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MINERAL METABOLISM ON DEPHYTINIZED BREAD

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Although by the year 1925 oatmeal and wheat had been clearly demonstrated by Mellanby to have rachitogenic properties, the discovery was grudgingly admitted to be true by a world which relied largely on cereals for its nutrition. E. & M. Mellanby found that the rachitogenic agent was destroyed by boiling the cereals with 1% HCl, and also by germination [M. Mellanby, 1929], but they were not able at that time to identify it. Bruce & Callow [1934], following up an observation of Steenbock, Black & Thomas [1930], showed that cereals were rachitogenic for rats on high-calcium low-phosphorus diets because a large part of the phosphorus in them was present as inositol hexaphosphoric, or phytic, acid, and hence was not so freely utilized as inorganic phosphate would have been. These findings at once received recognition. McCance & Widdowson [1935] then showed that phytate phosphorus was comparatively unavailable to man, a fact which had really been demonstrated by balance experiments on children as long ago as 1916. Ahlqvist [1916], however, who made these observations, had not designed her experiments for this purpose, and their significance seems to have escaped her. Nevertheless, she found that when 194 mg. of phytic acid phosphorus were added to diets which contained an average of 1510 mg. of phosphorus per day, the percentage of the intake absorbed fell from 65 to 58. The magnitude of this fall suggests that these children absorbed none of the phytic acid phosphorus, but this is probably an over-statement of the facts. Bruce & Callow [1934] also carried out some inconclusive experiments with low-calcium diets. They pointed out, however, that phytic acid had a calcium salt 'at least as insoluble as calcium phosphate', and that, therefore, it might be expected to lower the available calcium in such diets, possibly even to deficiency levels, by binding the metal in a precipitated form. McCance [1934] enlarged upon this property of phytic acid, and Mellanby recognized at once that it was likely to be the important one in the genesis of canine and human rickets, which are due to calcium rather than phosphorus deficiencies. As late as 1937, however, the considered opinion of the American 'Council on Foods' still was that 'there is no good evidence for the existence of a decalcifying factor in cereals'. Harrison & Mellanby [1939] took up the matter experimentally, and showed that diets could be made rachitogenic for puppies by incorporating sodium phytate into them. Mellanby [1941] has since demonstrated that as oatmeal is boiled with dilute HCl, the phytic acid in it is slowly destroyed, and its rachitogenic properties gradually diminish. Phytic acid, therefore, may be accepted as the ingredient which makes oatmeal so detrimental to the growing bones and teeth of dogs.

Returning to this problem because of some chance observations which had been made in an experimental study of rationing, McCance & Widdowson [1942] showed that healthy men and women absorbed calcium and magnesium much less readily from brown than from white bread dietaries. The subjects obtained 40–50% of their total calories from the staple cereal, the remainder from an ordinary mixed diet. It was considered that the reduced absorption was probably due to the large quantities of phytic acid in brown bread, and this view was enhanced by the fact that the effects of brown bread on mineral metabolism could be reproduced by adding sodium phytate to white bread. Nevertheless, the fact still remained that brown bread had a laxative action, and there was as yet no proof that this was not playing some part in hindering calcium absorption [Ascham, 1930–1; Aub, Tibbetts & McLean, 1937; Bloom, 1930]. It was felt that phytic acid could be even more definitely incriminated if experiments could be carried out with brown bread from which the phytic acid had been removed, and this investigation will now be described.

Methods

Subjects. There have been six subjects, three men and three women. One man, E.B., and one woman, A.M., had taken part in the previous investigation and were described in the report of it [McCance & Widdowson, 1942]. N.J., one of the new men, was a University teacher, aged 27 and weighing 142 lb., and the other, N.C., was a postgraduate student, aged 21, weight 140 lb. Of the new women, H.E. was a research student, aged 21, weight 118 lb., and W.Y. a doctor, aged 32, with a weight of 147 lb.

Number and order of the experiments. Each subject has carried out four experiments, a white bread control, a brown bread control, and experiments with two different kinds of dephytinized brown bread. The dietary and other technical arrangements were the same as those reported by McCance & Widdowson [1942], and, as before, the flour under investigation furnished 40–50% of the subjects' calories. The experiments began in September and ended just before Christmas. The first three lasted for 3 weeks and contained three analytical periods each of 1 week in length, but the December experiments only lasted a fortnight, for the results were a foregone conclusion by that time and several of the subjects were anxious to be free for Christmas. The preliminary periods were 3 days in length, and the usual after-periods were provided. Full metabolic discipline was rigidly enforced, including the prohibition of all tooth pastes [McCance & Widdowson, 1942]. In order to prevent seasonal or intrinsic fluctuations in calcium absorption from influencing the results [McCance & Widdowson, 1943], the subjects did not all do the experiments in the same order. Two began with the white bread control, two with the brown bread control and two with a dephytinized preparation. The later experiments were similarly arranged.

