

STUDIES ON THE NUTRITIVE VALUE
OF SOUTH AFRICAN STANDARD WAR BREAD
(MADE FROM 95 - 100 PER CENT EXTRACTION MEAL),
WITH PARTICULAR REFERENCE
TO THE METABOLISM OF CALCIUM BY HUMAN ADULTS.

THESIS
FOR THE DEGREE OF PH.D.

by

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INTRODUCTION.

INTRODUCTION.

THE IMPORTANCE OF BREAD.

Bread is the staple foodstuff of most of the civilized nations of the west, of the Americas, and of the British Dominions. From bread is obtained a large proportion of the daily requirement of carbohydrate (and hence calories); a fair proportion of protein, and of minerals (more especially phosphorus and iron, to a much lesser extent magnesium and calcium); it is also an important source of vitamins B Complex and E. In dealing with this foodstuff, it must be kept in mind that it fills a fundamental rôle in the nutrition of many millions of people; therefore the problem of its composition and nutritive value cannot be approached in a detached or academic manner.

HISTORICAL

To assist in the understanding of the problem to be investigated, a very brief historical outline will be given of the types of bread consumed during the last few decades. Although, for the sake of simplicity, the changes that have taken place in Great Britain only will be described, much the same pattern of changes has occurred in many other countries.

Prior to 1870, wheat was milled entirely in stone-grinding mills. The "bolted" flour from which the bread was made, had an extraction rate of 75 to 80 per cent (1). The light brown bread made from this flour (often admixed with rye) was consumed in large amounts, especially by the poorer classes: it is recorded that in 1840, the average daily consumption of this foodstuff amounted to 21 ozs. per head (2). From 1870-80 and onwards to the time of the Great War, the introduction of roller mills led to the production of refined white bread, made from straight-run endosperm flour of about 70 to 73 per cent extraction rate. This step was promoted by the rapid/...

rapid growth of urban centres which occurred at that period, and caused the need of a bread flour having better keeping qualities than the stone-ground flour. The change was further facilitated by the millers; for in seeking popularity for their products, they vied with each other in attempts at still further refinement. Thus, white bread ultimately became the staple.

THE INFLUENCE OF WAR ON THE COMPOSITION OF THE STAPLE BREAD.

A nation which in peace time has to supplement its wheat supply by importation, will obviously in war time be beset with the problem of transport. To meet this difficulty, such a nation will be prompted to eke out its wheat supply by incorporating more and more of the bran fraction into the bread, i.e. by raising the extraction rate of the flour. But the urge to obtain the maximum number of loaves is in practice countered by the fact that the bran fraction, to be diverted to human consumption, is normally fed as wheat offals to cattle and poultry. The deprivation of this feeding stuff to these livestock will, in the absence of alternative cereals, adversely affect the nation's supply of dairy produce. The government concerned must therefore strike a balance between making the wheat supply go as far as possible, and conserving and maintaining its supply of dairy produce.

During the Great War, toward the end of 1916, in Great Britain the extraction rate of the bread flour was raised progressively until by March 1918, when the wheat supply became very short, it had reached 92 per cent. Later, it fell to 87 per cent, and remained around that level for the rest of the period of control (1). But once restrictions were removed, the demand for white bread was strongly re-asserted. In the period between the two wars, at least 90 per cent of the bread consumed was of this type (3). The changes that occurred during the last war will be discussed later, but with certain limitations,

they/...

they followed the same course.

The foregoing may be summarized by stating that within the period of the last two to three generations, a dramatic change has occurred in regard to the type and amount of bread consumed. Formerly, bread made from stone-ground high extraction flour was the staple foodstuff, eaten and enjoyed in large amounts: nowadays that loaf has been almost entirely superceded by the refined white loaf, consumed however in much smaller amounts (4). Bearing in mind the fundamental rôle of bread in the diet of so many nations, such a change is of immediate concern to the nutritionist; for the question at once arises, how do the two types of loaf compare in nutritive value?

NUTRITIONAL CONSIDERATIONS

In a recent comprehensive review by Lepkovsky (5) entitled "The bread problem in war and peace", the nutritional aspects of this question are extensively discussed. In this thesis therefore, only the aspects relevant to the investigation to be carried out will be mentioned.

TABLE NO. 1.

ANALYTICAL DATA OF PRODUCTS USED FOR BREADMAKING (6).
(Figures expressed as mg. per 100g. fresh product).

<u>Type of Product</u>	<u>Calcium</u>	<u>Total Phosphorus</u>	<u>Phytic Acid Phosphorus.</u>
Wholemeal. 100 per cent extraction	36	343	246
National wheatmeal. 85 per cent extraction	27	203	123
White flour. 70 to 73 per cent extraction	15	101	35

It will be observed that the higher extraction meals contain more calcium and phosphorus than does white flour; according to the bare chemical figures, the former are nutritionally preferable. But such data do not of course provide an indication of the relative availability for metabolism of these elements, and it is with this aspect that this thesis is primarily concerned.

THE RÔLE OF PHYTIC ACID PHOSPHORUS.

It has been known for some time that a large part of the organic phosphorus of cereals, and of seeds in general, occurs in the form of phytic acid, inositol-hexaphosphoric acid. This acid is present, partly in the free state, and partly in combination with various bases (7). With certain bases of nutritional importance, e.g. calcium, magnesium, and iron, it forms salts which are almost insoluble at the range of pH values found in the digestive tract. In experiments on 4 adult humans, McCance and Widdowson (8) found that 36 to 63 per cent of the phytic acid phosphorus ingested (consumed in the form commercial "Phytin", also from Hovis bread) remained unchanged in the faeces. It was therefore feared that the utilization of the above bases would be seriously diminished should phytic acid phosphorus be present in the diet to any appreciable extent. At the same time, however, these workers showed that in pre-war Great Britain, this substance constituted less than 5 per cent of the total phosphorus content of the middle class diet; hence its ingestion in such small amounts was of little nutritional importance. But in war time, as will be appreciated from earlier considerations, the situation may become quite different; for nations with a restricted wheat supply may be forced to incorporate a portion of the bran fraction into their bread flour. Since the phytic acid phosphorus in cereals is largely located in this fraction, the more bran included, the greater will be the phytic acid content of the bread flour.

Early in the recent war, the probability of Great Britain having to introduce a higher extraction meal for breadmaking stimulated interest in the phytic acid phosphorus problem. In the first place, Widdowson (9) drew attention to the fact that the matter would be much more serious were it not that a certain amount of phytic acid phosphorus is destroyed during the baking of bread, this being accomplished

through/...

through the agency of an enzyme phytase present in the wheat meal. Notwithstanding this phenomenon, the phytic acid phosphorus content of a diet including high extraction bread is very much higher than when the diet includes white bread.

Early work by Mellanby (10, 11) and others, indicated that with animals, the adverse effects resulting from the ingestion of a large amount of phytic acid phosphorus could be nullified by increasing the calcium content of the diet. But the very fact of raising the extraction rate of the bread meal may well cause a decrease to take place in the quantity of wheat feeds available for dairy cows, thus leading to a decrease in the production of milk and cheese, both very rich sources of calcium. There would be considerable difficulty therefore in attempting to offset any adverse effects ascribed to a large ingestion of phytic acid phosphorus by raising the calcium content of the diet by dietetic means - indeed with many countries, in war time, it would be impossible.

ONE SOLUTION OF THE PROBLEM - THE ADDITION OF CALCIUM CARBONATE TO HIGHER EXTRACTION BREAD MEAL.

