CCLXXVIII. CEREALS AND RICKETS.[1]
VIII. THE HYDROLYSIS OF PHYTIN IN THE INTESTINE.

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INTEREST in the availability of phytin-P was stimulated by the report of Bruce & Callow [1934] that the rachitogenic manifestation of cereals was effected by the low availability of cereal P. McCance & Widdowson [1935] found that in man as much as one-half of the phytin-P was unavailable. The possible importance of this was revealed by their analyses which showed that phytin-P accounted for 46·4–66·0 % of the total P present in the common cereal grains such as wheat, maize and rolled oats.

Recently it was shown by Templin & Steenbock that immature maize [1933, 1] and germinated autolysed maize [1933, 2] were far less rachitogenic than the matured kernel. This difference was demonstrated by Lowe & Steenbock [1936] to be paralleled by an increase in inorganic P. Phytin, isolated from wheat bran, when fed as such was a poor source of phosphorus in a rachitogenic ration. The basal ration used in these experiments was Ration 2965 composed of 76 % yellow maize, 20 % wheat gluten, 3 % $CaCO_3$ and 1 % NaCl.

In view of these results, it seemed desirable to determine if phytin-P were generally unavailable or if its unavailability were but an artefact produced by an unusual diet. The high content of calcium in Ration 2965 might conceivably have altered relations so as to present an unusual picture of phosphorus availability. For this determination additional experiments have now been carried out using adult rats as the experimental animals.

EXPERIMENTAL.

Metabolism of phytin and inorganic P.

Both high and low calcium rations were fed. Four female rats weighing approximately 200 g. were used for each ration. Individual collections and analyses of excreta were made for two 4-day periods for each animal. All animals were kept on their respective rations for a preliminary period of not less than 4 days, but always until their food consumption was normal and their weights showed no decline. Food consumption was equalized so far as possible.

The urines were collected under toluene. The faeces were preserved by allowing them to drop into a collection chamber in which the air was saturated with vapour of formaldehyde. They were dried overnight at 100°, then finely ground for analysis. Both faeces and urine were analysed for inorganic and total P using essentially the method of Fiske & Subbarow [1925].

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The low calcium basal ration consisted of yellow maize 76, wheat gluten 20, sodium chloride 1, or, in other words, Ration 2965 from which the $CaCO_3$ had been omitted. Analysis showed it to contain 0·058 % Ca and 0·28 % P. When phosphorus was given it was added as disodium hydrogen phosphate or as phytin. Calcium was added as the carbonate. The phytin was prepared from wheat bran. It was free from inorganic P. It was mixed, as a fine powder, directly into the basal ration. The disodium hydrogen phosphate was added in solution, by dissolving it in a minimum of water and then evaporating it on the ration. The P supplements provided five times the amount of phosphorus present in the basal ration. The P supplements were made ample to reduce the proportionate effect of the non-phytin P compounds of the basal ration.

The most significant result from our feeding experiments when phytin was the source of P was the marked diminution in its hydrolysis effected by the presence of $CaCO_3$. The urinary P dropped to mere traces and the total excretion

Table I. *Effect of $CaCO_3$ on the retention and excretion of phytin-P.*

Averages of 8 analyses on 4 rats over two 4-day periods.

		Total P			Urinary P		Faecal P		
No.	Ration	Intake mg.	Output mg.	Balance mg.	Total mg.	% of output	Inorganic mg.	Total mg.	% of output
1	Basal	96	83	+ 13	45	54	21	38	46
2	Basal + phytin	493	450	+ 43	112	25	173	338	75
3	Basal + phytin + $CaCO_3$	486	450	+ 36	Trace	Trace	56	450	100
4	Basal + Na_2HPO_4, $12H_2O$	544	411	+133	347	84	37	64	16
5	Basal + Na_2HPO_4, $12H_2O$ + $CaCO_3$	510	424	+ 86	185	44	218	239	56

of inorganic P was reduced to about one-fifth of its former value. The P, in this case, was excreted entirely through the faeces and almost all in organic combination. In the absence of $CaCO_3$, phytin was hydrolysed to a small but very definite extent, as shown by the rise in urinary P over that resulting from the feeding of the basal ration alone.