Preparation and properties of the breads. A 'C roll' white flour, i.e. one of very low extraction, and bran from the same grist, ground to pass through a $\frac{1}{32}$ in. mesh, were obtained from the Research Association of British Flour Millers, and these formed the basis of all the flours used in these experiments. The white bread was made from the white flour, with yeast as the raising agent. The brown bread was made from a reconstituted flour containing 83 parts of white flour and 17 parts of bran. This bread was baked with sodium bicarbonate and acid potassium tartrate in order to avoid hydrolysis of the phytic acid [Widdowson, 1941] and contained about 0·1% of phosphorus in the form of phytic acid.

The other experimental breads were made in the following way. Bran was dephytinized enzymically by incubating it at 50° C. with 10 times its weight of water for 6 hr. at *p*H 4·5. The *p*H was adjusted by adding HCl, and at the end of the period of incubation this was neutralized with NaOH and the bran filtered off through a closely woven cotton cloth on a large Buchner funnel. The bran was then spread out to dry on a sheet of the same cloth tied over wires on a wooden frame set over an electric fire. The enzymic process broke down the phytic acid into inositol and inorganic phosphates. These, and the mineral ions with which the phytic acid had originally been combined, tended to pass into the liquid, as also did some of the more soluble nitrogenous and other organic compounds in the original bran. Consequently, the amount of dried bran recovered from the dephytinizing process was usually about 70% of the weight taken at the beginning. Of the water added to the bran, about 85% was usually recovered in the filtrate. The remainder stayed with the bran and was removed by volatilization as the bran was dried. Table 1 shows the composition of the original bran and the partition of its constituents by the dephytinizing process. Apart from the solids, nitrogen and phosphorus, to which reference has already been made, it will be noted that the metallic ions passed into the filtrate in very different amounts, and that the order of solubility was potassium, magnesium, calcium and iron. Practically none of the last left the bran. Whereas, therefore, the original bran contained more than enough phytic acid to combine with all the calcium, magnesium and iron, the dephytinized and demineralized bran did not, and the physico-chemical properties of magnesium and calcium phytates [McCance & Widdowson, 1942] indicate that most of the magnesium was in combination with some other acid radicle.

TABLE 1. The composition of bran and of its dephytinized products

*	Composition of the original bran g./1000 g.	Distribution of the solids and chemical elements in 1000 g. of bran between air-dried dephytinized bran and the filtrate separated from it: Dephytinized bran g.	Filtrate g.
Solids	1000	694	306
Total nitrogen	23·6	14·0	9·6
Total phosphorus	12·4	3·2	9·2
Phytic acid phosphorus	11·0	0·92	tr.
Calcium	1·1	0·75	0·35
Magnesium	5·8	1·6	4·2
Potassium	13·0	3·0	10·0
Iron	0·137	0·134	tr.

* Both the original bran and the air-dried dephytinized product contained small amounts of water, but these have been neglected in constructing this table.

The processed bran was used to reconstitute a brown flour, and since the incubation and filtration not only hydrolysed the phytic acid but also removed a large proportion of the resulting phosphates, potassium and magnesium, the bread so baked may best be described as a dephytinized and demineralized brown bread. The amount of the processed bran to be added to the white flour was determined by the weight which it had lost during its dephytinization. Thus if 17 g. of natural bran were used in order to reconstitute 100 g. of 'brown' flour, and if 100 g. of this bran lost 30 g. during dephytinization, then 13 g. of the product were used for the reconstitution of 100 g. of the dephytinized and demineralized brown flour. In order to study the effects of dephytinization without demineralization the liquid filtrate in the correct proportions was incorporated into the diets. Some of it was used to make up the dough from which the bread was to be baked, and the remainder was measured out in the correct quantities and drunk by the subjects as they ate the bread. The approximate compositions of the three kinds of bread and the bread-filtrate combination are given in Table 2. There are some rather interesting points about these figures. The only important difference between the brown and the dephytinized brown is the change of the phytic acid phosphorus to inorganic phosphorus, but there may have been changes in the state of combination of all the ions following the enzymic processes. The demineralized bread differed from the dephytinized bread in containing rather more than one-third as much phosphorus and magnesium and about four-fifths as much calcium. It is suggested that the magnesium was present as magnesium phosphate, maintained in solution during incubation of the bran at pH 4·5, but precipitated in, or with, the dephytinized bran when the NaOH was added before filtration. The white bread contained nearly as much phosphorus but considerably less magnesium than the demineralized bread.