McCance and Widdowson (12) from the results of balance experiments carried out on adult humans, concluded that the consumption of a large amount of bread made from 92 per cent extraction meal (involving the ingestion of a high intake of phytic acid phosphorus), reduced the amount of calcium absorbed, especially when the intake of this element was low. (These experiments, and the manner in which these workers interpreted their results are dealt with later). Krebs and Mellanby (13), repeated certain of these experiments using National Wheatmeal bread (made from 85 per cent extraction meal), and arrived at the same conclusion. The suggestion made by these groups of workers that higher extraction bread meal should be fortified by the addition of calcium carbonate, was ultimately adopted by the Medical Research Council (6), who recommended that 14 ozs. creta preparata should be added to each 280 lbs. sack

of National Wheatmeal. In May 1942, when the shipping position became acute, white flour and bread were completely withdrawn from sale; National Wheatmeal bread became the staple, and the British Government decided that creta preparata should be added to the bread meal, but at the rate of 7 ozs. per 280 lbs. sack, i.e. half the amount recommended by the Medical Research Council (14).

THE SITUATION IN SOUTH AFRICA.

South Africa, in regard to wheat production, is an intermediate country, for although the bulk of the cereal is locally grown, a certain amount always has to be imported. In 1940-41, due to the war situation, considerable difficulty was experienced (and more was anticipated) in obtaining the wheat requirement. To meet this difficulty, in May 1941, the Government introduced a standard war bread made from 95 to 100 per cent extraction meal (previously designated as No. 2 unsifted meal); the baking of all other types of bread was forbidden. Since alarm had been expressed overseas upon the adverse effects on mineral salt metabolism, likely to follow the adoption of bread of this character, nutritionists in South Africa had ample grounds for apprehension. But while there was some similarity to the situation in Great Britain, there were, however, many important points of difference.

1. In South Africa, unlike Great Britain, the change over was from a white loaf to a loaf, not of intermediate extraction, but made from almost wholegrain meal: in addition, as far as may be gathered from the Family Budget Enquiry Report of 1936 (15), the average European consumption of bread and of flour was considered to be appreciably higher in South Africa than it was in Great Britain. Hence, in the

first/...

first instance, the adverse effects in relation to calcium metabolism described by the British workers might be expected to be displayed to an enhanced degree in South Africa.

2. Early determinations by Hammersma (16) of the phytic acid phosphorus content of the standard war bread gave an average value of about 50 mg. per 100 g. fresh bread; this figure stands in marked contrast to the "about 100 mg." obtained by McCance and Widdowson (12) for the British 92 per cent extraction loaf; and indeed is not much more than the average figure of 38 mg., found by Pringle and Moran (17) for the 85 per cent British National Wheatmeal loaf. Hence the adverse effects associated with the consumption of the South African standard bread will be far less marked than the data in paragraph (1) would lead one to imagine.

3. In South Africa there are several differences in climatic conditions in comparison to overseas; for example in regard to the altitude, rainfall, humidity, and more particularly, the period of daily sunlight experienced. To illustrate this difference in sunlight, - perhaps the most influential of these factors, it need only be pointed out that in Johannesburg, the average daily period of sunshine throughout the year is about 8.8 hours; in London, the corresponding period is about 3.9 hours. The influence of this extra sunshine (and hence vitamin D) on the metabolism of calcium and phosphorus may well be profound. (This aspect will be considered in detail in the Discussion).

4. In South Africa, although there was a shortage of wheat, there was, unlike Great Britain, a fairly large amount of maize available: hence the diversion of bran from animal feeds to human consumption, did not lead to a falling off in the production of dairy produce, in fact the production of raw milk and cheese actually rose during the war years (18). Thus, in South Africa, there was no decline in the supply of

calcium rich foodstuffs following the introduction of the standard war bread.

5. Finally, in South Africa, before the introduction of the war bread, as much as a fifth of the total wheaten products consumed was of high extraction type. The bulk of the consumers were comparatively poor rural dwellers, whose average intake of calcium was likely to be on the low side. Yet in spite of these two factors, namely, a low intake of calcium and a high intake of phytic acid phosphorus, there were no reports of clinically diagnosable calcium deficiency diseases, such as would be anticipated to occur did the large amount of phytic acid phosphorus ingested seriously interfere with the utilization of calcium.

The National Nutrition Council therefore decided that before reaching any decision in regard to the addition of calcium carbonate to the war bread, experimental work on the following aspects should be undertaken:-

1. An investigation similar to that of McCance and Widdowson (12) should be carried out to learn whether under South African conditions, the consumption of the standard war bread (involving the ingestion of a large amount of phytic acid phosphorus) has a permanently adverse effect on the metabolism of calcium by humans.

2. An investigation should be carried out to determine whether the destruction of the phytic acid phosphorus which occurs during the baking of the bread, could be increased by any simple modification of the baking procedure.

3. Should the results of the experimental work carried out in (1) indicate the desirability of adding calcium carbonate to the standard meal, information will be required in regard to:-

- (a) What is the best available form of calcium carbonate to use, and how much should be incorporated in the meal.

- (b) Whether the addition will make the pH value of the bread medium sufficiently less acid

to increase the liability of "rope" development, an ever-present danger in the warmer parts of South Africa.

- (c) Whether the addition will adversely affect the baking quality, appearance, and palatability of the bread.

These investigations are described in the following pages.

P A R T I.

THE EFFECT OF SOUTH AFRICAN STANDARD WAR BREAD

(MADE FROM 95-100 PER CENT EXTRACTION MEAL)

ON THE CALCIUM METABOLISM OF ADULT HUMANS.

THE OBJECT OF THE INVESTIGATION.

The object of this section of the work is, as explained at the end of the Introduction, to determine whether the consumption of the almost wholegrain standard bread by adult South African Europeans, entailing the ingestion of a large amount of phytic acid phosphorus, has a permanently deleterious effect on the metabolism of calcium.

THE PLAN OF THE PRESENT STUDY.

1. The subject's calcium metabolism will be studied, firstly, on his usual or everyday diet, for a period of at least one week. There are two reasons for taking this step.

a) For at least two years, South Africans have been consuming high extraction standard bread to the exclusion of all other types of bread. It is probable that some adaptation to a fairly large intake of phytic acid phosphorus has already occurred. Balance data for the subject consuming his everyday diet should therefore indicate whether adaptation is actually in operation or not.

b) The analytical data on food consumption during the everyday diet period will provide the information required for fixing, altering, and (or) equalizing, the calcium intake during the experimental diet periods. The method by which this is accomplished is described on pages 19-25.

2. From the everyday diet, the subject will pass on to the First Experimental Diet, which includes the consumption of 1 lb. of standard bread daily: the calcium intake, by the above method, will be adjusted to be approximately equal to

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what is generally considered to be subject's requirement for maintenance, i.e. 10 mg. per Kg. body weight. The weekly experimental periods will be continued until a steady state of intake and excretion of calcium is reached. It will thus be possible to follow the various changes by which the subject deals with the disturbances occasioned by this type of diet.

It must be emphasized that the advantage of this experimental diet is that it resembles the diet consumed at present by a large bulk of the European population, i.e. it includes a large amount of standard bread (hence a large amount of phytic acid phosphorus), a small proportion of dairy produce (hence a comparatively low intake of calcium), and of course a modicum of meat, vegetables, etc. An inspection of the data given in the pre-war Family Budget Enquiry Report (15) shows that at least in so far as the intakes of bread and dairy produce are concerned, the supposition is valid. The behaviour, therefore, of the subject on this experimental diet, when given ample time for adaptation, should throw considerable light on what has occurred and is occurring in the bodies of the major proportion of European South African adults.

