CCCXX. PHYTIN IN HUMAN NUTRITION.

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It has long been recognised that a large proportion of the total phosphorus of cereals and other vegetable foodstuffs may be present in the form of phytin, the calcium magnesium salt of inositolhexaphosphoric acid. This compound, which is insoluble, is manufactured commercially, and has been widely recommended and accepted as a tonic [Ihm, 1929; Paulsen, 1929] and as a readily assimilable form of phosphorus for human nutrition [Starkenstein, 1910; Hutchison and Mottram, 1933]. On the other hand Plimmer [1913] showed that phytin was not hydrolysed by the intestinal enzymes and evidence has been accumulating in recent years that its phosphorus is not available. This is contrary to the results of earlier workers [see Plimmer, 1913] who found that the administration of phytin by mouth led to an increased excretion of inorganic phosphorus in the urine. Bruce and Callow [1934] however using a high Ca-low P diet for rats claimed that the rachitogenic effect of cereals was due to the fact that phytin-P was unavailable. Lecoc and Barban [1935] have shown that in rats aromatic phosphates do not have an antirachitic action, whereas carbohydrate phosphates have. Phytin-P was found to be quite unavailable. On the other hand Harris and Bunker [1935] found no correlation between the degree of rickets produced by a corn diet in rats and the absolute or relative amounts of phytin-P which the diet contained. The further question whether the absorption of calcium from the gut may not be interfered with by phytic acid was considered by Bruce and Callow [1934], but Forbes and Irving [1931] found that phytin-Ca was as available to rats as that of $CaCl_2$.

Many workers have determined the phytin-P of cereals, mainly by modifications of Heubner and Stadler's [1914] original method. This depended upon the extraction of phytic acid from the finely-ground cereal by means of HCl, and the titration of the extract with an acid solution of ferric chloride in the presence of ammonium thiocyanate. The phytin was precipitated as its insoluble iron salt, and the end-point determined by the appearance of the red colour. Later workers have experienced considerable difficulty in determining the end-point of the titration, and modifications have been introduced by Rather [1917], Averill and King [1926] and Harris and Mosher [1934]. Recent results have however fully confirmed the earlier view that phytin-P may form a large percentage of the total P in cereals [Andrews and Bailey, 1932; Knowles and Watkin, 1932; Harris and Mosher, 1934]. Nuts have also been investigated [Averill and King, 1926] but no systematic study appears to have been made of the phytin in other plant foods, and an attempt has been made in the present investigation to determine phytin in fruits, vegetables, nuts, cereals and cereal products commonly eaten in this country.

Method of determination.

Heubner and Stadler's [1914] method was first tried but was found to be unsatisfactory, partly owing to the difficulty of determining the end-point of the titration, even when modifications suggested by later workers were introduced, and partly because such large amounts of dried fruit or vegetable were sometimes required in order to obtain a reasonable titration.

Young [1935] has devised a method for the determination of phytin, which, like the earlier ones, is based on the precipitation of phytic acid by $FeCl_3$ from HCl solution: instead of using a titration method, the phytic acid solution is heated with a known amount of $FeCl_3$ and after removal of the precipitate the excess iron is determined colorimetrically as thiocyanate.

Similar precipitation with excess of $FeCl_3$ has been used in the present investigation, but the phytin has been directly determined by estimation of the amount of phosphorus present in the ferric phytate precipitate.

Reagents. HCl, $N/2$ and $N/6$.

Ferric chloride (A.R.) solution in N HCl containing 0·5 mg. ferric iron per ml.

NaOH 2 % (approx.).

Procedure. 5–10 g. of dried, finely-ground material were shaken in a small glass-stoppered bottle with 100 ml. $N/2$ HCl for 2 hours to extract the phytic acid; 10 to 40 ml. of the filtered extract were neutralised to phenolphthalein with NaOH, rendered slightly acid with HCl and made up to 50 ml.

Duplicate 20 ml. aliquots were treated in 50 ml. centrifuge-tubes, with 4 ml. of the $FeCl_3$ solution. The tubes were heated in a boiling water-bath for 15 min. to flocculate the precipitate of ferric phytate, cooled, centrifuged and the supernatant liquid poured off. The precipitate was washed with 5 ml. $N/6$ HCl, centrifuged again and the acid decanted.

