J. Physiol. (1942) 101, 44–85 612.015.31

MINERAL METABOLISM OF HEALTHY ADULTS ON WHITE AND BROWN BREAD DIETARIES

By R. A. McCANCE and E. M. WIDDOWSON

From the Department of Medicine, Cambridge

(*Received* 25 *October* 1941)

Apart from a few numerically unimportant races, such as the Esquimaux and Masai, humanity relies extensively for its nourishment upon starches and proteins of vegetable origin. Exceptionally, these may be furnished by the potato, as in Tristan da Cunha, or by kassava, as in certain parts of India and Africa, but cereals constitute the important supply for the great majority of mankind. There are at least seven used for human consumption in different parts of the world—wheat, rice, maize, millet, rye, barley and oats. Each of these may be processed in many different ways. There must therefore be problems galore about the merits and demerits of cereals; and yet, oddly enough, they have been relatively little explored. One of them was propounded by Mellanby [1925] when he discovered that there was some connexion between the severity of the rickets developed by a growing dog and the type of cereal upon which it had been fed. Oatmeal was highly rachitogenic, whereas white wheat flour was relatively innocuous. At the time, and subsequently, the general importance of this discovery was missed, mainly perhaps because the experiments centred upon the production of rickets in growing animals and its cure by vitamin D. The last was therefore seized upon as the important variable. Until very recently it would have been safe to say that in spite of a great deal of highly suggestive work, vitamin D has remained the centre of interest in calcium metabolism, and the nutritional properties of cereals have been regarded as beyond practical control.

Quite apart from Mellanby's work on rickets, our knowledge of cereal chemistry has undergone considerable expansion in the last 20 years, and striking differences have been discovered between the composition of different cereals, and also between the chemistry of the whole grain and most of the milled flours prepared therefrom. All these differences have nutritional significance. It has frequently been demonstrated, for instance, that white flour contains less phosphorus, iron, magnesium, vitamin E and the B group of vitamins than an equal weight of the whole wheat berry. It also contains less calcium and probably a less nutritious assortment of amino acids. It has, moreover, none of the laxative properties of wholemeal, owing to the fact that it contains practically no bran. The advocates of wholemeal flour have not been slow to seize upon these findings and to exploit them to the best of their ability, but they have said very little about the fact that a large part of the phosphorus in wholemeal flour is present as phytic acid. This compound, in which one molecule of inositol is linked with six phosphoric acid radicles, forms insoluble calcium, magnesium and iron salts and it is not hydrolysed by any of the enzymes produced by the human digestive tract. When, therefore, work which was carried out in this department in the first six months of the war, demonstrated the valuable nutritive properties of a wheatmeal containing 92 % of the original grain, but at the same time suggested that the consumption of large amounts of it might be unfavourable to calcium absorption, a comprehensive set of experiments was planned to try to clarify some of these interesting and practical aspects of cereal chemistry and human nutrition. The development of our knowledge of phytic acid has been traced in a recent editorial article in *Nature* [1941].

The experiments now to be described were designed to solve the following problems:

(1) Do men and women absorb calcium less freely from brown than from white bread dietaries?

(2) If this should prove to be the case

(*a*) Is phytic acid the noxious agent, and if so has it any effect on other metals?

(*b*) Will vitamin D restore the calcium absorptions and balances to their white bread levels?

(*c*) Will the fortification of wheatmeal flour with calcium salts overcome its bad effects on the absorption of this element?

And if so

(α) What is the best salt to use?

(β) How much should be added?

The methods and subjects

The work now to be described has been carried out by 'balance' experiments on normal healthy human subjects. Each experiment, i.e. each set of conditions, has lasted for at least 14 and usually 21 days. Some have lasted for 28 days. The control experiments have been as long as the experimental ones, and have been repeated if and when there seemed any need to do so. The subjects have been given at least 3 and often 7 preliminary days in which to adjust their metabolism to a change of conditions, and the experimental diet has never been stopped till the last collection of faeces has been made. Each experiment has been subdivided into two, three or four analytical periods of 1 week in length. If an unexpected answer has been obtained for any one set of analytical results, and such errors as sampling have been excluded, the question has always been raised as to whether this represents a genuine irregularity of metabolism or a technical error in the collection of the faeces. It has been found very helpful in this dilemma to have simultaneous balances of several minerals, and this procedure may be recommended to others. If, for example, the calcium balance of the first week of an experiment is unexpectedly negative but the magnesium balance is exact, and the iron balance also, then the result must be regarded as due to a metabolic cause if contamination of the excreta can be excluded. But if the faecal magnesium is also very high and the iron balance grossly negative, then the probability is that the faecal marking of this week has been incorrect, and it may be legitimate to disregard this set of results if two others are available.

The subjects

There have been four men and four women in each experiment. Their initials, ages, sex, and some further information are given in Table 1.

The dietary organization

At first all meals were served in a large room in the Department of Medicine, and the subjects always partook of them there till they were thoroughly versed in the routine. Owing to the prolonged nature of the experiments and of the whole study, it became apparent after some time that most of the subjects would be very much happier if they were allowed some measure of freedom, and accordingly each was encouraged to take one evening meal during the week at home. This meant taking away weighed amounts of prepared food, or cooking and weighing the food at home and bringing back duplicate portions for analysis. Both

TABLE 1

Initials	Sex	Age years	Weight lb.	Notes
E. B.	M.	31	152	A Spanish doctor with experience of research. Had the most regular metabolism of all the subjects. Readily absorbed calcium
C. B.	M.	29	176	An Antarctic explorer. Unfortunately had to leave the party after the first two experiments to take up work in Palestine
N. K.	M.	25	150	A botanist and spectrographist. Absorbed calcium less readily than some, and was generally in negative calcium balance
R. M.	M.	41	135	Took more physical exercise than the other subjects. His power to absorb calcium declined steadily through the 9 months of observation
P. S.	M.	32	176	A Czech surgeon. Replaced C. B. in the experimental party
B. A.	F.	26	117	Dietitian, took charge of all the catering and cooking
K. B.	F.	27	118	Zoologist, wife of C. B. Accompanied her husband to Palestine. Carried out the first two experiments only
A. M.	F.	26	164	A biologist. Calcium metabolism most consistent in spite of some irregular dietary practices partly due to severe dysmenorrhoea
E. W.	F.	33	124	Tended to gain weight throughout the experiment. Showed a progressive deterioration in calcium absorption similar to R. M.'s
R. W.	F.	25	142	Dietitian employed at Addenbrooke's Hospital. Replaced K. B. Had a less regular absorption of calcium than the other subjects

Note. The experiments were arranged without reference to the women's menstrual periods, which appeared to make no difference to their absorption and metabolism of calcium, magnesium or other minerals investigated.

were troublesome, but for most people the sense of escape made it well worth while. In the last few months the whole party took their evening meals home with them on Sundays.

In a prolonged study of this kind it is theoretically desirable that the basal diet eaten in all the experiments, the results of which are to be compared, should be exactly the same. This was an impossible goal owing to the limitations of war-time catering, but arrangements were made to maintain a roughly uniform dietary throughout the whole series of experiments, and when this was for some reason overborne, efforts were made to find out whether the change had had any demonstrable effect upon the metabolism of the subjects. A large proportion of each person's diet was automatically fixed, for it was planned in advance that flour should always furnish 40–50 % of the total calories. The flour, which was obtained from Foster Mills Ltd., Cambridge, was taken partly as bread, and partly as puddings, cakes and pastry. The amount of flour eaten was added up at the end of each week, and each person did his best to keep his flour consumption about the same from week to week. This was a surprisingly easy task, as Table 2 shows, although naturally some subjects were a good deal better at it than others. The remainder of the diet was as liberal as official rationing would allow, except that cheese was forbidden,

TABLE 2. Variations in the consumption of flour from week to week

	Exp. 2 (August)			Exp. 7 (Feb., March)		
	Week A	Week B	Week C	Week A	Week B	Week C
Subject	g.	g.	g.	g.	g.	g.
E. B.	2170	2124	2546	2389	2405	246
C. B.	2826	2812	2651	—	—	—
N. K.	3168	3463	3217	3616	3561	3487
R. M.	2736	2999	2808	2882	2846	2973
P. S.	—	—	—	2350	2280	2501
B. A.	2153	2401	2358	1820	1865	1770
K. B.	2114	2111	2034	—	—	—
A. M.	2157	2034	1980	2114	2463	2359
E. W.	2058	2103	1994	2048	2065	2087
R. W.	—	—	—	1955	1842	1980

and milk was restricted. The quantities of the various foods eaten were left to individual choice, but personal tastes and the repetitive tendency of the meals saw to it that there was not much variation from one week or from one month to another. Occasionally, someone was observed to be eating such large quantities of a particular food that there was a risk of his calcium intake becoming too high. He was then advised to reduce his intake, or he was given less milk. This applied particularly to R. M.'s green vegetables.

To assist in maintaining a uniform diet, enough fruit was bottled and enough jam was made to last throughout the series of experiments. Enough marmalade for the whole study was very kindly presented to us by Messrs Chivers Ltd. There was a limited allowance of fruit and jam containing seeds, since these are known to have phytic acid in them. There was also a regular issue of other fruits, but this was probably not very important since the experiences of the fresh-fruit season indicated that variations in the consumption of seedless fruits did not upset the balances (see Table 3). The vegetables eaten varied to some extent with the season, but legumes were eaten sparingly, since they have a certain amount of phytic acid in them. Spinach and rhubarb were avoided and any other plants known to contain oxalic acid, since this has been shown to precipitate calcium in the intestine and prevent its absorption [Fairbanks & Mitchell, 1938; Kohman, 1939; Logan, 1940]. McLaughlin's [1927] human experiments showed this also, but oddly enough the author interpreted her results in the opposite sense.

The first three experiments took place during July, August and September, and there were considerable variations in the consumption of raw plums, greengages, pears and damsons as these fruits became first plentiful and then scarce. It was feared, for a time, that these supposedly laxative fruits might upset the experiments, but inspection of the weekly results showed that variations in their consumption made so little difference to the output of faeces or to the calcium balances, that it was decided not to curtail them. Some of the data upon which this conclusion was based are given in Table 3. The variations, if any,

TABLE 3. Effect of variations in the consumption of fresh fruit upon the weight of the faeces and upon the metabolism of calcium

Subject	Week	Fresh fruit g./day	Faeces (wet weight) g./day	Calcium absorbed mg./day	Calcium balance mg./day
R. M.	1*a*	29	255	240	+14
	1*c*	160	268	203	-10
E. B.	1*a*	10	179	265	+27
	1*c*	147	136	274	+60
E. W.	1*a*	41	63	94	-17
	1*c*	250	76	111	-20
K. B.	1*a*	44	124	126	-34
	1*c*	177	143	180	+20

produced by alterations in the intake of fruit are quite trifling when compared with the differences produced by a change from white to brown bread, or by other changes which have deliberately been made.

All food was weighed on Hanson spring balances, on which readings could be made quite accurately to a gram. The bread was baked daily according to specification by Mr and Mrs Wilkin of Barrington, Cambs. Each person had a weighed '1 lb.' loaf at the beginning of the day, and was expected to eat the whole of it during the 24 hr. The men usually required a further half loaf to make up their day's quota. The amount eaten by each subject was totted up at the end of the day and $\frac{1}{10}$th as much set aside from the same baking for analysis. All other foods except butter and jam were weighed at the table as they were served, and the result entered at once in a book kept for the purpose. Waste on plates, such as stones of fruit, was weighed, and its weight deducted from the first weighing. The weight of bread crumbs was disregarded, as it had been found by experiment to be negligible.

After each meal $\frac{1}{5}$th as much as each person had eaten was set aside in an aluminium bowl, which was kept closely covered and which was large enough to hold one week's aliquots for analysis. Foods such as raw apples and meat were cut up finely with stainless steel knives before the $\frac{1}{5}$th portions were weighed. Butter and jam were weighed out for each person at the beginning of every week, and any left uneaten at the end of the week was weighed. Then $\frac{1}{5}$th of the amounts eaten were added to the bowl of food.