3. After a steady state or equilibrium on the First Experimental Diet is attained, the subject will pass on to the Second Experimental Diet, containing approximately the same calcium intake, but replacing the 1 lb. of standard bread by 1 lb. of low extraction white bread. This diet will be continued for a period to be determined as the experimental work proceeds. Here again, it is considered that this diet resembles that consumed by the major proportion of European South Africans prior to May 1941, when white bread was the staple: indeed it would seem legitimate to consider that the only major dietetic variable before and after the above date, was the type of bread consumed.

It/...

It is of importance to state that in this investigation, it was decided against equalizing the intakes of magnesium, phosphorus, and iron, in the different diets; for such a procedure would have created artificial dietetic conditions unrelated to the actual diets consumed by the public, and this the author was particularly anxious to avoid.

4. From the Second Experimental Diet, the subject will return to his normal or everyday diet. Since this return will take place in any case, it was considered that it may be of interest to continue further observations for a week or two.

EXPERIMENTAL.DATA ON THE SUBJECTS UNDERGOING THE DIETETIC TESTS.

All subjects were healthy adult males. Subjects F.F., A.W., and L.G., are research workers in biochemistry; subject L.O. is a reliable laboratory technician; all four, at the time of the investigation, were either working at, or associated with, the South African Institute for Medical Research, Johannesburg.

TABLE NO. 2.PHYSICAL DATA ON THE SUBJECTS.

<u>Subject</u>	<u>Age (Years)</u>	<u>Body Weight (Kg)</u>	<u>Height (cms)</u>	<u>Body Surface Area (sq.metres) *</u>	<u>Basal metabolism (Calories) **</u>
F.F.	52	65	180	1.82	1625
A.W.	31	51	163	1.53	1461
L.O.	30	64	168	1.71	1633
L.G.	33	75	168	1.83	1748

* Calculated by means of the formula of Dubois and Dubois(19).

** Calculated by means of the formula of Harris-Benedict(20).

Information about the effect of the diets on the subjects' health, weight, etc., is given on pages 26, 126 and 127.

DATA ON THE DIETS CONSUMED.

As will be gathered from what has been stated previously, there was a constant endeavour during the investigation to interfere as little as possible with the usual diet of the subjects concerned. Apart from the changes in the consumption of bread, and the slight dietetic changes required to alter the calcium intake, no other changes were instituted. It must be made clear, however, that excluding these limitations, subjects were requested to vary as little as possible the amount and type of food consumed during the various weekly periods/...

periods.

The foodstuffs contributing calcium to the diet may be classified under the following headings:-

1. Milk and cheese.
2. Bread.
3. The rest of the diet, made up of foods comparatively low in calcium.

If the calcium contributed by the rest of the diet is kept approximately constant, it should be possible, when the intake of the bread is known, to maintain the calcium intake at almost any particular level (within certain ranges), simply by varying the intake of milk and cheese. This principle forms the basis of the method which was used, firstly, to maintain the calcium intake at a level adequate for maintenance, and secondly, to equalize the calcium intakes during the Experimental Diet periods.

Before demonstrating how the method was applied, analytical data will be given on the breads used during the periods. Analytical determinations on the standard and white breads from the different bakeries serving the subjects, were carried out prior to the start of the investigation. The data on the breads actually consumed by the subjects (given in Table No. 3) were found to be almost identical with these earlier figures.

TABLE NO. 3.

TABLE NO. 3.THE AVERAGE COMPOSITION OF THE BREADS USED DURING
THE EXPERIMENTAL DIET PERIODS.

Results expressed as milligrams per 100 grams
moisture free bread.

<u>Subject</u>	<u>Type of Bread</u>	<u>Calcium</u>	<u>Magnesium</u>	<u>Iron</u>	<u>Phosphorus</u>	<u>Phytic acid Phosphorus.</u>
F.F.	Brown	62	128	5.7	295	75
A.W.	Brown	51	121	6.3	261	78
	White	24	23	1.2	78	3
L.G.	Brown	58	124	5.9	288	69
	White	22	17	1.7	74	2
H.G.	Brown	55	103	5.4	303	88
	White	21	19	1.4	81	Trace.

The Brown Bread was the South African standard war bread of 95 - 100 per cent. extraction rate.

The white bread was of low extraction rate, probably 70 per cent.

SUBJECT F.F.

Observations on subject F.F. were of a somewhat preliminary nature. During the short initial period when the usual diet was consumed, the food intake was weighed, but not sampled. During the Experimental Diet period, which included the daily consumption of 1 lb. standard war bread, F.F. consumed 100 ml. milk daily, but ate no cheese; the rest of his diet was not interfered with. This regimen was based on food consumption data given in McCance and Widdowson's paper (12).

SUBJECT A.W.Usual Diet Period (Weeks 1 and 2).

Analytical data: average daily calcium contributions.

<u>Usual Diet</u>	<u>Milk Ca</u>	<u>Bread Ca</u>	<u>Rest of Diet Ca</u>	<u>Total Ca intake.</u>
Week 1	840	80	240	1160
Week 2	840	85	250	1175

The calcium contributed daily by the rest of the

diet/...

diet was approximately constant, i.e. about 245 mg. Subject A.W. weighed 51 Kg., and thus required about 510 mg. calcium for maintenance.

First Experimental Diet Period (Weeks 3 to 10).

The calcium content of 1 lb. of A.W.'s standard bread was found to be 165 mg: the calcium contributed by the rest of the diet (assuming all was consumed) was about 245 mg., making a total of 410 mg. calcium. The amount of calcium to be made up by milk was therefore $510 - 410 = 100$ mg. For this purpose, A.W. was allowed to consume 100 ml. milk daily, containing about 120 mg. calcium. A.W., although a sparing eater, ate all the bread stipulated, and almost all the "rest of the diet" food.

Second Experimental Diet.

(a) Weeks 12 to 15. The purpose of this diet is explained later. The calcium content of A.W.'s white rolls and extra potatoes was estimated to be about 55 mg. This amount, with about 245 mg. from the rest of the diet, totalled 300 mg. calcium. The amount of calcium to be made up by milk was therefore $510 - 300 = 210$ mg. For this purpose, A.W. was allowed to consume 180 - 200 ml. milk daily.

(b) Weeks 16 to 19. The calcium content of 1 lb. of A.W.'s white bread was about 75 mg. This amount, with about 245 mg. from the rest of the diet totalled 320 mg. The amount of calcium to be made up by milk was therefore $510 - 320 = 190$ mg. For this purpose, the consumption of 180 ml. milk daily was allowed.

Return to Usual Diet (Weeks 20 to 22).

The diet consumed by A.W. during this period closely resembled that consumed during the initial usual diet period (weeks 1 and 2).

SUBJECT L.O.

SUBJECT L.O.Usual Diet Period (Weeks 1 and 2).Analytical Data: average daily calcium contributions.

<u>Usual Diet</u>	<u>Milk and Cheese Ca.</u>	<u>Bread Ca.</u>	<u>Rest of Diet Ca.</u>	<u>Total Ca intake</u>
Week 1	685	60	330	1075
Week 2	577	35	300	912

The calcium contributed daily by the rest of the diet was approximately constant, i.e. about 315 mg. Subject L.O. weighed 64 Kg., and thus required about 640 mg. calcium for maintenance.

First Experimental Diet Period (Weeks 3 to 10).

