophosphate it may be that the discrepancy between the results here presented and Mathieu's is due to a difference in the *pH* of the solutions injected.

A new solution with the same concentration of glycerophosphate was prepared, but was brought to *pH* 6 by addition of hydrochloric acid. When the experiments were repeated with this solution, the inorganic phosphate in the content remaining in the lumen after 1 hr. was only 0.5–0.9 mg. P in 5 rats. As would be expected, therefore the *pH* of the intestinal content governs the hydrolysis of phosphoric esters in the bowel. The *pH* in the small intestine ranges from 3 to 4 in the duodenum and up to about 6 in the ileum. In intact rats phosphoric esters ingested with the food should therefore be absorbed largely without hydrolysis.

SUMMARY

1. Potassium phosphate and sodium glycerophosphate are absorbed equally well in normal and vitamin D-deficient rats.
2. The rate of absorption of inorganic phosphate from isolated loops increases with increasing concentration. It is not influenced by iodoacetic acid poisoning.
3. Esterified phosphate is absorbed largely without hydrolysis. Appreciable hydrolysis in the intestinal lumen is observed only at a reaction more alkaline than that normally occurring in the small intestine.

REFERENCES

- Mathieu (1935). *Biochem. Z.* **276**, 49.
 Nicolaysen (1937, 1). *Biochem. J.* **31**, 122.
 — (1937, 2). *Biochem. J.* **31**, 323.
 Verzar (1935). *Biochem. Z.* **276**, 17.

Correction. In the previous paper [Nicolaysen, 1937, 2] all the values giving the absorbed amounts of xylose have to be reduced by 50%.