This method of sampling may be very accurate provided certain precautions are taken in the preparation of the food. Briefly, the principle to be adopted is either to serve and weigh each ingredient of a meal separately, or to make a sufficiently intimate mixture of the various components, so that an average helping is likely to be a representative sample of the whole dish. Sauces should be served and weighed as such, and not poured over food. Pastry is better to be made and weighed apart from the pie to which it gastronomically belongs. Green vegetables must be cut up before they are served so that each person, and his duplicate portion, receive a uniform proportion of inside and outside leaves, since these contain very different amounts of calcium.

Only glass-distilled water or soft artesian-well water was used for cooking and only glass-distilled water for drinking. Tea and coffee were measured in beakers marked with two rings. The volume to the lower was half the volume to the upper, and the latter was about a cupful. Half the volume measured to be drunk was set aside at once for analysis. Distilled water was not duplicated for analysis. Each person's ration of milk was measured out daily with a pipette into his or her personal milk vessel, and at the same time $\frac{1}{10}$th of the quantity was set aside in a bottle containing toluene. The orders were that all milk measured out must be consumed, and also the washings of the vessel. R. M. received 100 c.c. of milk a day, the others 150 c.c.

The analytical arrangements

As already described, each experiment was divided into weekly periods. At the end of these, each person's 'wet' food, which had been collected in the aluminium bowl, was weighed and pulped up to a uniform paste in a large mortar. His bread, which had been kept separately in a large beaker, was dried at 100° C., and then weighed and ground to a powder. His preserved drinks, themselves half the originals, were measured and $\frac{1}{5}$th of them added to his milk which, as already stated, was $\frac{1}{10}$th the volume actually consumed. $\frac{1}{100}$th to $\frac{1}{200}$th of the volume of the fluid sustenances taken by each person was then measured into each of two silica crucibles (max. external diameter 6·7 cm.) and the contents dried at 100° C. Similar aliquots of the wet mixed food were added and dried, and lastly the appropriate aliquots of the dried ground bread, so that the crucibles finally contained the same fraction of the wet food, fluids and bread consumed by the person in question during the week. By the same procedure $\frac{1}{50}$th of the week's fluids, wet food, and bread were collected in a 1 lb. jam jar and dried for permanent preservation. There was always some frothing while these foods dried off, and the pots required a little attention. The crucibles were next placed in an electric furnace [Haynes & McCance, 1939] and the contents ashed at about 450° C.

The faeces of each subject were collected throughout each week in a counterpoised glass bowl covered, when not in use, by a dinner plate. The periods were marked by carmine, taken before breakfast on the first day of each week. If one can believe the literature, 0·1 g. of carmine has generally been used for this purpose, but experience in this laboratory suggests that this is not nearly enough, and about 0·7 g. has always been employed. This was given in five capsules, and a similar one added to the duplicate food bowl to be incorporated in the analysis. At the end of the week the wet faeces were weighed, distilled water was added (if necessary) and about $\frac{1}{20}$th to $\frac{1}{10}$th of their weight of concentrated Analar HCl. They were then made into a uniform paste by pulping with a glass rod flattened at the end. The paste was weighed, and usually left overnight. It was then reweighed as a check on the original weighing and possible evaporation, stirred up once more and two samples of 50 g. taken from it and dried in silica crucibles. A further portion of the paste was preserved in a Forster fruit jar in case of analytical accidents or uncertainties, and the counterpoised glass bowl washed out. After the crucibles had been kept for 16 hr. at 100° C., 5 c.c. of concentrated Analar HNO_3 were added (1 c.c. at a time) to the hot contents, and the crucibles returned to the oven for a few hours. Some frothing sometimes took place during these manipulations, but was usually quite easy to control by tapping the crucibles gently on a cork or on the bench. The contents of the crucibles were ashed at 450° C.

The weekly urines were collected under toluene and $\frac{1}{10}$th retained for analysis. 100–200 c.c. were taken for analysis and dried off in silica crucibles, to which 1 c.c. of concentrated Analar HNO_3 had been added. The ashing was carried out at 450° C.

The ashes of the foods and faeces were treated with 5 c.c. of concentrated Analar HCl, and taken down just to dryness on a sandbath. Then a further 5 c.c. of concentrated HCl were added, a watch glass placed over the crucible, and the contents heated to boiling over a small flame. The inside of the watch glass was washed into the crucible with 10 c.c. distilled water; the contents were again boiled, and filtered through a Whatman paper no. 41 into a 100 c.c. graduated flask. The crucible was washed with four more additions of 10 c.c. of water, the washings being boiled each time before they were filtered. Any residual carbon was then transferred to the filter paper and repeatedly washed with boiling distilled water until the total volume was nearly 100 c.c. When cold, the solution was made up to volume.

The urine ashes were treated with 5 c.c. of concentrated Analar HCl and the contents of the crucible boiled. 10 c.c. of water were added, the mixture boiled and the extract filtered into a graduated 50 c.c. flask. Four subsequent extractions were made with 5 c.c. of water each time as described above, and the volume finally made up to 50 c.c.

The calcium, magnesium and other bases were determined as described by McCance, Widdowson & Shackleton [1936]. Phosphorus was determined in the unashed urines by Briggs's method [1922]. This element cannot be determined in foods by applying Briggs's method to the HCl extracts of a dry ash [McCance & Shipp, 1933], but it has been found practicable to use this method for faeces. A measured amount, usually 4 c.c., of the ashed extracts was heated for an hour at 100° C. to convert any remaining pyro- to ortho-phosphate, and was then washed out to 100 c.c. Briggs's method was applied to an aliquot of this. The phosphorus in foods was determined as described by McCance *et al.* [1936], on 0·5 g. of the ground-up samples of the dried mixed food which had been set aside week by week.

Two samples of all foods, faeces and urines were taken in parallel to be ashed so that duplicate extracts of each were obtained. A single chemical determination was made on each extract, and repeated if the agreement was not perfectly satisfactory. The procedure described has given excellent duplicates for calcium, magnesium, iron and potassium in faeces, and the same may be said of urines, although these contain very little iron. The calcium, magnesium and potassium duplicates in food have also been excellent, but the iron duplicates have been less perfect, and this is thought to have been due to the difficulty of obtaining a uniform distribution of meat, which is rich in iron, through the mass of food which had to be sampled for analysis. The differences were certainly not analytical in origin and were unlikely to have been due to contaminations.

RESULTS

The composition and properties of 69 *and* 92 % *flours*

92 % flour is generally recognized to contain somewhat less starch and more moisture, protein, fat, fibre, vitamins and minerals than 69 % flour. The present flours conformed closely to type. The 'brown' contained

61·5% starch, 15% water, 2·45% nitrogen and 3·06% fat; the 'white' 67·5% starch, 13% water, 2·12% nitrogen and 1·37% fat. The iron in the 92% flour amounted to 3·55 mg./100 g. as against 1·41 mg. in the 69%. There was also more lithium, boron, manganese and cobalt in the 92% flour [Kent & McCance, 1941*a*, *b*]. The minerals of direct importance to the present investigation are calcium, magnesium, potassium, total and phytic acid phosphorus, and the amounts of these in the two flours are given in Table 4.

TABLE 4. The calcium, magnesium, potassium and phosphorus in 69 and 92% flours

Mineral element	69% flour mg./100 g.	92% flour mg./100 g.
Calcium	18·5	34·8
Magnesium	38·0	127
Potassium	147	376
Phosphorus (total)	83	287
Phosphorus (as phytic acid)	56	214

In baking the usual type of bread, water, salt and yeast are added to the flour. The quantity of yeast is so small that it may be neglected for the moment. The moisture in a brown loaf, however, amounts to about 43% and in a white loaf to 34%. Bread, therefore, has correspondingly lower concentrations of nitrogen, magnesium, phosphorus, etc., in it than the flour from which it was baked. It has, however, more sodium chloride, and it often has more calcium in it, due to the introduction of this element in the water or as an impurity in the commercial salt. Bread, moreover, contains much less phytic acid phosphorus than can be explained by the changes in moisture. The reduction is due to the enzymic destruction of phytic acid when the bread is set to rise, and does not take place to any extent in the baking of 'soda' bread. In this process the dough is not set to rise at a temperature conducive to enzyme action, but is placed in a hot oven as soon as it is made. The *p*H, moreover, of the reacting mixture is less favourable for the action of phytase [Widdowson, 1941].

Flours of high extraction have laxative properties. This effect, which is generally attributed to the fibre [McCance & Lawrence, 1929], has been demonstrated repeatedly [Williams, 1927–8; Rose, MacLeod, Vahlteich, Funnell & Newton, 1932; Cowgill & Anderson, 1932], and was discussed by McCance & Widdowson [1940]. In the present experiments the change from 69 to 92% flour doubled the wet and dry weights of the faeces. These bulky stools were not analysed for nitrogen, fat and cellulose residues, but they may be assumed to have contained more of them all than those passed on the white bread diets [McCance & Widdowson, 1940].

It was thought that some or all of these physiological and chemical changes might influence the bacterial flora in a uniform manner, and that this in turn might have an important effect upon the absorption of some of the mineral elements (possibly by altering the intestinal *p*H). Accordingly, samples of four of the subjects' faeces were taken for bacterial examination at the end of two white and two brown bread experiments. One pair of these experiments lasted 3 weeks and the other 4 weeks. The examination was very kindly carried out by Dr Crowley. Direct smears were made, and anaerobic cultures of standardized faecal dilutions were set up in various ways, and the colonies counted after 5 days' incubation. The results are given in Table 5, and Dr Crowley considers that the figures show that neither

TABLE 5. Faecal bacteria on white and brown bread diets

Subject ...	N. K.		R. M.		B. A.		E. W.	
Bread ...	White	Brown	White	Brown	White	Brown	White	Brown
% total flora present as:								
Coliform bacilli	6	2	9	14	4	6	2	3
Faecal streptococci	3	5	11	9	0	5	7	13
Lactobacilli	63	63	57	69	85	77	70	63
'Bacteroides'	27	27	19	8	10	12	12	21
Other organisms	1	2	4	0	1	0	9	0

diet produced any gross alteration in the relative frequency of the organisms, and it is improbable, therefore, that bacterial variation lay behind any of the definite chemical changes now to be described.

The absorption and excretion of minerals from white and brown bread dietaries

The absorption of calcium. Table 6 shows the absorption of calcium by all the subjects from white and brown bread dietaries at two different levels of intake. Here, and throughout this paper, the amount of any mineral absorbed has been taken to be the amount in the food minus the amount in the faeces. It will be noted that (*a*) the intakes tended to be a little higher on brown than on white bread, (*b*) the absorptions were always lower. Some of the subjects, E. B. for example, absorbed calcium freely, others, such as N. K. and R. M., only with difficulty, but the same change in experimental conditions affected them all in the same way. Hence it may be concluded with confidence that there is something about flour of 92% extraction which has a deleterious effect upon the absorption

TABLE 6. The absorption of calcium from white and brown bread diets

	Low intakes				High intakes			
	69% flour		92% flour		69% flour		92% flour	
Period of observation ...	21 days		21 days		28 days		28 days	
Time of year ...	July		August		Oct., Nov.		Sept., Oct.	
Subject	Ca intake mg./day	Absorption mg./day	Ca intake mg./day	Absorption mg./day	Ca intake mg./day	Absorption mg./day	Ca intake mg./day	Absorption mg./day
E. B.	500	307	530	169	1030	403	1330	317
N. K.	482	130	560	27	1300	279	1470	185
R. M.	720	181	676	20	1390	198	1490	118
P. S.	—	—	—	—	1330	390	1365	253
C. B.	557	178	630	64	—	—	—	—
B. A.	380	121	495	46	1030	178	1030	107
A. M.	416	127	516	74	1150	273	1190	219
E. W.	450	118	550	50	1075	206	1110	114
R. W.	—	—	—	—	885	128	1000	85
K. B.	457	133	520	70	—	—	—	—

of calcium from the human intestine. These experimental findings recall those of Burton [1929–30], who carried out metabolic studies on six children and two adults. Her object was to compare the effects of oatmeal and white wheat flour on the absorption and excretion of calcium, and her work was partly inspired by Mellanby's. Unfortunately, her experimental periods were exceedingly short, the diets did not contain very much of the cereal, and the intakes were not similar enough in the periods to be compared. Nevertheless, her results showed that oatmeal depressed the absorption and retention of calcium, and this was no doubt due to the same phenomenon with which Mellanby had been dealing and which underlies the present results.