The calcium content of 1 lb. of L.O.'s standard bread was 175 mg. The calcium contributed by the rest of the diet (assuming all was consumed) was about 315 mg., thus making a total of 490 mg. calcium. The amount of calcium to be made up by milk was therefore $640 - 490 = 150$ mg. For this purpose, the consumption of 120 ml. milk was allowed daily.

L.O. was not fond of eating bread. During this diet period he had difficulty, not only in eating the 1 lb. of bread, but in consuming the "rest of the diet" food. In consequence his intake of calcium during the early weeks was too low. For weeks 8, 9, and 10, he was therefore allowed additional milk, the total consumption being increased to 200 ml. daily.

Second Experimental Diet Period (Weeks 11 to 13).

The calcium content of 1 lb. of L.O.'s white bread was about 65 mg; the calcium contributed by the rest of the diet (assuming all was consumed) was about 315 mg., thus making a total of about 380 mg. calcium daily. The amount of calcium to be made up by milk was therefore $640 - 380 = 260$ mg. For this purpose 200 ml. milk was ^{required} allowed daily, containing about 240 mg. calcium; L.O. was, however, allowed 250 ml. daily for the above reason.

Return/...

Return to Usual Diet (Weeks 14 and 15).

L.O. returned to much the same diet as he consumed during the first two weeks except that his consumption of milk was lower.

SUBJECT L.G.Usual Diet Period (Week 1).

Analytical data: average daily calcium contributions.

<u>Usual Diet</u>	<u>Milk and Cheese Ca.</u>	<u>Bread Ca.</u>	<u>Rest of Diet Ca.</u>	<u>Total Ca intake.</u>
Week 1	363	50	350	763

The calcium contributed daily by the rest of the diet was 350 mg. L.O. weighed 75 Kg. and thus needed about 750 mg. calcium daily for maintenance.

First Experimental Diet (Weeks 2 to 5).

The calcium content of 1 lb. of L.G.'s standard bread was about 160 mg. The calcium contributed by the rest of the diet (assuming all was consumed) was about 350 mg., thus making a total of 510 mg. calcium. The amount of calcium to be made up was therefore $750 - 510 \text{ mg.} = 240 \text{ mg.}$ For this purpose, the consumption of 200 ml. milk daily was allowed, containing 240 mg. calcium; alternatively, 1 oz. cheddar cheese containing about 260 mg. calcium was permitted. Unfortunately, in week 5 (also 6, see later), the calcium intake was too high: this was later traced to the fact that the cheese which L.G. elected to consume, contained almost double the usual calcium content.

Second Experimental Diet (Week 6).

The calcium content of 1 lb. of L.G.'s white bread was about 65 mg. The calcium contributed by the rest of the diet (assuming all was consumed) was about 350 mg., thus making a total of 415 mg. daily. The amount of calcium to

be made up by milk or cheese was therefore $750-415 = 335$ mg. For this purpose, the consumption of either 275 ml. milk (containing about 330 mg. calcium), or of $1\frac{1}{4}$ oz. cheddar cheese (containing about 330 mg. calcium), was allowed daily. As noted above, the calcium intake during this week was found to be too high: this was traced to the cheese being far richer than usual in calcium.

Return to Usual Diet (Week 7)

L.G., on his usual diet, was a comparatively small bread eater; on his return to the everyday diet, his consumption of bread fell to about 4 ozs. daily.

N.B. It may be mentioned that Hoff-Jorgensen and his associate workers (21), used much the same method as the author, in controlling the calcium intake in experiments on the effect of phytic acid phosphorus on the calcium metabolism of children: they kept the natural or everyday diet as uniform as possible during their 5 day periods, and varied the calcium intake by means of milk supplements.

The figures giving the amounts of the different foodstuffs eaten by the subjects during each weekly period, are available, together with the amounts of nutrients (protein, etc.) calculated from them. But it is not considered necessary to include them in this thesis, for the length would be increased by 100 pages without serving any useful purpose relevant to the object of the investigation. It may be stated however that the intakes of protein, fat, carbohydrate, calcium, phosphorus, iron, and vitamins A and C, during the various periods, exceeded the allowances recommended by the South African National Nutrition Council (22): the intake of thiamine was satisfactory except during the white bread diet period, when it was invariably too low,

i.e. less than 666. I.U. daily. In addition, speaking generally, it may be remarked that there was nothing unusual about the diet of F.F.; A.W. was very fond of eggs, but ate little meat; L.O. and L.G. were both partial to salads and fruit. Finally, it must be noted that throughout the investigation, no concentrated nutrients (e.g. vit^{vitamin} D preparations) were consumed whatever.

OBJECTIVE BEHAVIOUR OF THE SUBJECTS PARTICIPATING.

All subjects remained in excellent health throughout. There were no reports of digestion upsets, discomfort, or nausea. As will be observed from Tables Nos. 35-38 in the appendix, the weights of the subjects changed hardly at all.

While there was not undue difficulty in consuming the 1 lb. of standard bread daily, all subjects were glad when the period of the white bread diet was over; on this latter diet, constipation was reported occasionally by all subjects participating.

On changing from the standard bread to the white bread diet, the wet and dry weights of faeces were almost halved (a like observation has been made by many previous workers); the frequency of motions decreased; and the faeces, from being bulky, soft, and formless, became small, hard, and often expelled with difficulty. More detailed data on these aspects are given in the above-mentioned tables.

Apart from the understandable restraint associated with balance work, it can be stated that the subjects experienced no physical ill-effects whatsoever.

EXPERIMENTAL. (Cont'd).

The Collection of Urine, Faeces and Food for Analysis.

Urine. The collection of urine was begun at noon on the first day of each weekly period. It was collected throughout in Winchester Quart Bottles (3 or 4 being needed for the week's urine), and preserved with a thin surface layer of toluene.

Faeces. For the collection of the faeces, 2 large stone-ware jars of 3 - 4 gallons capacity were employed; one was kept at the subject's place of employment, and the other at his home. Each jar had a wide mouth 4 inches in diameter, which was provided with a tightly fitting cork bung, coated with paraffin wax. The robust character of the jars permitted the subject to sit while passing motions. The amount of toilet paper used each week was noted, samples being kept for analysis.

For the marking off of the weekly periods, carmine was used; 0.5g. either in gelatine capsules, or from a teaspoon, was taken on the first day of each weekly experimental period, before breakfast. The faeces began to be marked either in the evening motion or in the one excreted the following morning. The first motion (and if necessary the second one also) was passed into a shallow white porcelain evaporating basin 9 inches in diameter. If uncoloured, the motion was discarded : if coloured, a separation was effected with a stainless steel knife; the brown portion was thrown away, but the red-coloured portion was put into one of the faeces jars. Thereafter all motions were passed into the jars, until, on the morning of the eighth day, a further 0.5g. of carmine was taken before breakfast. The next motion (and the following one also if necessary) was passed into the porcelain basin as before. If it was uncoloured, it was added to the previous week's faeces collection : if it was coloured, a separation was effected; the brown portion was added to the previous week's faeces; the red portion formed the beginning of the new week's faeces collection.

There was, in general, little difficulty encountered in making these separations. The analytical figures, particularly those of iron, indicated that the separations were accurately showing the beginning and the end of the weekly periods.

Food. For analytical convenience, the different food samples of the diet, consumed during each weekly experimental period, were kept in separate containers. They were collected as follows:-

1. Milk. One tenth of each day's supply was pipetted out each morning into an 8 oz. reagent bottle, which contained a few ml. of 0.88 ammonia as a preservative. Throughout the week, the bottle was kept in the refrigerator.