TABLE 2. Composition of the four experimental breads

	Brown	Dephytinized brown (including the correct amount of filtrate draught)	Dephytinized and demineralized brown	White
Water, g./100 g.	36	35*	35	32
Total nitrogen, g./100 g.	1·52	1·52	1·44	1·56
Protein, g./100 g. ($N \times 6{\cdot}0$)	9·1	9·1	8·6	9·4
Total phosphorus, mg/100 g.	206	206	75	66
Phytic acid phosphorus, mg./100 g.	99	13	13	0
Calcium, mg./100 g.	25	25	19	15
Magnesium, mg./100 g.	85	85	30	17
Iron, mg./100 g.	2·3	2·3	2·3	1·0

* The water in the filtrate draught is not included in this table.

It is rather useful in considering the metabolic results to bear in mind the approximate proportions of the total dietary calcium, phosphorus and magnesium supplied by the three different breads and the bread-filtrate combination. The figures for the men are given in Table 3, and it will be

TABLE 3. Approximate percentages of the men's total dietary calcium, magnesium and phosphorus supplied by the four 'breads'

	Brown	Dephytinized brown (with filtrate draught)	Dephytinized and demineralized brown	White
Calcium	30	30	25	20
Magnesium	74	74	57	33
Total phosphorus	70	70	45	40

appreciated at once that most of the calcium came from foods other than the bread, and that all four breads supplied roughly the same percentage of the dietary calcium. 70 % of the dietary magnesium and phosphorus was provided by the brown and the dephytinized brown bread; much less was provided by the demineralized bread and only 33 % of the dietary magnesium by the white bread. Consequently, the magnesium and phosphorus results are more difficult to interpret than the calcium findings, and that for two reasons. First, the absorption of the magnesium and phosphorus present in the brown breads weighted the figures for the total absorptions much more than did the absorptions of these two minerals from white bread. Secondly, since the basal diet did not change from one experiment to the next, the total intakes of magnesium and phosphorus fell greatly on passing from the brown and dephytinized brown breads through the demineralized bread to white.

The reconstituted brown bread and the dephytinized and demineralized bread were good both in appearance and taste. The filtrate was slightly salty but, flavoured with lemon essence, it was not difficult to drink.

Metabolism results

Calcium

Table 4 shows the calcium intakes and absorptions of the six subjects on the four different diets. It will be noted that the intakes were a little higher on the brown bread and the dephytinized bread than they were on the demineralized and white breads, but that the intakes were of the same order and roughly comparable throughout. It will next be noted that on any given

Table 4. The intakes and absorptions of calcium

Type of bread	Intake mg./day	Absorption mg./day	Absorption % of intake	Intake mg./day	Absorption mg./day	Absorption % of intake
		Subject E.B.			Subject H.E.	
Brown	550	89	16	522	57	11
Dephytinized brown	590	231	39	566	169	30
Dephytinized and demineralized brown	472	236	50	490	192	39
White	488	250	51	478	219	46
		Subject N.C.			Subject A.M.	
Brown	558	74	13	556	7	1
Dephytinized brown	602	279	46	606	120	20
Dephytinized and demineralized brown	559	327	58	480	150	30
White	505	232	46	443	142	32
		Subject N.J.			Subject W.Y.	
Brown	610	−37	−6	575	23	4
Dephytinized brown	662	67	10	633	123	19
Dephytinized and demineralized brown	593	123	21	512	149	28
White	522	111	21	552	183	33