When disodium hydrogen phosphate was fed in place of phytin, the P was excreted, as was to be expected, chiefly through the urine. The addition of $CaCO_3$ also caused a marked reduction in the urinary P although this condition was not entirely analogous to that obtained when $CaCO_3$ was fed with phytin. In the case of the phytin-$CaCO_3$ ration, the urinary P was reduced to traces because of the greatly diminished hydrolysis of phytin as shown by the lowered inorganic P content of the faeces as well as of the urine. However, when $CaCO_3$ was given with disodium hydrogen phosphate, the total excretion of inorganic P remained practically the same, $CaCO_3$ presumably acting merely by reducing the absorption of P from the intestinal tract.

A consideration of the P balances on these rations also reveals some interesting information. It is apparent that the greatest retention of P occurred on the phosphate ration, yet even in the presence of $CaCO_3$ the retention of P was higher than it was on the phytin ration alone. Small differences in P retention probably would not be revealed because of the use of adult animals, but these differences appear to be significant.

From these data, it would seem justifiable to deduce two important generalizations; first, that phytin-P is not completely unavailable to the rat, and secondly that the small amount which might be available is rendered almost completely unavailable by the presence of $CaCO_3$.

Effect of other substances on the hydrolysis of phytin.

In view of the pronounced inhibitory effect of $CaCO_3$ on the hydrolysis of phytin, it appeared desirable to investigate the effect of other salts which have been shown to increase the severity of experimental rickets to varying degrees. These studies were limited to following the distribution of total and inorganic P in the faeces.

Table II. *Effect of various compounds on the hydrolysis of phytin*

		Faeces		
No.	Ration	Inorganic P %	Total P %	Inorganic % of total
6	Basal + phytin	2·9	6·7	43·3
7	Basal + phytin + 3 % $CaCO_3$	0·86	7·8	11·0
8	Basal + phytin + 3 % $MgCO_3$	0·89	7·3	12·2
9	Basal + phytin + 3 % $SrCO_3$	1·2	7·9	15·2
10	Basal + phytin + 3 % $BeCO_3$	1·2	7·8	15·4
11	Basal + phytin + 3 % Al_2O_3	2·3	6·3	36·5
12	Basal + phytin + 3 % Fe_2O_3	3·0	6·0	50·0

The rations were prepared by adding 3 % $MgCO_3$, $SrCO_3$, $BeCO_3$, Al_2O_3 or Fe_2O_3 in place of the $CaCO_3$. The consumption of the rations was kept relatively constant. The data presented in Table II show that $MgCO_3$, $SrCO_3$, $BeCO_3$ all produced an effect similar to $CaCO_3$ in inhibiting the hydrolysis of phytin as judged by the percentage of the total P of the faeces excreted in the inorganic form. The total amounts of P excreted remained practically constant except in the case of $BeCO_3$ where a lower food intake led to a corresponding drop in the P excretion. Al and Fe oxides caused only slight differences. Data were not obtained on the urinary P in these cases, so a comparison of the P balances could not be made. However, in view of the uniformity in P distribution in the faeces on all the rations, it appears likely that the modes of action of the other salts resembled that of $CaCO_3$.

Our attention is necessarily again directed to the means whereby the hydrolysis of phytin is effected. The intestinal mucosa is well known to be rich in a phosphatase which hydrolyses glycerophosphate and a wide variety of phosphoric acid esters. Armstrong [1935] has recently found that dog faeces are an excellent source for the preparation of a phosphatase; but Plimmer [1913] reported that extracts from the intestinal mucosa do not contain a phytin phosphatase—a fact which we have substantiated for both the chick and the rat. On the other hand, we have found both a glycerophosphatase and a phytin phosphatase present in faeces and in the contents of the small and large intestines of the rat. This faecal phytase showed an optimum activity distinctly on the acid side of neutrality.

Acid phosphatases have been reported by a number of investigators. Adler [1915] discovered a phytase in malt which showed an optimum activity at *p*H 5·4, and Kay & Lee [1931] reported a phosphatase in soy beans having an optimum *p*H at 5·2. Roche [1931] showed that the red blood corpuscles contained an acid phosphatase with an optimum *p*H between 6·0 and 6·8 and Davies [1934] found that extracts of both spleen and liver contained an acid as well as an alkaline phosphatase. The acid phosphatases, whether acting on phytin or glycerophosphate as a substrate, are quite distinct from the dominant phosphatase of mammalian tissues [Kay, 1932]. In considering the mode of hydrolysis of phytin

in the intestine, it is obvious that the phytase of the flora of the gastro-intestinal tract as well as the phytase ingested with the food warrant intensive investigation.