2. Drinks. These included water, tea, coffee, lemonade, alcoholic drinks, and so forth. One tenth was put aside for analysis on each occasion of drinking, and added to a Winchester Quart bottle. It must be noted that drinks needing milk (i.e. tea and coffee) were sampled before the addition of milk, otherwise the milk would appear twice in the final food sample.

3. Fatty Foods. These included cheese, butter, and cream. The samples of these foods were kept in separate containers, one fifth of the week's consumption being preserved for analysis in each case. Unless the same type of cheese was consumed throughout the week, the sampling was carried out at the time of consumption, because of the variable composition of this foodstuff.

4. Bread. One fifth of the consumption of bread was set aside each day, and stored in a cardboard box with a tightly fitting lid. One fifth also of cakes, pastry, propriety cereal foods, biscuits, etc., ^{was} were kept in a similar recepticle.

5. Eggs. One egg was set aside for every five eggs, or part of five eggs, consumed.

6. Other Foods. This group of samples was kept, together in a glass jar (a museum jar) and covered with a suitable plate. Into this container was put one fifth of the amount consumed of meat, boneless fish, and bacon, all of which were finely cut up : also one fifth of vegetables, fruit, puddings, soup, and so forth. There was added at the end of each weekly period 0.1 gram of carmine, i.e. one fifth of the amount ingested.

There was thus collected:-

One tenth each of milk, and drinks; also

One fifth each of fatty foods, bread, eggs and the remaining food.

Note.

1. Ordinary tap water was employed for drinks, not glass distilled water as many previous workers have used.

2. The minimum of tooth paste was used, the mouth being very carefully rinsed out after each occasion of cleaning teeth.

TREATMENT OF SAMPLES FOR ANALYSIS.

For the sake of simplicity, the following description reads as if one determination were being carried out; but it must be understood that all determinations were carried out in duplicate or triplicate.

Urine. The total volume of the week's urine was measured, using a 1000 ml. graduated cylinder; from this weekly volume, the average volume of urine excreted daily was obtained. The urine was decanted into a 40 litre capacity accumulator glass vessel. After thorough mixing, a litre sample was kept for analysis.

100 ml. urine were measured out and poured into a silica basin (8.5 cms. in diameter). After the addition of 1 ml. Analar HNO_3 , the liquid was evaporated to dryness on a waterbath. The viscid mass was first heated carefully on an electric hot plate at low heat until all the residual moisture was removed and charring commenced. It was then ashed slowly at 450°C . in an electric muffle furnace, the damper being left half open. After $1\frac{1}{2}$ to 2 hours, a white melt resulted, usually completely uncontaminated with carbon particles.

The urine ash was treated with 5 ml. conc. A.R. HCl ; a watch glass was put over the basin, and the contents boiled gently on the hot plate at low heat. The inside of the watch glass was washed into the basin with 10 ml. of distilled water; the contents were again boiled, and filtered through a Whatman Paper No. 41 into a 100 ml. graduated flask. The basin was washed with four more additions of 10 ml. of water, the washings being boiled each time before they were filtered. The volume was made up to the 100 ml. mark when cold. Aliquots of this solution were used for the determination of the various mineral constituents.

Faeces. The faeces in the stone-ware jars were washed out with distilled water into two large tared porcelain evaporating basins of 9 inches diameter. The total mass was weighed in each case; knowing the volume of washing water used, it was possible to determine the wet weight of the faeces for the week; and from this figure the average weight of wet faeces excreted daily was calculated.

Conc.A.R.HCl. was added to each basin, the amount being about one tenth of the wet faeces weight in each case. The basins were put on the waterbath : after about 4 days, the faeces were dry enough (containing up to 15 per cent moisture) to grind into a granulated material having the consistency of fine bread crumbs. The faeces were weighed; after accurately determining the residual moisture on a sample, the weight of the moisture free faeces, and hence the average weight of dry faeces excreted daily was calculated.

5 to 7 grams of faeces were accurately weighed into a silica basin (8.5 cms. in diameter); the contents were heated on a hot plate in a fume cupboard until the evolution of white fumes ceased. The material was then ashed slowly at 450°C. in the electric furnace, the draught being maintained as when ashing urines. The ashing took 1 to 2 hours. When cool, the mass was moistened with a few mls. of distilled water, and 5 ml. conc.A.R.HCl. were added. The ash was taken to dryness, first on the waterbath, and then on the hot plate at low heat. A further 5 ml. conc.A.R.HCl. were added, a watch glass placed on the basin, and the contents heated to boiling on the hot plate at low heat. The inside of the watch glass was washed into the basin with 10 ml. distilled water; the contents were again boiled, and filtered, as before, through a Whatman Paper No.41, into a 100 ml. flask. Thereafter, further extractions were made as with extracting the urine ash. Any

residual carbon was finally transferred to the filter paper and repeatedly washed with boiling distilled water. When cold, the volume was made up to the 100 ml. mark. Aliquots of this solution were used for the determination of the various mineral constituents.

Food. The volume of milk was first measured, and one tenth (i.e. one hundredth of the total volume consumed) was accurately pipetted into a silica basin, similar in dimensions to the above. The liquid was taken to dryness on the waterbath. The volume of drinks was next measured, and one tenth added to the silica basin, which was again taken to dryness. Any aqueous liquid in the food jar was decanted off, measured, and one twentieth of its volume dried in the silica basin. The food mass left was pulped in a mortar until a homogeneous paste was assured; one twentieth of the weight of this paste was next added to the basin. There was next added one twentieth of the fatty foods (butter, cheese, etc.). The amount of egg ingested (less shell of course) during the week was calculated : one hundredth of this weight of hard boiled egg, shelled, and pulped into a homogeneous paste, was added. Finally, the bread sample was broken down to fine crumbs, and one twentieth added to the basin's contents.

There was thus in the basin one hundredth of the total drinks and food ingested throughout the week.

The mass was dried, firstly on the waterbath, and then with increasing heat on the hot plate. When charring commenced, the basin was put in the furnace, and the material ashed very slowly at 450°C., with the draught damper half open. This operation took 2 to 3 hours. The basin was withdrawn on at least two occasions, and allowed to cool down; it was put on a sheet of white paper and the carbonaceous lumps broken down with a stout platinum wire.

The ash was moistened with cold water; 5 ml. conc. A.R.HCl. were added, and the contents taken to dryness, first on the waterbath, and then on the hot plate at low heat. After fixing the silica, the extraction procedure was the same as that followed with the ashes of the urine and faeces.

When the carbonaceous residue was considerable, only two preliminary extractions were made; the filter paper containing the carbon particles was put into the silica basin; 2 to 3 drops conc.A.R.nitric acid were added, and a further slow ashing carried out at 450°C. The ash from this second incineration was always free from carbon particles. It was moistened with water, 2 to 3 ml. conc.HCl. added, and taken to dryness as before. The ash was extracted in the usual way, the extractions being continued until the final volume approached 100 ml. When cool, the solution was made up to the 100 ml. mark, and aliquots were used for the various determinations.

6 silica basins, each containing one hundredth of the weekly food ingested, were prepared. They were proceeded with as follows:-

2 basin's contents were ashed and extracted as described above.

2 basins, after the contents had been dried, were kept for phytic acid phosphorus determinations.

1 basin's contents was kept in reserve in case of accidents.

In addition, 2 basins, each containing one two hundredth of the weekly food ingested, were prepared, and kept for the total phosphorus determinations.

METHODS OF ANALYSIS.

DETERMINATION OF CALCIUM.