The magnesium balances. Table 7 shows the magnesium balances of all the subjects on the two kinds of bread. The long periods of observation were due to the fact that changes in the calcium intakes could not be demonstrated to interfere with the magnesium metabolism, so that more experiments could be drawn upon for some of the magnesium data than for any one aspect of the calcium investigation. As may be seen, all the subjects reacted in the same way. They were 'in balance' on the white bread and remained so on the brown. Indeed, in spite of the greatly increased magnesium intake owing to the large quantities of this element in 92% flour, there was little absolute change in the amount absorbed or in the amount excreted in the urine. Both, however, formed a much smaller percentage of the intake when the basis of the diet was 92% wheatmeal. Before passing on from this table, there is one point which may be mentioned. It will be recalled that E. B. was the subject who

TABLE 7. The absorption and excretion of magnesium on white and brown bread diets

Subject	Bread	Period of observation days	Mg intake mg./day	Absorption mg./day	Absorption as % intake	Balance mg./day
E. B.	White	49	317	139	44	+ 3
	Brown	63	729	154	21	+ 2
N. K.	White	56	346	203	59	+ 9
	Brown	98	862	244	28	+ 8
R. M.	White	63	426	180	42	+ 2
	Brown	98	790	193	24	− 6
P. S.	White	42	378	172	46	+13
	Brown	63	719	158	22	− 3
C. B.	White	21	394	157	40	+14
	Brown	21	842	218	26	+42
B. A.	White	56	266	146	55	+12
	Brown	84	583	146	25	− 2
A. M.	White	49	271	131	48	+ 5
	Brown	84	660	174	26	+ 9
E. W.	White	63	318	128	40	+10
	Brown	105	650	145	22	+ 8
R. W.	White	28	213	89	42	−11
	Brown	63	510	115	23	− 6
K. B.	White	21	283	114	40	+16
	Brown	21	633	158	25	+ 2

absorbed calcium most readily, and that N. K. was the reverse. Table 7 shows that N. K. consistently absorbed magnesium better than E. B. This demonstrates very well the individual differences which may be found in mineral metabolism, and for which at present there seems to be no explanation.

The effect obtained when the magnesium intake was increased by adding magnesium carbonate to white bread is shown in Table 8. This

TABLE 8. The effect of adding magnesium carbonate to white bread

	White bread			White bread + $MgCO_3$			Brown bread		
Subject	Mg intake mg./day	Absorption mg./day	Urine mg./day	Mg intake mg./day	Absorption mg./day	Urine mg./day	Mg intake mg./day	Absorption mg./day	Urine mg./day
E. B.	310	127	131	590	239	176	568	127	133
B. A.	226	123	116	492	150	187	499	124	146
P. S.	391	169	156	678	237	210	670	171	149
R. W.	199	76	113	450	143	135	459	120	114

The periods of observation on which these results are based varied in length from 14 to 35 days.

gives the results of a small 14-day experiment on four of the subjects. It will be seen—and the results confirm those of Tibbetts & Aub [1937]—that in each subject the addition brought about an increased absorption and a corresponding increase in the urinary excretion. The percentage absorptions are not shown in the table, but E. B.'s did not alter, the others' fell, but not much. Table 8 also shows the absorptions and urinary excretions of these four subjects on brown bread diets at magnesium intakes selected so that they were comparable with those achieved when the white bread was fortified with magnesium carbonate. The absorptions were lower, and in three subjects they were almost the same as they had been on the unfortified white bread.

These results show that the magnesium in brown bread is not so well absorbed as inorganic magnesium added to white bread and probably less well than the magnesium in white bread itself. They do not, however, demonstrate as conclusively as the previous results did for calcium that there is something in the 92 % flour which inhibits the absorption of free magnesium ions. This is so because the 92 % flour itself introduced the extra magnesium into the diets, and it would be legitimate, therefore, to argue that all the magnesium therein was so encased by cellulose that it never went into solution in the stomach or gut, and so was never exposed to the chance of absorption. There was not the same ambiguity in the calcium results, for little of the calcium in the diet came from the breads, whether they were made from wheatmeal or from white flour. Further discussion will be deferred till more evidence has been brought forward.

The potassium balances. Table 9 shows some of the potassium balances on white and on brown bread diets. The subjects were essentially 'in balance' throughout, but it will be seen that the change from white to brown bread raised the intakes. As in the case of magnesium, this was due to the fact that the 92 % flour had much more potassium in it than the white flour. More potassium was absorbed, and in consequence more was excreted in the urine. The faecal excretions were also increased both

TABLE 9. The potassium balances on white and brown bread

			Excretion			
Subject	Bread	K intake mg./day	Urine mg./day	Faeces mg./day	Faeces % of intake	Balance mg./day
N. K.	White	3820	3030	728	19	+272
	Brown	6230	4760	1440	23	+ 30
C. B.	White	4640	3500	636	14	+504
	Brown	6430	4070	1790	28	+670
K. B.	White	3160	2830	585	18	−255
	Brown	4340	3000	1250	29	+ 90
E. W.	White	3980	3240	443	11	+297
	Brown	5350	4500	950	18	−100

Periods of observation: N. K. and E. W., 14 days; C. B. and K. B., 21 days.

absolutely and as a percentage of the intakes. These findings corroborate the preliminary data of McCance & Widdowson [1940], and recall the magnesium results which have just been discussed. Similar reasoning may be applied, but since it is improbable that wheatmeal contains any substance which could precipitate potassium ions from aqueous solution, in the same way that phytic acid precipitates calcium and magnesium, the increased amounts in the faeces may be attributed with more confidence to the indigestible nature of the branny particles, or to their laxative action. It is interesting in this connexion to mention that lithium in natural diets, and particularly these brown bread diets, was very poorly absorbed, whereas added lithium salts were quantitatively absorbed and excreted [Kent & McCance, 1941*a*].

The intakes and absorptions of phosphorus. Table 10 shows the intakes, absorptions, and the percentage absorptions of phosphorus from

TABLE 10. The absorption of phosphorus from white and brown bread dietaries

	White bread (periods of observation 21 days)			Brown bread (periods of observation 21 days)		
Subject	P intake mg./day	Absorption mg./day	Absorption % of intake	P intake mg./day	Absorption mg./day	Absorption % of intake
E. B.	1230	900	73	1970	1060	54
N. K.	1270	978	77	2400	1310	55
R. M.	1530	897	59	2320	1030	44
C. B.	1480	1020	69	2340	1250	53
B. A.	995	705	71	1850	920	50
A. M.	880	620	71	1660	880	53
E. W.	1120	744	67	2120	1170	55
K. B.	1140	760	67	1800	998	55

TABLE 11. The absorption of phosphorus from diets with and without added inorganic phosphate

	White bread				Brown bread			
	Low P intake 7-day periods		High P intake 14-day periods		Low P intake 14-day periods		High P intake 7-day periods	
Subject	Intake mg./day	Absorption % intake	Intake mg./day	Absorption % intake	Intake mg./day	Absorption % intake	Intake mg./day	Absorption % intake
E. B.	1130	69	1810	66	2360	50	2830	52
N. K.	1310	70	1730	72	2620	48	2910	56
R. M.	1400	55	1890	59	2490	41	2770	48
P. S.	1370	61	1910	66	2340	47	2860	56
B. A.	1080	63	1510	62	1760	42	2100	44
A. M.	1000	66	1410	66	1750	47	2040	55
E. W.	1270	60	1650	60	1980	46	2320	49
R. W.	990	57	1230	63	1680	46	2020	48

white and brown bread diets, and Table 11 the intakes and percentage absorptions from similar diets to which calcium carbonate or calcium monohydrogen phosphate had been added. The balances have not been given for they have shown no alterations, any increase or decrease in the amounts absorbed being followed by a corresponding change in the amount excreted in the urine.

It will be noted that all the subjects had larger absorptions of phosphorus on the brown bread diets than they had on the white, but that these represented a smaller percentage of the intakes. When inorganic phosphate was added to the diets, the subjects absorbed this as freely as the phosphorus in white bread and more so than the phosphorus in brown. This is shown in Table 11 by the fact that the percentage absorptions did not change when the additions were made to white bread, but all rose when the additions were made to brown. Evidently, therefore, much of the phosphorus in brown bread is less readily absorbed than added inorganic phosphorus, or than the phosphorus in white bread. These observations should probably be explained as follows: The phytic acid phosphorus in brown bread is partially broken down in the gut to inorganic phosphorus, which is then absorbed. This explains most of the increased absorption, but some is no doubt due to the fact that brown bread contains not only much more phytic acid but also a little more inorganic phosphorus than does white. The smaller percentage absorption is due to two causes, of which the relative unavailability of phytic acid phosphorus is the main one, and the laxative effect of brown bread a subsidiary one.

The role of phytic acid

The addition of sodium phytate to white bread. In the experiment now to be discussed eight people have eaten diets similar to those already described, but 40–50% of their calories were derived from white bread to which sodium phytate had been added. Thus it was possible to investigate the effect of phytic acid on the absorption of calcium, magnesium and phosphorus without the complicating laxative action of the brown bread. The sodium phytate was prepared and used as follows: 120 g. of commercial 'Phytin' (Messrs Ciba Ltd.) were dissolved in warm water with the addition of 10 c.c. of concentrated HCl. To this were added 48 g. of sodium oxalate dissolved in 100 c.c. of boiling water. The *p*H of the mixed solutions was adjusted to 5 by the addition of (about) 20 c.c. of *N*/10 NaOH. After allowing 2 hr. for the precipitate to settle, this latter was collected on a fluted paper and washed with hot water. The *p*H of the filtrate was next adjusted to 7 by the further addition of (about) 19 c.c. of *N*/10 NaOH. If a cloud formed, this was filtered off; if not, this step was omitted, and the solution made up to a total volume of 2000 c.c. After making up to volume, the solution was tested with ammonium oxalate to make sure that there was still a faint trace of calcium in the preparation, and hence that no oxalate was present. The yield of phytic acid phosphorus was almost quantitative and the solution contained 1·1 % phytic acid phosphorus.

This solution of sodium phytate was used for preparing the dough when the bread was being baked. 50 c.c. (0·55 g. of phytic acid phosphorus) were incorporated into each 1 lb. loaf, and the bread was baked with sodium bicarbonate and potassium hydrogen tartrate. This was done for reasons of economy because baking with yeast destroys part of the phytic acid [Widdowson, 1941]. The bread so prepared contained about 130 mg./100 g. of phytic acid phosphorus. This was rather more than the brown bread previously used, for which the corresponding figure was about 100 mg. The sodium phytate solution was also used in some of the puddings, cakes and pastries at the rate of 100 c.c. (1·1 g. of phytic acid phosphorus) to 1–1½ lb. of flour. Had the puddings and pastries not been so treated, the daily intake of phytic acid would have been roughly the same as it had been on the brown bread. As it was, it was somewhat higher.

When the decision was taken to bake the bread with baking powder instead of yeast it was not appreciated that citrates and tartrates had been shown to prevent the high-calcium low-phosphorus type of rickets in rats [Shohl, 1937; Hamilton & Dewar, 1937; Hathaway & Meyer, 1939; Day, 1940]. If their action were due to the unionized complexes which they may form with calcium salts, then presumably such acids might influence unfavourably the absorption of calcium from low-calcium high-phosphorus diets. There is, however, no evidence that they do any such thing [Bauman & Howard, 1912; Hess & Matzner, 1924; Chaney & Blunt, 1925; Weissenberg, 1924; Siwe, 1938; Shohl & Butler, 1939]. In fact, citrates seem to promote calcium absorption in man, and therefore they would probably minimize rather than exaggerate the bad effects of phytic acid about to be described. As a final precaution, however, the bread was baked for one week with an acid phosphate instead of tartrate, and no differences could be detected.