The precipitate was then stirred up with 2 ml. distilled water and heated in a boiling water-bath for a few minutes. 2 ml. of 2 % NaOH were then added, and the heating continued for a further 15 min. The solution containing the phytin as sodium phytate was filtered into a Kjeldahl flask, the precipitated ferric hydroxide was well washed with hot water and the washings were added to the filtrate in the flask.

1 ml. of conc. H_2SO_4 and 1 ml. of 65 % $HClO_4$ (A.R.) were added, and the mixture incinerated very gently until completely digested. It was then heated strongly for 60 min. to drive off any residual $HClO_4$. When cool, about 20 ml. of water were added and the contents of the flask just neutralised to phenolphthalein with 40 % NaOH. The solution was then made up to 100 ml. An aliquot of 5 or 10 ml. was taken in a test-tube and the volume made up to 10 ml. in every case. A blank solution containing 1 ml. conc. H_2SO_4 almost neutralised with 40 % NaOH and made up to 100 ml. was used for the dilution. Standards containing 0·025, 0·05, 0·10 and 0·20 mg. of P were prepared by diluting 0·25, 0·50, 1·0 and 2·0 ml. of a standard solution (containing 0·1 mg. of P per ml.) to 10 ml. with the blank solution. The subsequent procedure was exactly as described by Briggs [1922].

Various steps in the method have been tested as follows:

(*a*) Preliminary experiments were carried out on a 0·1 % solution of commercial phytin in $N/2$ HCl in order to determine whether slight variations in the p_H of the solution affected the amount of ferric phytate precipitated, and to ensure that the presence of inorganic P did not increase the apparent phytin-P of the solution.

10-ml. samples of the standard phytin solution were treated exactly as described above, but the p_H at which the ferric phytate was precipitated varied from very faintly acid to the acidity of the solution usually employed ($N/6$). Aliquots of the phytin solution were similarly treated

in the presence of an approximately equal amount of inorganic P. All the results agreed to within 2%, indicating that phytin was quantitatively precipitated at all the degrees of acidity tested whilst the inorganic P remained in solution.

(*b*) The phytin-P in the phytin solution, determined as described above, was 19·0 mg. per 100 ml. The phytin-P calculated as the difference between the total and the inorganic P was 20·3 mg. per 100 ml. showing a recovery of 94%. Since commercial phytin is not pure and may well contain traces of other phosphorus-containing substances, this result was regarded as satisfactory.

(*c*) In order to determine whether all the phytin was extracted in 2 hours and to study the effect of varying the amounts of extracted material in relation to the volume of acid used, four samples of oatmeal, two of 5 g. and two of 10 g., were shaken with 100 ml. *N*/2 HCl for 2 hours. One of the 5 g. samples and one of the 10 g. samples were filtered immediately, whilst the other two were left soaking in the acid overnight and then shaken for a further 2 hours before being filtered. The phytin-P of each extract was determined in duplicate as already described. The results are shown in Table I. They indicate that the extraction of phytin was complete after 2 hours, and that the whole of the phytin was extracted by 100 ml. of acid whether 5 g. or 10 g. of dried material were used.

Table I. *Effect of different methods of extraction on the phytin-P content of oatmeal.*

					Phytin-P mg. per 100 g.
10 g. oatmeal	extracted	2	hours with	100 ml. *N*/2 HCl	223
10 g.	,,	20	,,	,,	229
5 g.	,,	2	,,	,,	229
5 g.	,,	20	,,	,,	224

Determination of total P. 0·2 g. of dried ground material was incinerated with H_2SO_4-$HClO_4$, the mixture neutralised, washed out and analysed for P as already described.

The phytin content of foods.

Each food to be analysed was purchased from 3 or more different shops in order to obtain an average sample. The mixed material was dried at 100° and ground as finely as possible. The analytical results in Table II, in agreement with previous analyses, show that phytin is a characteristic and abundant constituent of whole cereals and grains and of foodstuffs prepared therefrom, in some cases 40–50% of the total P being present in this form. Removal of the husk and germ considerably reduces the total and relative amounts of phytin-P. Thus, wholemeal flour contained nearly half its total P as phytin-P, whilst white flour contained very much less. It should be noted however that in spite of its high content of phytin-P wholemeal flour still contained almost twice as much non-phytin- or "available" P as white flour.