Reference:- McCance.R.A. and Shipp.H.L.(1933).

Spec. Rep. Series. Med. Res. Coun. London. No.187, Page 37.(23)

The method referred to was modified as follows.

For the calcium determination in the urine, 10 ml. of urine extract were used. For the determinations in the faeces and food, 2 ml. of the extract solutions were used : for this purpose, 10 ml. of each extract were pipetted into 50 ml. graduated flasks, made up to the mark with distilled water, and 10 ml. of these diluted solutions were taken, i.e. 2 ml. of the original solutions.

The above three liquids were pipetted into 100 ml. pyrex beakers. 0.2 ml. of phenol red (0.02 per cent solution) was added to each. This was followed by a 10 per cent ammonia solution, added drop by drop, until the colour changed slowly from brick-red to yellow, and then sharply to purple. Next, one to two drops of glacial acetic acid were added, just sufficient to change the purple colour back to bright yellow. 5 ml. of saturated ammonium oxalate solution were then added. The beakers were covered and left overnight.

The precipitates were filtered off through Whatman No.541 filter papers, and washed with 1 per cent ammonia solution; they were then washed through with about 100 ml. of 2N. sulphuric acid. The resulting solutions were heated to 80°C., and the oxalate titrated against $\frac{N}{100}$ potassium permanganate. The latter solution was made up freshly for each batch of determinations.

Calculations.

1 ml. $\frac{N}{100}$ potassium permanganate is equivalent to 0.2 mg. calcium.

$$1. \text{ Urine titre} \times 0.2 \times \frac{100}{10} \times \frac{\text{daily vol. urine.}}{100}$$

= milligrams calcium excreted daily in the urine.

$$2. \text{ Faeces titre} \times 0.2 \times \frac{100}{2} \times \frac{\text{wt. dry faeces excreted daily.}}{\text{wt. taken for analysis}}$$

= milligrams calcium excreted daily in the faeces.

$$3. \text{ Food titre} \times 0.2 \times \frac{100}{2} \times \frac{100}{7}$$

100 related to the fraction of the total food taken for analysis and 7 is the number of days in the experimental period.

= milligrams calcium excreted daily in the food.

DETERMINATION OF MAGNESIUM.

Reference:- McCance.R.A. and Shipp.H.L.(1933).

Spec.Rep. Series Med. Res. Coun. London. No.187. Page 38.(13)

The method referred to was modified as follows.

[The preparation of the extractions and the determinations of calcium on the urine, faeces, and food samples, were normally as much as the author could cope with for one week, before the next week's samples came in. The determinations of magnesium were therefore not proceeded with after the removal of calcium from the extracts, simply because of lack of time.]

10 ml. of the urine extract, and 10 ml. each of the diluted faeces and food extracts (prepared as for calcium determinations and containing 2 ml. of the original extracts) were pipetted into 50 ml. centrifuge tubes. The calcium was precipitated as oxalate (see previous page for details).

After leaving for at least an hour, the tubes were centrifuged. 10 ml. of the supernatant liquor were withdrawn in each case and run into 100 ml. beakers. 2 ml. of 2 per cent ammonium

phosphate, and 5 ml. of ammonia (S.G. 0.880) were added. The mixtures were covered and then left overnight. Next morning, the precipitates were filtered off and washed with 10 per cent ammonia solution. They were then washed through with $\frac{N}{10}$ HCl., into 200 ml. graduated flasks. When the washings had reached about 100 ml., the solutions were made alkaline to phenolphthalein with 20 per cent NaOH, and then just re-acidified with dil.HCl. When cool, the volumes were made up to the mark with distilled water. 10 ml. of the solutions were used in the colorimetric determinations.

The total phosphorus content of the solutions (and hence by calculation the magnesium previously in combination) was determined colorimetrically by the McCance and Shipp modification of Brigg's method. This method will now be described.

DETERMINATION OF PHOSPHORUS.

Reference:- McCance R.A. and Shipp H.L. (1933).

Spec. Rep. Series. Med. Res. Coun. London. No.187 Page 28.(23).

Reagents needed.

1. Standard phosphate solution. 0.4394 g. of pure KH_2PO_4 was dissolved in water and made up to 1 litre. The solution was preserved with chloroform. 1 ml. of this solution contains 0.1 milligrams of phosphorus.
2. Molybdic acid reagent. 25 g. of ammonium molybdate were dissolved in 300 ml. of water without heat, and to this solution 200 ml. of water were added and 25 g. of conc. sulphuric acid.
3. 2 per cent hydroquinone. 1 drop of sulphuric acid was added to each 100 ml. The solution was rejected when it became brown.
4. 20 per cent solution of sodium sulphite. This solution was kept tightly stoppered, and renewed once a month.

Reagents 2, 3, 4, were tested by mixing some of each.

No blue or green colour should develop.

Determination.

10 ml. of the unknown solution were pipetted into a test tube. 10 ml. of the standard phosphate solution were diluted to 100 ml. and 10 ml. of this solution (containing 0.1 milligram P) were pipetted into a second test tube. 1 ml. of molybdate solution was added to each, followed by 0.5 ml. sulphite solution, and finally 0.5 ml. hydroquinone solution. After a period of between 1 and 3 hours, the colours were matched in a colorimeter.

DETERMINATION OF MAGNESIUM. (Continued from above).

Calculations.

For greater clarity, let the original 10 ml. of extract (or diluted extract), after the addition of the reagents for the removal of calcium, be diluted to say 16.0 ml. Then since 10 ml. of this solution were taken for the magnesium determinations, the conversion factor to the original volume is $\frac{16.0}{10}$.

The colorimetric standard used contained 1 ml. of standard phosphate solution, equivalent to 0.1 milligram phosphorus, and also equivalent to 0.0784 milligram of magnesium.

$$\text{Let the colorimeter reading be } \frac{\text{Standard}}{\text{Unknown}} = \frac{15.0}{12.5}$$

Urine determination.

$$0.0784 \times \frac{15.0}{12.5} \times \frac{200}{10} \times \frac{16.0}{10} \times \frac{100}{10} \times \frac{\text{daily vol. urine}}{100}$$

= milligrams magnesium excreted in the urine daily.

Faeces determination.

$$0.0784 \times \frac{15.0}{12.5} \times \frac{200}{10} \times \frac{16.0}{10} \times \frac{100}{2} \times \frac{\text{Wt. dry faeces excreted daily}}{\text{Wt. taken for analysis}}$$

= milligrams magnesium excreted in the faeces daily.

Food determination.

$$0.0784 \times \frac{15.0}{12.5} \times \frac{200}{10} \times \frac{16.0}{10} \times \frac{100}{2} \times \frac{100}{7}$$

where 100 relates to the fraction of the total food taken for analysis, and 7 is the number of days in each experimental period.

= milligrams magnesium excreted in the food daily.

DETERMINATION OF IRON.Determination in the urine.

The method of Kennedy was employed.

Reference:- Kennedy, R.P. (1927). J. Biol. Chem. 74. 385. (24).

Reagents.

1. Standard iron solution. 0.700 g. of pure ferrous ammonium sulphate was weighed out, dissolved in water, and made up to 100 ml. 1 ml. of this strong stock solution contained 1.0 mg. of iron. For use 5 ml. were diluted to 1 litre. 1 ml. of the diluted standard contained 0.005 mg. of iron.
2. 20 per cent potassium thiocyanate.

Determination.