The effect of sodium phytate on the absorption of calcium and other bases. Table 12 shows how the absorptions and urinary excretions of calcium were changed when sodium phytate was added to white bread. All the subjects reacted in the same way. Their urinary calcium fell, their absorptions went down so much that a number of the subjects (notably N. K., R. M. and E. W.) actually showed quite large negative quantities in this column. This means that there was more calcium in their faeces

TABLE 12. The effect of sodium phytate on the absorption and excretion of calcium

	White bread				White bread + sodium phytate			
Subject	Period of observation days	Ca intake mg./day	Absorption mg./day	Urine mg./day	Period of observation days	Ca intake mg./day	Absorption mg./day	Urine mg./day
E. B.	21	500	307	254	21	453	107	155
N. K.	14	540	141	214	14	540	-111	128
R. M.	14	645	36	97	21	630	-105	46
P. S.	14	578	216	216	21	597	- 9	168
B. A.	21	510	63	109	21	436	- 11	57
R. W.	14	525	157	139	21	432	13	64
A. M.	21	416	127	122	21	518	6	86
E. W.	14	440	41	84	21	476	- 46	46

than there had been in their food, and it is explained by the fact that they must have failed to reabsorb much of the calcium in their intestinal secretions. It is obvious that if a person's faeces contain more calcium than his food he must be in negative balance. As a matter of fact all these subjects were in negative balance when their bread contained sodium phytate, for their urinary output was always greater than their alimentary absorption. The whole question of calcium balances will, however, be deferred for the present and discussed fully when calcium requirements are being considered.

The addition of sodium phytate to white bread, therefore, reproduced in an exaggerated degree the effect of brown bread on calcium metabolism. In these experiments, however, there were none of the laxative effects of brown bread. In fact, none were expected, for, in spite of some earlier confusion [Jordan, Hart & Patten, 1906; Mendel & Underhill, 1906; Williams, 1927–8], it now seems to be established that the laxative action of whole cereals is due to the indigestible nature of the branny particles and not to phytic acid [Cowgill & Anderson, 1932]. Hence the whole of the changes in the metabolism of calcium shown in Table 12 may be attributed to the chemical action of the added sodium phytate.

Table 13 shows how the metabolism of magnesium was altered by sodium phytate. As with calcium, the absorptions and the urinary excretions fell. Obviously sodium phytate was affecting magnesium metabolism in very much the same ways as calcium metabolism. (This additional evidence, if such were needed, indicates that the calcium effects were not due to the tartrate used in the baking powder.) The balances, however, were not made negative, and there was never any suggestion that the amount of magnesium in the faeces might exceed that in the food. Again, sodium phytate had reproduced the effects of brown bread.

TABLE 13. The effect of sodium phytate on the absorption and excretion of magnesium

	White bread				White bread + sodium phytate			
Subject	Mg intake mg./day	Absorption mg./day	Urine mg./day	Balance mg./day	Mg intake mg./day	Absorption mg./day	Urine mg./day	Balance mg./day
E. B.	310	127	132	− 5	308	86	90	− 4
N. K.	365	218	229	−11	439	129	125	+ 4
R. M.	447	214	182	+32	490	140	137	+ 3
P. S.	392	169	156	+13	419	92	100	− 8
B. A.	304	146	162	−16	296	116	97	+19
A. M.	333	153	156	+ 3	347	103	99	+ 4
E. W.	324	138	125	+13	350	83	82	+ 1
R. W.	229	103	103	∓ 0	251	83	72	+11

The periods of observation on white bread were in general longer than those in Table 12, those on white bread + sodium phytate were the same length.

In one way, however, sodium phytate did not mimic the effects of brown bread. Table 14 shows that the phytate had no inhibitory effect on the absorption of potassium. None was expected, but the negative result makes a satisfactory control. Moreover, it enhances the probability (*a*) that sodium phytate inhibits the absorption of calcium and magnesium by a specific chemical action, and (*b*) that the inhibitory effect of brown bread on the absorption of potassium is due to some less specific effect—probably to the indigestible nature of the bran.

TABLE 14. The effect of sodium phytate on the absorption of potassium

	White bread		White bread + sodium phytate	
Subject	K intake mg./day	Potassium in the faeces as % of intake	K intake mg./day	Potassium in the faeces as % of intake
E. B.	3700	16	4010	16
N. K.	3970	19	5010	17
R. M.	5550	23	5490	28
P. S.	4920	13	5380	16
B. A.	3080	15	3360	12
A. M.	3270	13	3740	18
E. W.	4160	13	4470	14
R. W.	2530	19	2460	17

The periods of observation varied from 14 to 35 days.

The metabolism of phytic acid phosphorus. Table 15 shows the intakes, absorptions and percentage absorptions of phosphorus from white bread and from white bread to which sodium phytate had been added. The subjects were in balance on both diets, so that the urinary excretions closely followed the absorptions, and have been omitted from the table. If it be assumed that the increased intake of phosphorus shown by all the subjects was due to the added sodium phytate, which is approximately true, it is possible to make an estimate of its absorption, and this has been

TABLE 15. The absorption of phytic acid phosphorus

Subject	White bread: Total P intake mg./day	White bread: Absorption mg./day	White bread: Absorption % of intake	White bread + sodium phytate: Total P intake mg./day	White bread + sodium phytate: Absorption mg./day	White bread + sodium phytate: Absorption % of intake	% absorption of P added as sodium phytate
E. B.	1230	900	73	1840	1150	62	41
N. K.	1270	978	77	2670	1700	64	51
R. M.	1390	870	63	2480	1350	55	44
P. S.	1290	870	67	2260	1300	57	44
B. A.	1000	750	71	1750	1130	65	56
A. M.	995	620	71	1790	1130	63	56
E. W.	1160	765	66	2010	1230	61	55

done in the last column of the table. The figures show quite clearly that the addition of sodium phytate was followed by an increased absorption of phosphorus. Some of the phytic acid phosphorus, therefore, was certainly broken down in the intestine, and became available. A comparison of columns 3, 6 and 7 suggests that the added phytic acid phosphorus was not so readily absorbed as the phosphorus in white bread, and the approximation may be made that 70 % of the phosphorus in the diets was absorbed, and 50 % of the phosphorus introduced as sodium phytate. In general these results uphold the view that the phosphorus in phytates is relatively unavailable [McCance & Widdowson, 1935], but they also show that phytates cannot be disregarded as a source of phosphorus. McCance & Widdowson found that, of the phytic acid phosphorus ingested by three adults, 36–63 % was excreted unchanged in the faeces. The present results provide evidence as to the fate of the remainder. The extent to which phytates are broken down and their phosphorus absorbed probably depends upon many things, but the intestinal flora and the amount of calcium in the diet [Lowe & Steenbock, 1936] are probably the two that matter most.

Putting the calcium, magnesium and phosphorus results together, it is interesting that sodium phytate should be able to prevent the absorption of so much calcium and magnesium, and yet itself be broken down freely enough to allow half of its phosphorus to be absorbed. This suggests that the two metals, and particularly the calcium, can only be absorbed high up in the intestine. Once past the level at which the metals can pass through the wall of the gut, the phytates may be destroyed and the phosphorus absorbed, leaving the metals to be excreted in the faeces.

Phytate equilibria in vitro. Harrison & Mellanby [1939] concluded their paper with a thoughtful discussion of the rachitogenic actions of commercial 'Phytin', sodium phytate, and oatmeal. They made the point that for dogs and humans the rachitogenic action of any compound or foodstuff depended upon the amount of phytic acid and the amount of calcium in it. A low calcium/phytic acid ratio was the real criterion of whether a substance would produce rickets. In their own words 'the degree of active interference with calcification produced by a given cereal will depend upon how much phytic acid and how little calcium it contains'.

Consideration of the present results and the known properties of phytic acid suggested that this was inherently sound reasoning, but that it did not go quite far enough. Phytic acid forms insoluble magnesium, zinc, iron and copper salts as well as an insoluble calcium salt, and insoluble mixed salts are also well known. The ferric salt is highly insoluble, even in $N/6$ HCl, and is used for the isolation and determination of phytic acid. The calcium salt is soluble at *p*H 2·8, but precipitation begins when the *p*H rises to 3·0. The magnesium salt is soluble up to about *p*H 5. These facts raise two questions. First, to what extent can magnesium and other metals in a natural diet saturate the phytic acid and so prevent it exerting its rachitogenic effect? Secondly, why does the presence of phytic acid in a diet not produce severe anaemia, or even a zinc deficiency? Experiments have been undertaken in vitro to try to answer these questions.

In practice there is not enough iron or zinc in a diet to influence the precipitation of calcium by phytic acid. There is, however, enough magnesium, for some of the foods which contain phytic acid also contain really large concentrations of magnesium, whole wheat being an excellent example. Further, the magnesium results which have just been given show that the metabolism of this element is affected by phytic acid.

Even if experiments in vitro are restricted to calcium, magnesium and phytic acid, matters are extremely complicated, for the number of variables is so great. The relative concentrations of calcium, magnesium, total base and phytic acid, and the *p*H, all affect the amount and the composition of the phytates which are precipitated. These variables have been investigated, although naturally not in every particular, but the presentation of all the results in quantitative tabular form is out of the question. Many of the experiments were carried out at *p*H 6·5, which may be assumed to be the approximate *p*H of the intestine. As a matter of fact, the truth of what follows would not be materially altered if the actual *p*H were 6 or 7. It has been found that at this *p*H the composition of the mixed phytates varies with the relative concentrations of the bases and the ratio of total base to phytic acid in the solution in which the precipitates are formed. If this solution contained twice as many equivalents of magnesium as calcium, and if the bases were in excess by three to one, the whole of the phytic acid was precipitated and equal equivalents of calcium and magnesium with it. As the phytic acid in such a solution was reduced, less total base was, of course, precipitated, but the ratio of calcium to magnesium in the precipitate tended to rise. As the phytic acid was increased, the whole of it continued to be precipitated fully saturated with base, so that when equal equivalents of total base and of phytic acid were present in the original solution, for practical purposes the precipitate contained them all, and the calcium/magnesium ratio in the precipitate was 1/2. As the phytic acid in the original solution was further increased, the organic phosphate continued to be precipitated fully saturated with base, but the quantity of the precipitate decreased, so that when the ratio of total base to phytic acid phosphorus in the original solution was 1/3 only 85 % of the calcium was precipitated and 58 % of the magnesium, and the calcium/magnesium ratio in the precipitate was 1/1·6.

At *p*H 6·5 no excess of magnesium ions likely to be achieved in practice will prevent small quantities of calcium being precipitated. When 11·65 equivalents of magnesium, 1·12 of calcium and 6 of phytic acid phosphorus were mixed together, 90 % of the calcium was precipitated. Yet calcium never completely displaces magnesium from precipitation, for when the equivalents of calcium were raised to 11·65 and the original amounts of magnesium and phosphorus retained, 23 % of the magnesium was still precipitated and the calcium/magnesium ratio in the precipitate was 1·8/1. This demonstrates the immobilizing action of phytic acid on small amounts of calcium, but it also shows that calcium will never be completely precipitated in the presence of abundant magnesium ions.

When the *p*H was reduced below 6·5 in the presence of a total base/phytic acid phosphorus ratio of 3/1, and a magnesium/calcium ratio of 2/1, the whole of the phosphorus was precipitated down to *p*H 6, and below this progressively less. At the same time the calcium/magnesium ratios in the precipitate tended to rise.