diet the subjects tended to have absorptions very different from one another, even if their intakes were nearly the same. E.B. and N.J. make an excellent contrast in this respect, for N.J. consistently absorbed very much less calcium, although he always had a somewhat larger intake. Nevertheless, the same alterations in diet, i.e. the same changes in external conditions, changed the absorptions of all the subjects in the same direction. This is a satisfactory feature of the results. Finally, and this is the most important point demonstrated by the table, the absorptions of calcium were all at their worst on brown bread. In fact, all the subjects had negative balances on these diets. The calcium absorptions were all improved by the hydrolysis of the phytic acid, but further improvement took place when the phosphates were removed. Four of the subjects absorbed calcium slightly better when white replaced the dephytinized and demineralized bread. One subject did not, and on the whole the differences between the calcium absorptions on these two diets were small. The interest of this observation lies in the fact that the dephytinized and demineralized bread was highly laxative. Indeed, as might have been anticipated from the work of Falcon-Lesses [1929–30] on rats, the processed was really as laxative as the original bran. The average weights of the fresh faeces passed daily were 116 g. on white bread, 209 g. on the dephytinized and demineralized bread, 226 g. on the dephytinized bread, and 224 g. on the brown bread. Comparison of these faecal weights with the calcium absorptions (Table 4) shows that it was not the increased bulk of the faeces on brown bread diets which made the absorptions of calcium so much lower than they were on white. The poor absorptions, therefore, of calcium from brown and dephytinized brown bread must be attributed to the specific action of the phytates and phosphates [McCance & Widdowson, 1942]. This conclusion is in keeping with the findings of Aub *et al.* [1937] in human subjects, and substantially, also, with the work of Ascham [1930–1] on dogs and of Bloom [1930] on rats.

Magnesium

Table 5 shows the intakes and absorptions of magnesium on the four different diets. Some of the results are like those of calcium and are equally conclusive, and a comparison of the absorptions from the brown and from the dephytinized brown bread diets shows that phytates interfered with magnesium

TABLE 5. The intakes and absorptions of magnesium

Type of bread	Intake mg./day	Absorption mg./day	Absorption % of intake	Intake mg./day	Absorption mg./day	Absorption % of intake
		Subject E.B.			Subject H.E.	
Brown	720	120	17	650	163	25
Dephytinized brown	704	214	30	524	152	28
Dephytinized and demineralized brown	370	143	39	352	124	35
White	307	140	45	270	160	59
		Subject N.C.			Subject A.M.	
Brown	754	47	6	592	140	24
Dephytinized brown	764	208	27	564	154	27
Dephytinized and demineralized brown	505	207	41	318	126	39
White	320	163	51	230	109	47
		Subject N.J.			Subject W.Y.	
Brown	740	166	22	638	176	28
Dephytinized brown	748	207	28	618	217	35
Dephytinized and demineralized brown	480	177	37	378	176	46
White	324	164	50	341	193	57

as they did with calcium absorption. There are, however, some interesting differences between the magnesium and the calcium results. The first is the great fall in the magnesium intake brought about by the demineralization of the bran. Such a fall may have combined with the removal of phosphates in helping to improve the percentage absorptions on the demineralized diets, for there is some evidence that as magnesium intakes rise the percentage absorptions tend to fall [Tibbetts & Aub, 1937]. Secondly the magnesium in the demineralized bran (see Table 1) was less readily absorbed than the magnesium in the white bread diets. Taking the intakes and subjects as a whole, the differences in the amounts ingested on these two diets were scarcely enough to have accounted for this. There are good reasons, moreover, for thinking that it was not due to the laxative properties of the demineralized breads. It can hardly have been caused by the traces of residual phytic acid, for calcium, which forms a more insoluble phytate than magnesium, was almost as well absorbed from the demineralized as from the white bread diets. It may be that the magnesium in this dephytinized and demineralized bread was combined with phosphate, and that the two ions were mutually preventing each other's absorption (see later), but it is possible that the magnesium in the bran was in some unknown but stable combination. Lithium seems to form such salts [Kent & McCance, 1941].

Phosphorus

Table 6 shows the phosphorus absorptions of the six subjects on the four different kinds of bread. The change from brown to dephytinized brown much improved the absorptions of all the subjects, and the average rose from 48 to

TABLE 6. Intakes and absorptions of phosphorus (including phytic acid phosphorus)