20 ml. of urine extract were pipetted into a large test tube. 2 ml. of the diluted iron standard (containing 0.01 mg. of iron) were pipetted into a second test tube, and the volume made up to 20 ml. 0.2 ml. of conc. nitric acid was added to each tube. The tubes were then heated for 40 minutes on a boiling waterbath to oxidize the ferrous to ferric iron. The heating also converted the pyrophosphate which interferes with the determination, into orthophosphate, which is harmless. At the end of the 40 minutes the tubes were removed and allowed to cool, and 10 ml. of amyl alcohol followed by 5 ml. of 20 per cent potassium thiocyanate were added to each tube. The tubes were well shaken to extract all the red colour from the aqueous layer. After separation, the amyl alcohol layer

was removed with a large test pipette into dry colorimeter cups, and the unknown matched against the standard colour in the usual way.

Calculation.

Let the colorimeter reading be $\frac{\text{Standard}}{\text{Unknown}} = \frac{15.0}{12.5}$

$$\frac{1}{100} \times \frac{15.0}{12.5} \times \frac{100}{20} \times \frac{\text{Vol. urine excreted daily.}}{\text{Vol. taken for analysis i.e. 100 ml.}}$$

= milligrams iron excreted in the urine daily.

Determination in the food and faeces.

The method of Lyons () as modified by McCance, Widdowson and Shakleton, was employed.

Reference:- McCance.R.L., Widdowson.E.M., and Shakleton.L.R.B.(1936).

Spec. Rep. Series. Med. Res. Coun. London. No.213. Page 21.(25).

Reagents.

1. Thioglycollic acid.
2. Approximately 7 N. ammonia solution.
3. A standard solution of ferrous ammonium sulphate, containing 0.1 mg. of ferrous iron per ml.

Determination.

10 ml. of the food or faeces extract were pipetted into a test tube. A standard containing 0.1 mg. of iron was prepared from the stock solution of ferrous ammonium sulphate, and made up to 10 ml. with approximately $\frac{N}{2}$ HCl. To the tubes, 4 drops of thioglycollic acid were added from a pipette, and the contents mixed. 2 ml. of 7 N. ammonia solution were added, and the contents again thoroughly mixed. The unknown solution, which developed a precipitate on the addition of ammonia, was filtered, and the colour of the filtrate was matched against the standard.

Calculation.

Let the colorimeter reading be $\frac{\text{Standard.}}{\text{Unknown}} = \frac{15.0}{12.5}$

For the food.

$$\frac{1}{10} \times \frac{15.0}{12.5} \times \frac{100}{10} \times \frac{100}{7}$$

where 100 relates to the fraction of the total food taken for analysis, and 7 is the number of days in the experimental periods.

= milligrams iron ingested in the food daily.

For the faeces.

$$\frac{1}{10} \times \frac{15.0}{12.5} \times \frac{100}{10} \times \frac{\text{Wt. dry faeces excreted daily.}}{\text{Wt. taken for analysis.}}$$

= milligrams iron excreted in the faeces daily.

DETERMINATION OF TOTAL PHOSPHORUS.Determination in the urine.

The procedure described by McCance and Widdowson⁽¹²⁾₍₂₃₎ was followed.

5 ml. of urine were diluted to 500 ml. with distilled water, and 10 ml. of this solution (containing 0.1 ml. urine) was pipetted into a test tube. 10 ml. of the diluted standard phosphate solution (containing 0.1 mg.P) were pipetted into a second test tube. The requisite amount of the three reagents, molybdate, sulphite, and hydroquinone, were added, and the colours matched as described previously.

Let the colorimeter reading be $\frac{\text{Standard}}{\text{Unknown}} = \frac{15.0}{12.5}$

Calculation.

$$\frac{1}{10} \times \frac{15.0}{12.5} \times \frac{\text{Vol. urine excreted daily.}}{0.1}$$

= milligrams phosphorus excreted daily in the urine.

Determination in the faeces.

The procedure described by McCance and Widdowson (¹²/₂₃) was followed.

5 ml. of the faeces extract were pipetted into a 300 ml. Erlenmayer flask, and about 200 ml. of distilled water added. The liquid was boiled for an hour to convert all pyrophosphate into orthophosphate. The residual liquid was washed into a 200 ml. graduated flask, and when cool, made up to the mark.

10 ml. of this solution, and 10 ml. of the diluted standard solution (containing 0.1 mg.P.), were treated with the requisite reagents, and the colours matched.

Let the colorimeter reading be $\frac{15.0}{12.5}$

Calculation.

$$\frac{1}{10} \times \frac{15.0}{12.5} \times \frac{200}{10} \times \frac{100}{5} \times \frac{\text{Wt. dry faeces excreted daily.}}{\text{wt. taken for analysis.}}$$

= milligrams phosphorus excreted daily in the faeces.

Determination of total phosphorus in the food.

Reference:- McCance.R.A., Widdowson E.M. and Shackleton.L.R.P. (1936).

Spec. Rep. Series. Med. Res. Coun. London. No.213 Page 16.(25).

McCance and Widdowson (¹²/₂₃) stated that for the determination of the total phosphorus in the food, 0.5 g. of the ground-up food sample should be used. This 0.5 g. has to be a representative sample of the week's food, so that any errors in failing to obtain a homogenous food sample, or in the analytical determination, become considerably magnified in the final answer. The author was unable to obtain consistent results by taking so small a sample : it was found much better to use a larger amount.

One two-hundredth of the weekly food was weighed out,

as described earlier, in duplicate, in silica basins, and thoroughly dried. The masses were well ground in a mortar, and half of each mass, i.e. one four-hundredth of the weekly food, was introduced into 100 ml. pyrex Kjeldahl flasks. 5 ml. conc. H_2SO_4 and 5 ml. A.R. perchloric acid (60 per cent), were added to each flask. The black masses were left to digest for 2 hours on a boiling waterbath. The flasks were next heated VERY CAREFULLY on a sand bath. If the heating is too rapid, explosions occur very readily, and material is lost. When the viscid liquids became brown in colour, much stronger heating was gradually applied, until finally all the residual perchloric acid was driven off, and colourless liquids remained.

When cool, 30 ml. water were added to each flask. The contents were then made alkaline to phenolphthalein with 50 per cent NaOH , and then just re-acidified with dil. HCl . The solutions were washed out into 200 ml. graduated flasks, and when cool, made up to the mark.

10 ml. of each solution were diluted to 100 ml., and 10 ml. of such solutions (containing 1 ml. of the prepared 200 ml. solutions) were pipetted into test tubes. Into a third test tube was pipetted 10 ml. of the diluted standard phosphate solution, containing 0.1 mg. P. The reagents were added and the colours matched in the usual manner.

Calculation.

Let a colorimeter reading be $\frac{\text{Standard}}{\text{Unknown}} = \frac{15.0}{12.5}$

$$\frac{1}{10} \times \frac{15.0}{12.5} \times \frac{200}{1} \times \frac{400}{7}$$

where 400 relates to the fraction of the total food taken for analysis, and 7 is the number of days in the experimental period.

= milligrams phosphorus ingested in the food daily.

DETERMINATION OF PHYTIC ACID PHOSPHORUS.

Reference:- McCance.R.A., Widdowson E.M., and Shackleton,L.R.B. (1936).

Spec. Rep. Series. Med. Res. Coun. London. No.213. Page 17.(25).

For the determination on the food, a one-hundredth of dry ground sample ^{was} were used; for the faeces determination, 5 g. of the finely ground dry material were used.

In order to extract the phytic acid from a sample, the sample was introduced into a 500 ml. glass-stoppered bottle, 100 ml. $\frac{N}{2}$ HCl. were added, and the mixture shaken in a machine for a period of 3 to 4 hours.