These rather complicated effects all go to show that there are two main clues to the composition of mixed phytates, and indeed to the general physiological behaviour of phytic acid. The first is that the magnesium salt is somewhat more soluble than the calcium salt at *p*H's between 6 and 7, and that it becomes more soluble still as the *p*H falls below 6. The second is that mass action among the reactants plays a very large part in determining the composition of the products. These two general principles are well illustrated by another experiment which has been carried out. Solid magnesium phytate was added to a large excess of calcium chloride at *p*H 6·5. The suspension was well shaken and left to stand for 24 hr. At the end of this time the precipitate was separated, washed and analysed, and was found, within the limits of experimental error, to consist of pure calcium phytate. Complete replacement had taken place. When, however, the converse experiment was performed, the precipitate analysed after 24 hr. contained 61 equivalents of calcium and 49 equivalents of magnesium per 100 equivalents of phytic acid phosphorus. Replacement of calcium by magnesium had taken place, but it had been incomplete.

One observation has been made in studying the mixed phytates which cannot be explained along these lines. When the ratio of total base to phytic acid phosphorus in the original solution was about 1, the equivalent weights of calcium plus magnesium in the precipitate were equal to those of phosphorus (assuming phytic acid phosphorus to be divalent). This is the expected composition of a saturated phytate. Yet when the bases were present in excess, the equivalent weights of calcium plus magnesium invariably exceeded those of phytic acid phosphorus, and the effect was progressive. When, for example, the original solution contained 23 equivalents of base and 6 of phytic acid phosphorus, the precipitate contained 8 equivalents of base and 6 of phosphorus. It is not easy to picture the way in which the excess of base is combined with the phytic acid. It can hardly be attached to OH groups in the benzene ring of the inositol, for these are occupied by phosphorus.

To make the conditions in vitro a little more realistic solutions of calcium, magnesium and sodium phytate were prepared and mixed in the proportions in which these reactants were present in one of E. W.'s brown bread diets and in B. A.'s white bread diet to which sodium phytate had been added. The precipitates which formed at *p*H 6·5 were analysed, and the results are shown in Table 16. Considering the brown

TABLE 16. The precipitation of phytates from artificial diets

Type of diet		Composition of diet, i.e. of original solution mg./day	Composition of diet, i.e. of original solution m. eq./day		Composition of precipitate m.eq./day	
Brown bread	Calcium	505	25·2	79·8	17·4	36·2
	Magnesium	655	54·6		18·8	
	Phytic acid P	480		31·0		30·4
White bread + sodium phytate	Calcium	478	23·9	53·0	23·2	49·4
	Magnesium	350	29·1		26·2	
	Phytic acid P	766		49·5		48·8

bread diet first, it will be seen that in it there was an excess of base of nearly 3 to 1 and an excess of magnesium over calcium of slightly more than 2 to 1. (In 100 g. of brown bread itself there are 6·45 m. eq. of phytic acid phosphorus, 6·9 m. eq. of magnesium and 1·8 m. eq. of calcium, so that the bases slightly exceed the acid, and magnesium is present in great excess.) 99 % of the phytic acid phosphorus was precipitated, and slightly more magnesium than calcium. The excess of base over acid in the precipitate will also be noted. This reproduction of actual conditions demonstrates rather well that, owing to the greater insolubility of the calcium salt, even a large excess of magnesium will not prevent most of the calcium being precipitated, so that on such diets phytic acid must be expected to depress the absorption of free calcium ions to a greater extent than it depresses the absorption of free magnesium ions. The practical difficulty of testing this lies in the fact that all the magnesium ions in 92 % flour may not be 'free'. Turning to the other diet, it will be seen that there was a bare excess of base over acid in the original solution, and only a slight excess of magnesium over calcium. There was, moreover, more phytic acid than in the brown bread diet. Almost the whole of this last was precipitated, and 97 % of the calcium. Owing to the excess of base being so small, there was also room on the precipitate for 90 % of the magnesium, and the whole of this came down, so that the precipitate contained more equivalents of magnesium than calcium. This excess, moreover, slight though it was, was greater than it had been on the brown bread precipitate, in spite of the fact that the latter had been formed in the presence of a much greater magnesium preponderance. It is in conditions such as these that phytic acid might be expected to exert its maximum effect in depressing the absorption of free magnesium and calcium ions, and the metabolism experiments bore this out.

The second question which has been raised, namely, what is the effect of phytic acid on the metabolism of iron and divalent metals other than calcium and magnesium, will be discussed on another occasion.

Cereal diets and calcium requirements

If the calcium in the faeces is subtracted from the calcium in the food, the remainder represents the amount 'absorbed and available for the body's use'. If this quantity is as great as the amount of calcium in the urine, the person is in 'balance', and his immediate needs for calcium are being met. The smallest absorption, therefore, which will maintain balance may be defined as the true or internal requirement. The magnitude of this figure depends only upon an individual's intermediary calcium metabolism, and is independent of the food. The smallest amount of calcium which has to be taken by mouth to maintain balance, i.e. to provide the true or internal requirement, is often spoken of as the minimum requirement. Its value depends partly upon the individual's internal requirement and partly upon his ability to absorb calcium, but fully as much upon the nature of his food. He will absorb calcium badly if his diet contains much bread made from 92% flour. All dietary requirements for calcium, therefore, must be defined in terms of the staple cereal. This has not been appreciated, and has never up till now been done. Table 17, however, shows how essential it is to do so. In

TABLE 17. Calcium balances on white and brown bread diets

	White bread		Brown bread	
Subject	Ca intake mg./day	Balance mg./day	Ca intake mg./day	Balance mg./day
E. B.	500	+ 54	530	− 10
P. S.	642	+ 1	550	− 97
N. K.	482	−118	560	−190
R. M.	720	− 43	676	−150
B. A.	380	− 9	495	− 74
C. B.	557	− 27	630	−111
K. B.	457	− 23	520	− 54
A. M.	416	+ 4	516	− 26
E. W.	450	− 4	550	− 73

Periods of observation 21 days.

considering these results it is best to disregard small positive and negative balances, for they may have been technical or fortuitous. The difficulty is to know what limits to put upon this range, but assuming that balances between + and −25 mg./day are not significantly positive or negative, then Table 17 shows that seven subjects were in satisfactory balance on the white bread diets. There was, therefore, enough calcium in this food. Only R. M. and N. K. were well outside the range of ∓25 mg./day, and these two clearly needed more calcium. In contrast, Table 17 shows that only one person remained in balance when 92% flour was substituted for the 69%. The intakes were higher in nearly every case, but they did not satisfy the subjects' requirements. E. B. was able to obtain enough calcium from these diets, and he must be regarded as exceptionally good at this, for his percentage absorptions were always very high as judged by others in this investigation, and by data culled from the literature.

To change a nation's dietary from white to brown bread, and at the same time to reduce their milk and cheese supply, would probably mean that nine adults out of ten would begin to lose calcium. No data are available for children, but it may be predicted that if such a change were made their storage of calcium would deteriorate. Rickets might increase in young children and growth become slower at all ages.

Vitamin D and calcium absorption

Mellanby [1925, 1934] has always stressed the great value of vitamin D in correcting the rachitogenic action of oatmeal diets in puppies, and in improving the calcium metabolism of children. Others have shared this view, and when the bad effects of 92% flour on the absorption and balance of calcium were first observed it was thought that vitamin D would probably put them right. Accordingly, the effect of vitamin D was investigated. Throughout this 3-week experiment and for 7 days before it, 2000 I.U. of vitamin D as calciferol were given daily to each subject. The drug, which was given to us by Messrs Glaxo Ltd., was made up in arachis oil and eaten at lunch-time on a crust of bread. 92% flour was used as the basal cereal because it was thought that on it vitamin D would have a chance to show what it could do. Special 14-day control periods were necessary for some of the subjects, whose calcium metabolism had changed since their original 3 weeks on brown bread 6 months before. The results are shown in Table 18, and will

TABLE 18. The effect of vitamin D on the calcium absorptions and balances

	Brown bread				Brown bread + 2000 I.U. calciferol/day			
Subject	Ca intake mg./day	Absorption mg./day	Urine mg./day	Balance mg./day	Ca intake mg./day	Absorption mg./day	Urine mg./day	Balance mg./day
E. B.	530	169	179	- 10	476	131	179	- 47
P. S.	550	23	120	- 97	540	57	111	- 54
N. K.	662	4	131	-127	610	31	127	- 96
R. M.	656	-97	27	-124	701	-53	29	- 82
B. A.	499	16	74	- 59	501	7	46	- 39
A. M.	516	74	100	- 26	607	69	99	- 30
R. W.	472	-33	86	-119	463	-73	64	-137
E. W.	518	-33	41	- 74	522	41	47	- 6

Periods of observations on brown bread alone 21 or 14 days and on vitamin D 21 days.

disappoint those who regard vitamin D as a universal restorative. Four of the subjects—E. B., B. A., A. M. and R. W.—absorbed less calcium under the influence of vitamin D, and E. B., A. M. and R. W. had worse balances. The others absorbed more and had rather less negative balances, but only E. W. showed any real betterment from D, and even her increased absorption was not enough to produce a positive balance. A study of the literature then showed that this was really the result to have been expected, taking into account the dosage of D and the age and health of the subjects. Our knowledge about the action of the vitamin in adults may be summarized as follows. Whatever the other effects of vitamin D there is no doubt that even small doses greatly improve the calcium absorptions of adults with osteomalacia. Chu, Liu, Yu, Hsu, Cheng & Chao [1940] found that even one egg a day made a difference to the calcium absorptions, and 500 I.U./day produced a large effect, which lasted for some weeks after the drug was stopped. Chu, Yu, Chang & Liu [1939] showed that sunlight and irradiation had good effects in quite moderate dosage, and there is an extensive literature on the beneficial effects of larger doses [Hannon, Liu, Chu, Wang, Chen & Chou, 1934; Liu, 1940; Liu, Hannon, Chu, Chen, Chou & Wang, 1935; Liu, Hannon, Chou, Chen, Chu & Wang, 1935; Liu, Su, Wang & Chang, 1937; Liu, Chu, Su, Yu & Cheng, 1940; Liu, Chu, Hsu, Chao & Chen, 1941]. In some of this work the periods have been too short and the subjects have not always been given enough time to come into equilibrium after a change of diet. (See particularly Liu, Hannon, Chu *et al.* [1935].) Nevertheless, the osteomalacia results as a whole are indisputable. Bauer & Marble [1932] showed that 5 mg. of irradiated ergosterol per day produced a very slight improvement in the calcium absorption of a patient with osteoporosis; 8 and 20 mg./day produced very definite effects, but these were enormous doses. Adams, Boothby & Snell [1935–6] claimed to get an improved absorption on giving 30 minims of viosterol per day to a patient with osteoporosis, but their results are not convincing. Most people have given large and variable doses of calcium as well as vitamin D [Meulengracht & Meyer, 1937], and their results are of therapeutic interest but of no value to the present discussion. On the other hand, all those workers who have administered physiological doses of vitamin D to normal adults have found that it did not alter the calcium absorptions, urinary excretions, or balances. Hunscher, Donelson, Erickson & Macy [1934] used 15 g. of cod-liver oil per day as the source of vitamin. Kroetz [1927] used quartz lamp irradiation, but he had only one subject (himself). Hart, Tourtellotte & Heyl [1928] are often quoted as having found that neither cod-liver oil nor irradiation affected the calcium balance of adults, but they had only one subject, and although the periods were long they unfortunately irradiated the subject in the first period and used the second as their control. The prolonged action of vitamin D makes their experiment of little value. Henderson & Kelly [1929–30*a*, *b*] and Kelly & Henderson [1929–30] used African natives as subjects, and gave each of them 15 c.c. of cod-liver oil per day. The experiments were well conceived, but the metabolic periods were too short. Nevertheless, their results seem definite enough. Buttner's [1939] experimental periods were long. The excretion of calcium by the kidney was quite unaffected by a dose of cod-liver oil containing 100 I.U. per day, but no other data were given. Johnson [1937] gave 3 c.c. of viosterol, which apparently contained 750 units of D, to patients with ileostomies. Some changes in the ileal discharges were noted, but no improvement in the absorption of calcium. Bauer, Marble & Claflin [1932] found that 5–20 mg. of irradiated ergosterol produced only slight and indefinite effects on the calcium metabolism of normal persons. They stated in their paper that 0·0001–0·00025 mg. of their preparation of ergosterol protected rats against rickets, so that they seem to have been giving 50,000–200,000 I.U. of the vitamin per day, doses which were quite outside physiological possibilities. When they raised the dose of irradiated ergosterol still further to 30 mg./day they noted that less calcium was excreted in the faeces and more in the urine, but that the balances remained unchanged. This dose was so colossal that the results have really no bearing on the present issue.