The availability of phytin-P in different cereals.

In line with the premise that the gastro-intestinal environment can affect the hydrolysis of phytin, the question arose as to whether cereals might not differ in the extent to which they promote this action. The activity of the intestinal flora produced on one cereal might be substantially different from that produced on another.

To study this question, the yellow maize component in our basal diet was replaced in one case by rolled oats and in another by whole wheat. Each was fed with and without $CaCO_3$ to adult female rats. The rats were kept on their respective rations for a preliminary period of 5 days. The urines were not collected because we were concerned primarily with the comparative hydrolysis of the phytin in the rations and not with differences in P retentions. With our adult rats, retention of phosphorus would naturally be low.

Table III. *Effect of varying the cereal component in Ration 2965.*

		Faeces		
No.	Modification of Ration 2965	Inorganic P %	Total P %	Inorganic % of total
13	No modification	0·71	2·13	33·5
14	Same as 13 minus $CaCO_3$	0·60	1·25	47·8
15	Rolled oats substitued for yellow maize	0·83	2·44	33·8
16	Same as 15 minus $CaCO_3$	0·77	1·68	45·9
17	Whole wheat substituted for yellow maize	0·69	2·06	33·7
18	Same as 17 minus $CaCO_3$	0·45	1·11	40·1

The results are presented in Table III. They afford no basis for assuming any differential action of maize, rolled oats or whole wheat. They do offer additional evidence in support of the fact that $CaCO_3$ has a definite inhibitory action on the hydrolysis of phytin. This action was noted without exception with each of the cereals studied.

Various other modifications of the basal ration which might be expected to change the intestinal flora and so alter the degree of hydrolysis of phytin were made. Lactose, lard and vitamin D were added with and without $CaCO_3$. The results from the effect of these additions are presented in Table IV. They show

Table IV. *Effect of various modifications of Ration 2965.*

		Faeces		
No.	Modification of Ration 2965	Inorganic P %	Total P %	Inorganic % of total
19	No modifications	0·71	2·13	33·5
20	Same as 19 minus $CaCO_3$	0·60	1·25	47·8
21	Plus 1250 U.S.P. units vitamin D/day	0·57	1·75	32·6
22	Same as 21 minus $CaCO_3$	0·45	0·74	60·8
23	Plus 10% lactose	0·77	1·95	39·5
24	Same as 23 minus $CaCO_3$	0·35	0·72	48·6
25	Plus 30% lard	0·51	1·54	33·1
26	Same as 25 minus $CaCO_3$	0·26	0·59	44·0

that the percentage of the total P excreted in the faeces as inorganic P was uniform with all rations containing $CaCO_3$. In every case, these values were lower than those from corresponding rations without $CaCO_3$. The vitamin D

supplement in the absence of $CaCO_3$ gave an unusually high proportion of total P present as inorganic P. This value stands as an isolated observation.

The possible relation of our findings on the hydrolysis of phytin to rachitogenic diets used for assay purposes bears mention. Variability in the utilization of phytin-P may be a factor of no small significance in explaining the irregularities reported in the literature in the production of experimental rickets [Harris & Bunker, 1931; 1934; 1935]. It is obvious that our rachitogenic diets as used for assays should contain their P in the form of compounds of invariable nutritive value. Whether this can be achieved in the presence of a substantial content of organic P compounds appears doubtful.

SUMMARY.

1. Phytin when fed to rats on a low Ca and low P ration was hydrolysed to a substantial though incomplete degree.

2. The hydrolysis of phytin was greatly diminished by including 3 % $CaCO_3$ in the ration. This same effect was observed with the use of various other salts known to have a rachitogenic action.

3. The substitution of whole wheat or rolled oats for yellow maize in the basal ration or the addition of lard or lactose effected no significant change in the excretion of P.

4. The role of intestinal flora must be given more consideration in the solution of problems in nutrition than has hitherto been the case.

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