All legumes investigated contained phytin-P, which in dried pulses ranged between 38 and 50% of the total. Fresh legumes, on the other hand, whether raw or cooked, contained considerably less of their total P in this form (5–20%). The reason for this difference is not apparent, but it must be remembered that dried butter beans, blue peas *etc.* are still alive, and that metabolic changes may have taken place within them during storage. Tinned peas are cooked while fresh, and it is to be expected that these will contain about the same amount of phytin-P as fresh peas.

Phytin was present in carrots and parsnips, whilst turnips, swedes and onions contained none. Potatoes and Jerusalem artichokes contained about 20% of their total P as phytin-P.

Table II. *Phytin content of foods.*

Edible portions only have been analysed. The results have been expressed on a fresh weight basis.

Food	Total P mg. per 100 g.	Phytin-P mg. per 100 g.	Phytin-P as % total P
Cereals, wheat and wheat products:			
Whole wheat	361	168	46·4
Wholemeal flour	355	166	46·8
White flour	102	15	14·7
Wholemeal bread	237	87	36·5
Hovis bread	211	90	42·5
Turog bread	127	35	27·6
Brown bread—mixed sample	198	82	41·5
White bread	59	3	5·1
Grape Nuts	255	86	33·7
Shredded Wheat	173	79	45·3
Post Toasties	50	8	16·0
Vitawheat	340	140	41·2
Digestive biscuits	134	40	30·0
Rusks	81	9	11·0
Other cereals:			
Rice, unpolished	350	240	68·5
Rice, polished	99	41	41·5
Whole oats, including husk	350	182	52·0
Rolled oats	339	224	66·0
Scotch oatmeal	380	160	42·0
Whole barley, including husk	335	211	63·0
Pearl barley	354	78	22·0
Maize (yellow)	363	210	58·0
Millet, whole	350	191	55·5
Tapioca	42	0	0
Sago	38	19	50·0
Ryvita	336	100	29·7
Swedish hard bread	360	90	25·0
Cocoa and chocolate:			
Cocoa	675	162	24·0
Plain chocolate	139	82	58·5
Milk chocolate	215	38	17·6
Nuts:			
Almonds (shelled)	442	188	42·5
Barcelona nuts (shelled)	299	113	37·8
Brazil nuts ,,	592	133	22·4
Chestnuts ,,	74	9	12·2
Cobnuts ,,	229	104	45·5
Coconuts ,,	94	41	44·0
Peanuts ,,	365	210	57·5
Walnuts ,,	510	120	23·5
Legumes:			
Beans, baked, tinned	184	27	14·6
Beans, broad, boiled	108	5·4	5·0
Beans, butter, raw*	318	147	46·3
Beans, Haricot, raw*	309	154	50·0
Lentils, raw*	243	93	38·3
Peas, fresh, raw	105	11	10·8
Peas, blue, raw*	303	150	49·5
Peas, split, raw*	268	124	46·3
Peas, tinned	168	29	17·0
Root, leaf and stem vegetables:			
Carrots, raw	20·9	3·3	15·8
Parsnips, raw	69·0	21·6	31·4
Potatoes, old, boiled	31·0	6·0	19·3
Potatoes, new, boiled	35·7	8·2	23·0
Jerusalem artichokes, boiled	37·0	9·2	25·0
Onions, raw	30·0	0	0
Swedes, raw	19·0	0	0
Turnips, raw	27·5	0	0
Cauliflower, boiled	35·7	0	0
Spinach, boiled	98·0	0	0
Mushrooms, raw	136·5	0	0
Celery, raw	31·7	0	0
Fruit:			
Apples	8·5	0	0
Bananas	28·1	0	0
Blackberries	25·9	4·2	16·2
Figs, dried*	91·5	11·9	13·0
Prunes*	83·0	0	0

* The results of these "dried" foods have been expressed on a purchased weight basis.

As reported by Averill and King [1926] phytin was found to be present in nuts, and in peanuts, cobnuts, almonds and coconuts about half the total P was present in this form. Cocoa and chocolate also contained phytin, 24 % of the total P in cocoa occurring as this compound.