Although some have argued otherwise, our subjects must be regarded as having been normal. Even R. M. and N. K., who had been in negative calcium balance for some months, only lost 10 or 12 g. of calcium in the whole series of experiments, and these amounts could hardly be considered to have depleted their body stores to a serious extent.

The present results therefore confirm those of Kroetz [1927], Henderson & Kelly [1929–30*a*, *b*], Hunscher *et al.* [1934], and Buttner [1939]. Vitamin D, given in physiological doses, does not improve the calcium absorptions of normal adults. The results, of course, do not suggest that vitamin D has no action upon adults, or that adults do not require vitamin D. The vitamin's action on the absorption and excretion of calcium is the only one which has been, and possibly ever can be, studied directly in man. Many have thought this the only, or certainly the primary action of the vitamin [Harris, 1934; Nicolaysen, 1937 *a*, *b*, *c*], but some have always believed that the important action of D was on the internal metabolism of bone [Light, Miller & Frey, 1929; Brown & Shohl, 1930; Taylor & Weld, 1932; Schneider & Steenbock, 1939]. This opinion has been reiterated by Cohn & Greenberg [1939] and Morgareidge & Manly [1939], who have been working with radioactive phosphorus in rats. The present results have no bearing on this aspect of vitamin D's activity.

The fortification of bread with calcium

In an experimental study of rationing which was carried out by McCance & Widdowson [1940] in the first 6 months of the war, small amounts of precipitated chalk were added to the bread. This was done to prevent the intakes of calcium being curtailed by the experimental restrictions of milk, cheese and eggs. This fortification of the bread did not impair its palatability, but it was not possible to find out its effect upon calcium absorption, because metabolism experiments did not form part of that investigation. When, therefore, brown bread was found to depress the absorption of calcium, it was natural to try whether fortifying it with calcium carbonate would overcome this obvious defect. The situation became more complicated when the Government proposed to fortify white flour with a calcium salt, for the suggestion aroused noisy, if not perhaps very scientific, opposition. Some workers, with experience of calcium and phosphorus metabolism in rats, suggested that the proposal to use the carbonate was an unwise one. They argued that such an addition would merely defeat its own ends by raising the intake of calcium, but not of phosphorus, and by so doing make the calcium/phosphorus ratio in the diet less favourable to calcium absorption and to bone formation than before. If, therefore, the object of the Government's move was to improve the absorption and metabolism of calcium and phosphorus, then calcium mono-hydrogen phosphate was the salt to use. Accordingly, in September 1940, the programme of these experiments was extended to include a fortified white bread, and to compare the merits of the carbonate and phosphate.

There seemed to be three main questions to be answered:

(1) What were the relative advantages of calcium carbonate and calcium phosphate?

(2) Did the absorptions of calcium satisfy the requirements of the subjects when the bread was fortified?

(3) Should calcium be added to the nation's bread, and if so, how much?

Carbonate or phosphate? Table 19 shows the answer which was obtained to this question. The calcium salts were added to the flour so that 100 g. of bread contained 0·1 g. of added calcium. The results have been condensed by separating the four men from the four women and averaging each set of data. Since brown bread is known to inhibit the absorption of calcium it was necessary to see that the proportions of calcium and of flour in the diets did not vary enough to vitiate the

TABLE 19. The absorption of calcium from flours fortified with calcium carbonate and calcium monohydrogen phosphate

	Carbonate				Phosphate			
Type of flour and designation of subjects	Ca/flour ratio	Average intake of calcium mg./day	Average absorption mg./day	Average absorption % of intake	Ca/flour ratio	Average intake of calcium mg./day	Average absorption mg./day	Average absorption % of intake
69% flour:								
4 men	2·76	1250	340	26	2·82	1270	294	23
4 women	2·91	1040	189	18	2·87	1030	202	20
92% flour:								
4 men	2·93	1330	201	15	3·25	1430	202	14
4 women	3·26	1015	110	11	3·49	1110	142	13

Note. In calculating the calcium/flour ratio, the calcium was reckoned in milligrams and the flour in grams. The periods of observation varied from 14 to 28 days.

comparisons. The calcium/flour ratios, therefore, as well as the calcium intakes are given in the table. It will be seen that the absorption of calcium was equally good, whether the bread was fortified with the carbonate or the phosphate, so that there was nothing in this respect to choose between them. This being so, there are two good reasons for preferring the carbonate. The first is its abundance, of which no more need be said. The second is its action in the baking and cooking processes. In our experience the mono-hydrogen phosphate does not go so well into the bread. The phosphated white loaf has never been quite so appetizing as its unfortified counterpart, whereas the carbonated loaf has been, if anything, more so. Phosphated brown bread is similar in taste to unfortified bread but its appearance has betrayed it, for even when the salt has been added to the flour as a fine anhydrous powder, white specks have always been observed in the finished article, so that the adulteration has been easily detected by the naked eye. For puddings and pastries made from white flour, there is again a case for the carbonate. Phosphate added to brown flour used for puddings and pastries did not spoil the taste but the white specks were always visible. Carbonate seemed to improve the taste and certainly did not detract from the appearance.

Calcium absorptions on fortified bread. Table 20 gives the intakes, absorptions and balances of the subjects on white and brown breads which had been fortified so that 100 g. contained 0·1 g. of added calcium. The flour used for other cooking was also fortified, and to the same extent. The carbonate and phosphate periods have been combined in making up this table since the two salts seemed equally efficacious. If this table is compared with Tables 6 and 17 it will be seen how fortifying the bread altered the intakes, absorptions and balances.

TABLE 20. Calcium intakes, absorptions and balances on diets fortified with calcium salts

	White bread			Brown bread		
Subject	Ca intake mg./day	Absorption mg./day	Balance mg./day	Ca intake mg./day	Absorption mg./day	Balance mg./day
E. B.	1030	403	+ 49	1330	317	+20
N. K.	1300	279	+ 20	1470	185	−46
R. M.	1390	198	+ 14	1490	118	− 2
P. S.	1330	390	+ 14	1365	253	−68
B. A.	1030	178	+ 23	1030	107	−12
A. M.	1150	273	+120	1190	219	+58
E. W.	1075	206	+ 95	1110	114	+27
R. W.	885	128	− 28	1000	85	−48

Periods of observation on white bread 28 days. Periods of observation on brown bread 28 days (E. B., B. A., P. S., R. W.), 49 days (N. K., R. M., A. M., E. W.).

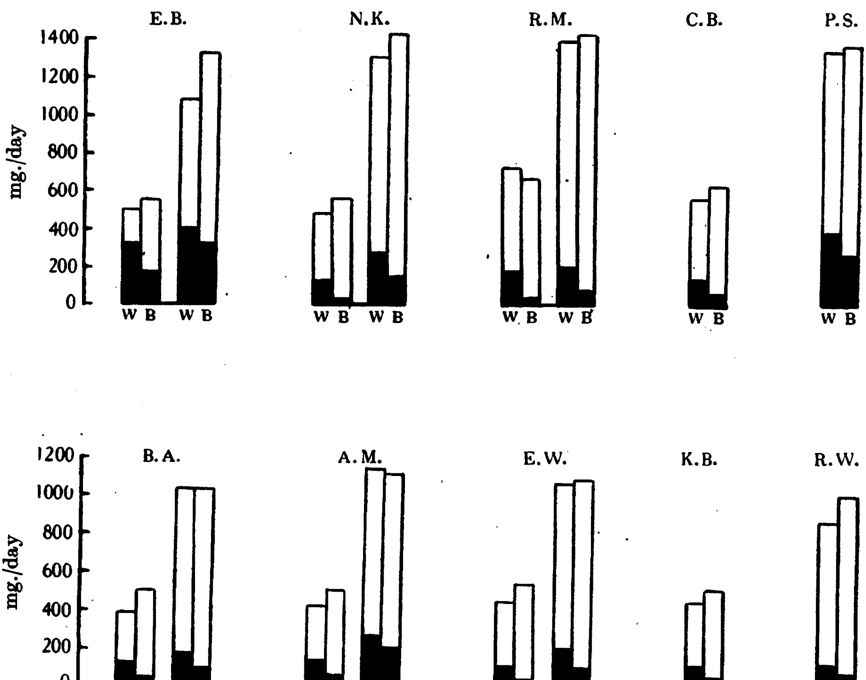

Fig. 1. The absorptions of calcium from unfortified and fortified white and brown bread. W = white bread. B = brown bread. The total heights of the columns represent the intakes, the heights of the solid portions, the absorptions.

Adopting the same convention as before, that persons gaining or losing less than 25 mg. calcium per day should be regarded as being in balance, the fortification of white bread can be said to have maintained all the subjects (even N. K.) in balance or positive balance, with the possible exception of R. W. Fortifying the brown bread did not quite suffice to keep N. K., P. S. and R. W. in balance. The contrast between Tables 17 and 20 in this respect is striking. Fig. 1 sets out graphically the intakes and absorptions on the white and brown breads, fortified and unfortified. The balances have had to be omitted for the sake of clarity, but the effects of fortification on the absorptions can now be seen at a glance. Two points stand out clearly. The first is that fortification always raised the absorptions and improved the balances if the latter required it. This is so interesting if one recalls that vitamin D failed to do so, and Henderson & Kelly [1929–30] must be given the credit of reaching the same conclusion on the basis of much less satisfactory experimental results. The second is that only a small proportion of the added calcium was absorbed, so that quite a bold addition must be made to effect any real improvement in a person's absorption. There appears, however, to be an interesting difference in this respect between white and brown breads. When white bread was fortified with calcium the percentage of the intake which was absorbed invariably fell. It is impossible to say why this should have been so, but there are several possible explanations, all rather speculative. When, however, brown bread was similarly fortified the percentage absorption rose in all subjects except E. B., who, as already mentioned, absorbed calcium so well from a low intake that he could scarcely have maintained the same percentage absorption when the intake was raised. By taking the differences between the intakes and absorptions on white and brown bread diets, it may be shown that the calcium added as an inorganic salt was equally well absorbed from brown and from white bread diets. The data upon which

TABLE 21. The percentage absorption of calcium from unfortified and fortified diets

	% of dietary calcium absorbed							
	White bread		Brown bread					
Subject	Un-fortified	Fortified	Un-fortified	Fortified	Calcium added to white bread diets mg./day	% of added calcium absorbed	Calcium added to brown bread diets mg./day	% of added calcium absorbed
E. B.	61	39	32	24	530	17	800	19
N. K.	27	21	5	13	818	18	910	17
R. M.	27	14	3	8	710	2	824	12
P. S.	40	29	4	19	688	19	815	28
B. A.	32	17	9	10	650	9	535	11
A. M.	31	24	15	18	734	20	675	22
E. W.	26	19	9	10	615	14	560	11

these statements are based are given in Table 21. Taken together, these results indicate that brown bread inhibits, as white bread does not, the absorption of small amounts of calcium. The inhibitory effect, however, is no longer to be observed after a certain level of intake has been reached. These facts are in keeping with the view that phytic acid is the inhibitory agent. This substance, potent though it may be, is limited in its action by its powers of chemical combination. Once these are exceeded, it ceases to exert any harmful effect, and the intestine deals with the excess as though there was no inhibitory agent present at all. It was for some such reason no doubt that Harrison & Mellanby [1939] found that commercial 'Phytin' cured rather than promoted rickets.