Type of bread	Intake mg./day	Absorption mg./day	Absorption % of intake	Intake mg./day	Absorption mg./day	Absorption % of intake
		Subject E.B.			Subject H.E.	
Brown	1940	900	46	1650	819	50
Dephytinized brown	1900	1230	65	1550	980	63
Dephytinized and demineralized brown	1200	810	68	1110	724	66
White	1260	923	73	1090	875	80
		Subject N.C.			Subject A.M.	
Brown	1920	840	44	1490	680	46
Dephytinized brown	2070	1400	68	1500	914	61
Dephytinized and demineralized brown	1450	1010	69	890	560	63
White	1290	1010	78	880	620	70
		Subject N.J.			Subject W.Y.	
Brown	2050	965	47	1850	980	53
Dephytinized brown	2030	1250	61	1720	1080	63
Dephytinized and demineralized brown	1440	890	62	1080	655	61
White	1220	847	70	1180	842	71

64 % of the intakes. This was expected, for phytic acid phosphorus was already known to be less readily absorbed than inorganic phosphorus [McCance & Widdowson, 1942]. It is of some interest to refer here to a remark of Blatherwick & Long [1922] that the phosphorus of washed bran is unavailable to man, whereas a certain part of the phosphorus of unwashed bran is assimilated. It is easy to see now that the large amount of phytic acid phosphorus in bran was the basis for this observation, although in the light of recent work their statement must be regarded as somewhat of an exaggera-

tion. On the demineralized bread the intakes fell to a little over half what they had been before, but the percentage absorptions remained virtually unchanged. The intakes on demineralized bread and on white bread were about the same, but the absorptions on white bread were better. The absorptions averaged 65 and 74% of the respective intakes. The small difference in the amount of phytic acid phosphorus in the two breads is not quite enough to account for the whole of this further, although admittedly smaller, improvement in absorption. If, however, the magnesium and phosphate ions in the demineralized bran were combined with each other and mutually preventing each other's absorption, then the absence of bran and consequently the smaller magnesium intakes on the white bread diets probably contributed to the improved absorptions of phosphorus.

Discussion

The results of these experiments speak for themselves and they require little discussion. Taken in conjunction with the results of the experimental work previously reported [McCance & Widdowson, 1942], they leave little room for doubt that phytic acid is the agent in whole wheat primarily responsible for the poor absorptions of calcium and magnesium. They confirm also that the phosphorus in phytic acid is absorbed less freely than inorganic phosphorus. They show, however, that mere hydrolysis of phytic acid will not permit calcium and magnesium to be absorbed as freely from brown flour as from white. Some of the products of hydrolysis also interfere to some extent, and the whole trend of work on calcium and phosphorus metabolism and the present results themselves suggest that the inorganic phosphates are responsible. It is evident, therefore, that even if the processing of brown flour could so be organized that all the phytic acid were hydrolysed [Widdowson, 1941], the resulting bread would still compare unfavourably with white bread so far as its effects upon calcium absorption were concerned. This is not to say that wholemeal bread is a worse food for man than white bread. The evidence generally is that it is better, except for the fact that it contains so much phytic acid. The addition of calcium to the brown flour would at once correct this defect.

The whole of this work on human metabolism should be regarded as confirming, expanding and in part explaining the pioneer work of the Mellanbys on puppies [Mellanby, 1925; M. Mellanby, 1929; Harrison & Mellanby, 1939]. It is satisfactory to have been able to extend their work to the species to which it was always intended to apply, and to have been able to show that the growing puppy, deprived of vitamin D, was a better indicator of what went on in the human intestine than many people supposed.

SUMMARY

1. The calcium, magnesium and phosphorus absorptions of three men and three women were studied on diets in which 40–50% of the total calories were supplied in the form of:

(*a*) a 'brown' flour reconstituted from white flour and bran;

(*b*) a flour mixture similar to (*a*) in which the phytates had been hydrolysed enzymically to inorganic phosphates and inositol;

(*c*) a mixture of white flour and bran from which nearly all the phytates and most of the products of hydrolysis had been removed;

(*d*) white flour.

The laxative properties of (*a*), (*b*) and (*c*) were approximately the same.

2. The calcium absorptions were worse in (*a*) than (*b*) and in (*b*) than (*c*), but almost the same in (*c*) and (*d*).

3. The magnesium absorptions improved progressively in passing from (*a*) to (*d*).

4. The phosphorus absorptions were worse in (*a*) than in (*b*) or (*c*), and a little worse in (*b*) or (*c*) than in (*d*).

Miss B. K. Alington has played a very large part in making this investigation a success. Those who have taken part in similar studies will appreciate how much has been due to the good will and self-discipline of the six subjects. The work has largely been financed by the Medical Research Council, and E. M. W. is in the whole-time service of the Council.

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