Green-leaf and stem vegetables contained no phytin and none was found in mushrooms. It was entirely absent from the pulp of fruit, whether fresh or dried, but fruits with a large number of "edible" seeds, *e.g.* figs and blackberries, contained small amounts.

The fate of ingested phytin.

Experiments have been carried out on 4 subjects, the authors (M.) and (W.), a healthy woman (L.) and a boy (S.) aged 4½, who had been operated on for an inguinal hernia 6 days previously. The basal diet was not weighed but was

chosen to be as far as possible phytin-free, and on this diet it was ascertained that the faeces contained only very small amounts of phytin. W. and S. ate weighed amounts of Hovis bread as their sole source of phytin, and samples were set aside daily for analysis. M. ate Hovis bread and blackberries, and L. took 2 g. of phytin (Messrs Ciba, Ltd.) daily in divided doses. The three adults consumed almost exactly the same amount of phytin on each day of their experiment, but the boy's intake was rather more variable. After a fore period of 2–3 days, faeces were collected in 2-, 3- or 4-day periods, well mixed, and duplicate aliquots of 50 to 100 g. taken. The latter were dried at 100°, ground up in a mortar and analysed for total P and phytin-P as already described.

The results are given in Table III.

Table III. *The excretion of ingested phytin.*

	Average daily intake phytin-P mg.	Average daily output in faeces		% of phytin-P ingested which was recovered in faeces
		Phytin-P mg.	Total P mg.	
M. Period 1 (2 days)	416	181	950	43·4
M. „ 2 (2 „)	436	206	975	47·5
M. „ 3 (3 „)	436	192	860	44·0
W. „ 1 (2 „)	330	150	765	45·5
W. „ 2 (3 „)	336	120	635	35·8
W. „ 3 (2 „)	336	134	830	39·8
L. „ 1 (3 „)	370	194	765	52·5
L. „ 2 (4 „)	370	231	790	62·5
S. „ 1 (3 „)	101	25	505	25·0
S. „ 2 (3 „)	114	24	840	21·4

These experiments show that the three adults excreted 36–63 % of the ingested phytin unchanged in the faeces. Of these, L. who took commercial phytin excreted the most. The child (S.) excreted a much smaller percentage, but this experiment, which was carried out in a children's ward, was not so accurate as those on the adults. A variable fraction of the phytin therefore proved to be absolutely unavailable in every case, but the fact that the remainder did not appear again in the form in which it was eaten is no proof that the phosphorus in it was available even to this extent, for the phytin may have been broken down by the intestinal flora at a level below that at which absorption could take place. The total P in the faeces was far in excess of the amount of phytin-P ingested so this may have been the case.

The present experiments therefore provide concrete evidence that as much as half the phytin-P eaten may be unavailable, but they should not be considered to provide any definite evidence as to the fate of the remainder.

Dietetic importance of phytin.

A study has recently been completed on the individual, freely-chosen diets of 63 men and 63 women of the English middle class. By means of the figures in Table II the phytin-P intakes of these men and women have been calculated and compared with the total P in their diets.

Table IV. *Total P and phytin-P intake of men and women.*

(Results expressed as g. per day.)

	Men Mean of 63	Women Mean of 63
Total P	1·61	1·13
Phytin-P	0·04	0·04
Non-phytin-P	1·57	1·09
Non-phytin-P as percentage of total P	98	97

No individual took more than 20 % of his total P in the form of phytin. Relative amounts as high as this were the exception, and only occurred if large quantities of brown bread were being eaten. In most cases less than 10 % of the total food P was phytin-P. In this country therefore, where we live on a varied diet and derive most of our phosphorus from animal and not from vegetable sources, 80–100 % of the total phosphorus eaten is in available form (average 97·5 %). In other countries, however, where cereals, either whole or milled, constitute by far the largest portion of the diet, the total P may be a wholly incorrect guide to the available P intake.

SUMMARY.

1. A method for estimating small amounts of phytin is described. The phytin was extracted by HCl, precipitated as the ferric salt and the P in the precipitate estimated after sulphuric-perchloric acid incineration.

2. The phytin in 64 foodstuffs has been determined.

3. The fate of ingested phytin in the human body has been investigated in 3 adults and a child, and it has been shown that 20–60 % is excreted unchanged in the faeces.

4. Phytin-P constitutes less than 5 % of the total P of the average middle-class diet in this country.

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