Phosphorus absorption from fortified flour. The surest way of producing rickets in rats is to feed them on a diet in which the ratio of calcium/phosphorus is very high—at least 3/1. The absolute amount of phosphorus in the diet need not be very low, but the rickets which results is of the low phosphorus type, and the accepted explanation is that the excess of calcium in the gut precipitates the phosphorus there, and prevents its absorption. There is a great deal of evidence in favour of this view, and it was an intimate experience of rickets in rats, rather than in man, which prompted the suggestion that calcium hydrogen phosphate and not calcium carbonate should be used to fortify the nation's bread. It has just been shown that calcium, a shortage of which is the main cause of human rickets, is absorbed equally well from either salt, so that it is unnecessary to fortify with the phosphate if the only object is to increase the absorption of calcium. It may readily be shown that fortification with the phosphate increases the absorption of phosphorus, and the data have already been given in Table 11. Table 22 shows the effect of fortifying the bread with calcium carbonate on the absorption of phosphorus. It will be seen that, whether the bread was white or brown, fortification invariably reduced the percentage of the phosphorus which was absorbed. This is unquestionably the same mechanism which produces rickets in rats, but it is no new observation in human metabolism. Schabad [1910] seems to have been the first to record it as the result of a balance experiment on a single healthy child. Orr, Holt, Wilkins & Boone [1924] made a similar observation on two babies, neither strictly normal, and Bowditch & Bosworth [1917] have also noted it. In the present experiments the absorption of phosphorus was only slightly reduced, and the reduction would never be of any dietetic importance to persons taking brown bread as the basis of their diets, for these contain so much phosphorus that anyone living on them would always have enough and to spare. The reduced absorption on the white bread diets requires a little more consideration, for they originally contain much less phosphorus. The present observations might be used as an

TABLE 22. The effect of calcium carbonate on phosphorus absorption

	White bread				Brown bread			
	Unfortified		Fortified		Unfortified		Fortified	
Subject	P intake mg./day	Absorption % of intake	P intake mg./day	Absorption % of intake	P intake mg./day	Absorption % of intake	P intake mg./day	Absorption % of intake
E. B.	1230	73	1130	69	1970	54	2360	50
N. K.	1270	77	1310	70	2400	55	2620	48
R. M.	1530	59	1400	55	2320	44	2490	41
P. S.	1290	67	1370	61	1650	48	2340	47
B. A.	995	71	1080	63	1850	50	1760	42
A. M.	880	71	1000	66	1660	53	1750	47
E. W.	1120	67	1270	60	2120	55	1980	46
R. W.	990	57	1230	50	1300	47	1680	46

argument against the use of white flour, but among the experimental subjects at least 50% of the dietary phosphorus was always absorbed, even from the fortified diets, and this should have provided for all the requirements of metabolism. Furthermore, in these experiments, 132 mg. of calcium were added to every 100 g. of white flour. Had the smaller quantity recommended by us and by the Medical Research Council [1941] (see below) been added, the effect on phosphorus absorption would have been smaller and probably quite insignificant.

IMPLICATIONS OF THIS WORK AT THE PRESENT TIME.

The question of whether calcium should be added to flour at the present time raises some rather difficult issues. They would appear to be: (*a*) Was the calcium intake of the population of this country deficient before the war? It is impossible to be dogmatic about this because so little is known about the calcium intakes of individual members of the community. There is also the uncertainty about what constitutes a person's calcium requirement—but see below. According to Orr [1937], Khan [1940], and others, many of the population were living below their optimum dietary requirements, although it must be admitted that there was little clinical evidence in adults of calcium deficiencies of an uncomplicated dietary type. Some people can only be convinced by evidence of this nature, but these people are apt to forget that it is not the adults that matter, but the children, and that they matter even before they are born. Judging by the published and unpublished work of this department, many adults and children, even of the middle class, were taking less calcium than the amounts which seem to be desirable. (*b*) Have the calcium intakes of the people of this country been reduced by the war, and are they likely to be further reduced? The richest sources of calcium in English diets are milk and cheese, but eggs and green vegetables are also important. The first of these has been restricted so far as adults are concerned, and the second and third have been severely rationed. Obviously the intakes of calcium have been reduced: the extent of their further reduction depends mainly upon the future supplies of milk and cheese. (*c*) What percentage of the whole wheat grain is to be used for human consumption? We have no official information about the food position, or the cereal reserves of Great Britain. There is evidently opposition in certain quarters to the introduction of a flour of high extraction, but the Government's hand may be forced—just as it was in the last war—and flours of high extraction may become the staple. It may still just be possible to make out a case for not adding calcium salts to white flour. Those afraid or unwilling to take the step can still shelter behind the consolation that the health of the people is not likely to deteriorate much below pre-war standards so long as the milk supply can be maintained. If, however, the public are to be invited to partake of flour containing 92% of the original wheat, then, in our opinion, the Government would be neglecting its duty if it failed to fortify these flours with calcium. (*d*) Can a moderate excess of calcium do any harm? The answer must surely be—certainly not. If the body were so constituted that it was unable to correct minor excesses in the food supplied to it from time to time, we should none of us be alive to-day. Furthermore, the calcium intakes of the English are far below those of other nations, such as the Finns, whose physique, longevity and health are quite as good as our own.

Reference must be made at this point to a thoughtful article by Nicholls & Nimalasuriya [1939], in which they put forward the view that the human being has such powers of adaptation to a change in his calcium intake that to worry unduly over such a matter is to make much ado about nothing. They point out that Ceylonese children often get no more than 0·2–0·4 g. of calcium per day. Yet they grow and look normal, and their bones resemble those of European children in structure and composition. The short stature of the Ceylonese seems to be the weak point in this argument. The authors themselves give the average female height to be less than 5 ft. and the male 5 ft. 4¾ in. Furthermore, Nicholls & Nimalasuriya state that the better class are taller and heavier than their poorer neighbours. The height of these children, therefore, is probably less than that to which they would grow if their calcium intakes and general nutrition were improved.

Turning now to the matter of how much calcium carbonate should be added to the bread, this clearly depends upon whether the present milk supplies can be maintained, upon how much of the nation's calorie intake will be derived from the staple cereal, and upon what the nature of that cereal is likely to be. Only those with the necessary inside information can make these forecasts. Assuming, however, that conditions will be similar to those created for the present experiment, viz. milk supplies reduced and wheat providing 40–50% of the total calories, then data derived from this study can be used as a guide. In point of fact the experimental conditions were not unlike what the national situation may yet become, so that this is probably as good a way as any other of assessing the amount of calcium to add. Accordingly, all the experimental data have been assembled and inspected, and the results are summarized in Table 23. The experimental periods upon which these figures are based

TABLE 23. The assessment of calcium requirements.

		White bread		Brown bread	
Subject	Absorption needed to maintain Ca balance mg./day	Intake by mouth needed to maintain balance mg./day	Intake by mouth needed to promote + balance mg./day	Intake by mouth needed to maintain balance mg./day	Intake by mouth needed to promote + balance mg./day
			Men		
E. B.	169	450	500	530	1420
N. K.	229	1250	1350	1600	>1700
R. M.	214	745	>1400	1500	1600
P. S.	260	644	1330	1530	>1600
C. B.	222	585	—	—	—
Average	218	734	>1140	1290	>1600
			Women		
B. A.	146	394	1030	1000	>1100
A. M.	129	415	1140	530	1220
E. W.	119	450	1070	785	1220
R. W.	157	526	> 872	1050	>1100
K. B.	136	500	—	572	—
Average	136	457	>1030	785	>1160

were not all of equal length, and a modicum of judgement has been exercised in their selection and presentation. Exceptional results, for example, have been excluded, and a certain amount of averaging has been done. The men and the women have been separated for the obvious reason that their requirements, both nett and gross, were consistently different. No record has been found of a similar observation, although such a finding has been foreshadowed by the tendency in recent years to express calcium requirements as so many mg. per kg. of body weight. It will be seen from the table that the men needed to absorb about 220 mg.

of calcium per day to maintain themselves 'in balance', the women only 136 mg. Naturally, the individuals differed somewhat among themselves, but there was no overlapping of the sexes. These were the subjects' true, i.e. internal, calcium requirements. A figure for the intake of calcium necessary to maintain balance on a white bread diet was obtained from five men and five women. The figures averaged 734 and 457 mg./day respectively, and these may be said to have been their minimum food requirements. Taking into account the differences in the diets and methods of assessment, these figures agree very well with those of Outhouse, Breiter, Rutherford, Dwight, Mills, & Armstrong [1941], and of Steggerda & Mitchell [1941]. Positive balances were obtained at the figures shown for three men and three women, but one man and one woman failed to retain calcium at their highest experimental intakes. The averages, therefore, for what may be termed these men's and women's luxury food requirements are lower than they ought to be. Assuming the working dietary requirement (or optimum dietary requirement) to be the mean of the figure which maintains the subjects in balance, and the one which promotes calcium storage, then the optimum calcium requirement on white bread diets was 947 mg./day for the men of the experimental party, and 744 mg./day for the women.

Turning to the brown bread diets, it is obvious that much higher intakes were required to maintain calcium equilibrium. The average figure for minimum food requirement was 1300 mg./day for the men and 785 mg./day for the women. Positive balances were obtained from so few of the subjects on the brown bread diets that it was impossible to form any estimate of the luxury requirements of the party by averaging the limited data. Working therefore from the minimum/luxury ratios for A. M. and E. W. on brown bread diets, and from the minimum/luxury ratios for the whole party on white bread diets, a figure of 2100 mg./day has been arrived at for the men's luxury requirement on brown bread diets and one of 1400 mg./day for the women's. Taking as before the optimum dietary requirement to lie midway between the minimum and the luxury requirement, then the optimum requirement on brown bread diets was about 1700 mg./day for the men and 1100 mg./day for the women.

Had 65 mg. of calcium been added to every 100 g. of white flour, and 200 mg. to every 100 g. of 92% flour, these requirements would have just about been met. To persons eating 1 lb. of white or brown bread per day, these additions of calcium would be no more than the amounts in 1/3 or 9/10 of a pint of milk respectively.

In all fairness it must be stated that this method of assessing requirements has been criticized. Nicholls & Nimalasuriya [1939] state: 'If a person is accustomed to a calcium intake of 0·6 g. daily his metabolic processes will become adapted to this amount.... If suddenly his intake is reduced to 0·3 g., his metabolism will not be adapted to this amount for some time, and consequently this intake will be followed by negative balances.... Provided the calcium balances of an individual are determined over a sufficiently long period of time, all that such experiments will reveal is the daily amount of calcium the individual is accustomed to take; and calculation from these of optimum requirements cannot be made.'

This criticism can be met from the present results themselves. The experimental diets contained less calcium than those to which the subjects had been accustomed. Yet their calcium balances were not uniformly negative at the beginning, nor did any of the subjects show any signs of adaptation over a period of 9 months. In fact some of their absorptions showed every sign of deterioration, not improvement, as the time passed and the low intakes were maintained.

There is another way of assessing how much more calcium should be added to 92 than to 69 % flour. Assuming that the breads are baked with yeast in the usual way, and the normal amount of phytate is destroyed in the process, 158 mg. of calcium would have to be added to every 100 g. of 92 % flour to precipitate all the phytic acid in it as the calcium salt. There is so little phytic acid in white bread that it may be considered for this rough calculation to contain none. 100 g. of brown bread therefore might require to contain 158 mg. more calcium than white before it became metabolically equivalent. This is in very fair agreement with the figure of 135 mg. derived from the balance experiments, for some of the phytic acid in the brown bread would certainly be precipitated as the magnesium salt, and hence the figure of 158 is somewhat too high.

When these experiments were planned the Ministry of Food had not yet given birth to the National Wheatmeal Loaf. It is obvious that if 69 % flour requires to be fortified in the interests of general health, 85 % flour must be fortified all the more. It stands midway between the 69 and 92 % flours so far as its phytic acid is concerned. Assuming that phytic acid is the main agent in all these flours which inhibits calcium absorption, and knowing that it is destroyed in baking bread with 85 % flour, as it is in baking with 69 and 92 % flours [Widdowson, 1941], then 120 mg. of calcium added to every 100 g. of 85 % flour should just about have met the optimum calcium requirements of the experimental subjects, had they been tried on such a diet.

It may be recommended, therefore, that 65 mg. of calcium be added to every 100 g. of 69 % flour to be used in this country during the war, and that 120 mg. should be added to 100 g. of the National 85 % flour. It may further be suggested that if it is at any time desirable to introduce a 92 % flour for general use, 200 mg. of calcium should be added to every 100 g.

In making these recommendations, it seems wise at the same time to sound a note of warning. The Ministry of Food thinks—and must think—in terms of national requirements. If the nation is short of calcium because its milk supplies have been curtailed, the simplest way of putting this right (from the Government's point of view) is to add calcium to bread—because everyone eats bread. But people will not change their dietary habits to please the Ministry of Food. The large milk consumer may—or may not—become the big bread eater when his favourite beverage is restricted. Hence, many who were well supplied with calcium may go short, and, vice versa, many who were taking very little may suddenly find themselves with more than they had before. It is clear, therefore, that although the addition of calcium to bread may maintain the nation's calcium intake at its previous level, there will be a great deal of watchful care needed on the part of those responsible for the well-being of individuals.

There is one further point to be mentioned. Unfortified brown bread depresses the absorption of calcium. The more one eats, the worse one's calcium balance is likely to become. If phytic acid is the main inhibitory agent, and just enough calcium has been added to inactivate it, the bread may be described as neutral, and the calcium balances should not be affected by the amount of bread consumed. If more than enough calcium has been added to precipitate all the phytate, the bread will supply free calcium available for absorption, and balances are likely to improve as consumption rises. White bread contains so little phytate that the unfortified material is probably fairly neutral, and any degree of fortification would probably make the bread a source of available calcium.

Summary

1. Balance experiments have been carried out over a period of 9 months on five healthy men and the same number of women.

2. The absorption and excretion of minerals have been studied when 40–50 % of the calories in these subjects' diets were provided by wheat

flours of the following types: 69% extraction; 92% extraction; 69% extraction fortified with calcium carbonate or mono-hydrogen phosphate; 92% extraction fortified with the same salts; 69% extraction with the addition of sodium phytate; 92% extraction with a supplement of 2000 I.U. of calciferol per day.

3. The following conclusions have been reached:

(*a*) The calcium, magnesium, phosphorus and potassium in diets made up with 92% flour were less completely absorbed than the same minerals in diets made up with 69% flour. Hence in defining calcium requirements it is essential to state the nature of the cereal in the diets.

(*b*) Sodium phytate added to 69% flour depressed the absorption of calcium and magnesium, but not of potassium. About 50% of the phosphorus in sodium phytate was absorbed.

(*c*) Vitamin D did not materially improve the absorption of calcium from diets made up with 92% flour.

(*d*) Fortifying the bread with calcium salts improved the absorptions of calcium, and prevented a loss of calcium from the body if this had been taking place. The carbonate and phosphate were equally efficacious. The addition of calcium carbonate slightly depressed the absorption of phosphorus.

(*e*) It has been recommended that flours for national use during the present emergency should have calcium carbonate added to them in the following proportions: white flour, 65 mg. of calcium per 100 g.; National 85% wheatmeal, 120 mg. of calcium per 100 g.; 92% wheatmeal, 200 mg. of calcium per 100 g.

The present work really represents the united efforts of the ten subjects, B. A., C. B., K. B., E. B., N. K., A. M., R. M., P. S., E. W. and R. W. The authors have merely had the pleasant task of committing the results to paper. The Medical Research Council made the investigation possible by their financial help.

REFERENCES

Adams, M., Boothby, W. M. & Snell, A. M. [1935–6]. *Amer. J. Physiol.* **114**, 383.
Bauer, W. & Marble, A. [1932]. *J. clin. Invest.* **11**, 21.
Bauer, W., Marble, A. & Claflin, D. [1932]. *J. clin. Invest.* **11**, 1.
Baumann, L. & Howard, C. P. [1912]. *Arch. intern. Med.* **9**, 665.
Bowditch, H. J. & Bosworth, A. W. [1917]. *Boston med. surg. J.* **177**, 864.
Briggs, A. P. [1922]. *J. biol. Chem.* **53**, 13.
Brown, H. B. & Shohl, A. T. [1930]. *J. biol. Chem.* **86**, 245.
Burton, H. B. [1929–30]. *J. biol. Chem.* **85**, 405.
Buttner, E. E. [1939]. *S. Afr. Med. J.* **13**, 251.
Chaney, M. S. & Blunt, K. [1925]. *J. biol. Chem.* **66**, 829.

Chu, H. I., Yu, T. F., Chang, K. P. & Liu, W. T. [1939]. *Chin. med. J.* **55**, 93.
Chu, H. I., Liu, S. H., Yu, T. F., Hsu, H. C., Cheng, T. Y. & Chao, H. C. [1940]. *J. clin. Invest.* **19**, 349.
Cohn, W. E. & Greenberg, D. M. [1939]. *J. biol. Chem.* **128**, xvi.
Cowgill, G. R. & Anderson, W. E. [1932]. *J. Amer. med. Ass.* **98**, 1866.
Day, H. G. [1940]. *J. Nutrit.* **20**, 157.
Editorial Article [1941]. *Nature, Lond.*, **147**, 225.
Fairbanks, B. W. & Mitchell, H. H. [1938]. *J. Nutrit.* **16**, 79.
Hamilton, B. & Dewar, M. M. [1937]. *Amer. J. Dis. Child.* **54**, 548.
Hannon, R. R., Liu, S. H., Chu, H. I., Wang, S. H., Chen, K. C. & Chou, S. K. [1934]. *Chin. med. J.* **48**, 623.
Harris, L. J. [1934]. *Ann. Rev. Biochem.* **3**, 282.
Harrison, D. C. & Mellanby, E. [1939]. *Biochem. J.* **33**, 1660.
Hart, M. C., Tourtellotte, D. & Heyl, F. W. [1928]. *J. biol. Chem.* **76**, 143.
Hathaway, M. L. & Meyer, F. L. [1939]. *J. Nutrit.* **17**, 419.
Haynes, A. & McCance, R. A. [1939]. *Analyst*, **64**, 584.
Henderson, J. M. & Kelly, F. C. [1929–30*a*]. *J. Hyg., Camb.*, **29**, 429.
Henderson, J. M. & Kelly, F. C. [1929–30*b*]. *J. Hyg., Camb.*, **29**, 439.
Hess, A. F. & Matzner, M. J. [1924]. *J. Amer. med. Ass.* **82**, 1604.
Hunscher, H. A., Donelson, E., Erickson, B. N. & Macy, I. G. [1934]. *J. Nutrit.* **8**, 341.
Johnson, R. M. [1937]. *J. clin. Invest.* **16**, 223.
Jordan, W. H., Hart, E. B. & Patten, A. J. [1906]. *Amer. J. Physiol.* **16**, 268.
Kelly, F. C. & Henderson, J. M. [1929–30]. *J. Hyg., Camb.*, **29**, 418.
Kent, N. L. & McCance, R. A. [1941*a*]. *Biochem. J.* **35**, 837.
Kent, N. L. & McCance, R. A. [1941*b*]. *Biochem. J.* **35**, 877.
Khan, N. U. [1940]. *A Study of the Diet and Social Conditions of the Working Class Families in Newcastle-upon-Tyne.* Newcastle-upon-Tyne: Crane, Mann and Son.
Kohman, E. F. [1939]. *J. Nutrit.* **18**, 233.
Kroetz, C. [1927]. *Arch. exp. Path. Pharmak.* **120**, 351.
Light, R. F., Miller, G. & Frey, C. N. [1929]. *J. biol. Chem.* **84**, 487.
Liu, S. H. [1940]. *Chin. med. J.* **57**, 101.
Liu, S. H., Chu, H. I., Hsu, H. C., Chao, H. C. & Chen, S. H. [1941]. *J. clin. Invest.* **20**, 255.
Liu, S. H., Hannon, R. R., Chu, H. I., Chen, K. C., Chou, S. K. & Wang, S. H. [1935]. *Chin. med. J.* **49**, 1.
Liu, S. H., Hannon, R. R., Chou, S. K., Chen, K. C., Chu, H. I. & Wang, S. H. [1935]. *Chin. J. Physiol.* **9**, 101.
Liu, S. H., Su, C. C., Wang, C. W. & Chang, K. P. [1937]. *Chin. J. Physiol.* **11**, 271.
Liu, S. H., Chu, H. I., Su, C. C., Yu, T. F. & Cheng, T. Y. [1940]. *J. clin. Invest.* **19**, 327.
Logan, M. A. [1940]. *Physiol. Rev.* **20**, 522.
Lowe, J. T. & Steenbock, H. [1936]. *Biochem. J.* **30**, 1991.
McCance, R. A. & Lawrence, R. D. [1929]. *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 135.
McCance, R. A. & Shipp, H. L. [1933]. *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 187.
McCance, R. A. & Widdowson, E. M. [1935]. *Biochem. J.* **29**, 2694.
McCance, R. A. & Widdowson, E. M. [1940]. *Spec. Rep. Ser. med. Res. Coun., Lond.* (In the Press, but publication deferred for political reasons.)
McCance, R. A., Widdowson, E. M. & Shackleton, L. R. B. [1936]. *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 213.
McLaughlin, L. [1927]. *J. biol. Chem.* **74**, 455.
Medical Research Council [1941]. *Lancet*, **1**, 703.
Mellanby, E. [1925]. *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 93.
Mellanby, E. [1934]. *Nutrition and Disease.* London: Oliver and Boyd.

Mendel, L. B. & Underhill, F. P. [1906]. *Amer. J. Physiol.* **17**, 75.
Meulengracht, E. & Meyer, A. R. [1937]. *Acta Med. Scand.* **92**, 584.
Morgareidge, K. & Manly, M. L. [1939]. *J. Nutrit.* **18**, 411.
Nicholls, L. & Nimalasuriya, A. [1939]. *J. Nutrit.* **18**, 563.
Nicolaysen, R. [1937*a*]. *Biochem. J.* **31**, 107.
Nicolaysen, R. [1937*b*]. *Biochem. J.* **31**, 122.
Nicolaysen, R. [1937*c*]. *Biochem. J.* **31**, 323.
Orr, J. B. [1937]. *Food, Health and Income*, 2nd ed. London: Macmillan.
Orr, W. J., Holt, L. E., Wilkins, L. & Boone, F. H. [1924]. *Amer. J. Dis. Child.* **28**, 574.
Outhouse, J., Breiter, H., Rutherford, E., Dwight, J., Mills, R. & Armstrong. W. [1941]. *J. Nutrit.* **21**, 565.
Rose, M. S., MacLeod, G., Vahlteich, E. M., Funnell, E. H. & Newton, C. L. [1932]. *J. Amer. diet. Ass.* **8**, 133.
Schabad, J. A. [1910]. *Arch. Kinderheilk.* **54**, 83.
Schneider, H. & Steenbock, H. [1939]. *J. biol. Chem.* **128**, 159.
Shohl, A. T. [1937]. *J. Nutrit.* **14**, 69.
Shohl, A. T. & Butler, A. M. [1939]. *New Engl. J. Med.* **220**, 515.
Siwe, S. [1938]. *Scand. Arch. Physiol.* **80**, 385.
Steggerda, F. R. & Mitchell, H. H. [1941]. *J. Nutrit.* **21**, 577.
Taylor, N. B. & Weld, C. B. [1932]. *Brit. J. exp. Path.* **13**, 109.
Tibbetts, D. M. & Aub, J. C. [1937]. *J. clin. Invest.* **16**, 491.
Weissenberg, H. [1924]. *Arch. Kinderheilk.* **84**, 200.
Widdowson, E. M. [1941]. *Nature, Lond.*, **148**, 219.
Williams, G. A. [1927–8]. *Amer. J. Physiol.* **83**, 1.