25 ml. of the filtered extract were pipetted into a 50 ml. centrifuge tube. The solution was made alkaline to phenolphthalein with a few drops of 40 per cent NaOH., and then just re-acidified with dil.HCl. 4 ml. of ferrie chloride solution were added. (A.R. FeCl₃ solution in N.HCl., containing 0.5 mg. ferrie iron per ml.).

The centrifuge tube was heated in a boiling waterbath for half an hour, to flocculate the precipitate of iron phytate. After cooling, the tube was centrifuged, and the supernatant liquor poured off into a second centrifuge tube. To the supernatant liquor was added a further 4 ml. of ferrie chloride solution, to ensure that the range of the method had not been exceeded. The iron phytate precipitate was washed with 10 ml. $\frac{N}{6}$ HCl., centrifuged again, and the acid decanted off.

The precipitate was stirred up with 10 ml. distilled water, and heated on a boiling waterbath for a few minutes. The iron phytate was taken into solution as sodium phytate by the addition of 5 ml. of 2 per cent NaOH.; the heating was continued for a further 15 minutes to flocculate the ferrie hydroxide precipitate.

The solution of sodium phytate was filtered into a 100 ml. pyrex Kjeldahl flask, and the precipitated ferric hydroxide well washed on the filter paper with hot water; the washings were of course added to the filtrate in the flask.

1 ml. each of perchloric acid (60 per cent) and conc. sulphuric acid were added, and the determination of the phosphorus proceeded with as described previously for total phosphorus. Of the final 200 ml. solution, 10 ml. were used for the colorimetric determination.

Calculation.

Let the colorimeter reading be $\frac{\text{Standard}}{\text{Unknown}} = \frac{15.0}{12.5}$

For the food.

$$\frac{1}{10} \times \frac{15.0}{12.5} \times \frac{200}{10} \times \frac{100}{25} \times \frac{100}{7}$$

where 100 relates to the fraction of total food taken for analysis, and 7 is the number of days in the experimental period.

= milligrams phytic acid phosphorus ingested in the food daily.

For the faeces.

$$\frac{1}{10} \times \frac{15.0}{12.5} \times \frac{200}{10} \times \frac{100}{25} \times \frac{\text{Wt. dry faeces excreted daily.}}{\text{Wt. faeces taken i.e. 5g.}}$$

= milligrams phytic acid phosphorus excreted in the faeces daily.

N.B. Phytic acid phosphorus was never found in the urine.

DECISION TO INTERPRET METABOLISM CHANGES IN TERMS OF "BALANCES"
RATHER THAN "ABSORPTIONS".

It has occasionally been considered that the difference between the amount of a substance ingested and that appearing in the faeces provides an indication of the digestibility or utilization of that substance. It is probable that there is a measure of truth in this belief when certain very large complex molecules, e.g. cellulose, are concerned: but in the case of mineral salts like calcium, the situation is not quite so simple. The above method of interpreting balance data is favoured by McCance, Widdowson, and their associate workers, in their several studies, for they consider the amount of calcium excreted into the gut to be insignificant (26). Duckworth and Warnock (27), while using the same terminology as these workers, take a slightly different view: they state "It is well known that the quantity of calcium secreted into the gut may be as high as 1g. daily, and this is subject to re-absorption together with that contributed by the diet. Where differences in net absorption (intake calcium minus faecal calcium) are interpreted as evidence of improved intestinal absorptive powers, the implicit assumption is that secreted calcium has not varied in amount during the study". In discussing this problem, Shohl (28) has drawn attention to the difficulty of interpretation which arises on occasions when faecal calcium is greater than intake calcium: clearly, in such instances at least, some excretion or secretion into the gut must occur.

This method of interpretation of metabolism data has recently been strongly criticized by Steggerda and Mitchell (29); adducing a large amount of evidence from experimental work undertaken on both animals and man, they stress the fact that the intestinal mucosa have the capacity to both excrete and absorb. In the above paper they describe experiments which demonstrate that the calcium was

passing/...

passing in large amounts from the blood stream to the lumen of the gastro-intestinal tract, and that under these conditions, faecal calcium is obviously much more than unabsorbed food calcium.

The difference between the intake and output of a mineral salt, however, is a factor which is not subject to any equivocal interpretation; in view of the sources of error pointed out above, the data in this study will thus be considered solely in terms of balances, and not absorptions.

Table No. 4

The Intake, Excretion, and Retention, of various Elements
during Normal, and high extraction Standard Bread Diets.

Subject: F.F. Weight: 65 Kg.

<u>Length of periods</u> <u>in days</u>	<u>Element</u> <u>studied</u>	<u>Diet</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>Excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
3	Calcium	Normal	-	225	914	1139	-
4		Standard	535	192	504	696	-161
7		Bread	501	235	512	747	-246
7		"	490 ⁵¹⁰	210 ²¹⁴	350 ⁴³⁷	560 ⁶⁵¹	-70 ⁻¹⁴¹
4		"	533	205	390	595	-62
3	Magnesium	Normal	-	98	300	398	-
4		Standard	490	108	372	480	+10
7		Bread	480	89	395	484	-4
7		"	535 ⁵¹⁴	91 ⁹⁴	440 ⁴¹⁵	531 ⁵⁰⁹	+4 ⁺⁵
4		"	560	93	450	543	+17
3	Iron	Normal	-	1.0	20.5	21.5	-
4		Standard	26.0	0.3	24.2	24.5	+1.5
7		Bread	24.6	0.3	23.3	23.6	+1.0
7		"	26.5 ^{26.1}	0.5 ^{0.4}	25.0 ^{24.6}	25.5 ^{25.0}	+1.0 ^{+1.1}
4		"	28.0	0.6	26.5	27.1	+0.9
3	Phosphorus	Normal	-	1520	650	2170	-
4		Standard	1700	1360	360	1720	-20
7		Bread	1900	1510	440	1950	-50
7		"	2100 ¹⁸⁸⁵	1590 ¹⁴³⁵	490 ⁴⁵⁴	2080 ¹⁸⁸⁹	+20 ⁻⁴
4		"	1670	1110	510	1620	+50
3	Phytic	Normal	-	Nil	135	135	-
4	Acid	Standard	380	Nil	75	75	-
7	Phosphorus	Bread	420	Nil	40	40	-
7		"	460 ⁴²⁵	Nil	55 ⁵⁶	55 ⁵⁶	-
4		"	420	Nil	65	65	-

Table no. 5

The Intake, Excretion and Retention, of CALCIUMduring the Normal, Brown Bread, and White Bread Diets.Subject: A.W. Weight: 51 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet - including about 250 g. brown bread, and 600 ml. milk daily	1160	246	848	1094	+66
2			1175 ¹¹⁶⁷	256 ²⁵¹	865 ⁸⁵⁶	1121 ¹¹⁰⁷	+54 ⁺⁶⁰
3	B ₁	Experimental Diet I, including about 1 lb. brown bread, and 100 ml. milk daily	510	194	402	596	-86
4			512	203	387	590	-78
5			524 ⁵¹²	220 ²²¹	373 ³⁶⁵	593 ⁵⁸⁶	-69 ⁻⁷⁴
6			501	269	297	566	-65
7	B ₁	Ditto, same as B.	509	225	255	480	+29
8			515	217	320	537	-22
9			509 ⁵¹⁶	201 ²¹²	321 ²⁸⁹	522 ⁵⁰¹	-13 ⁺¹⁵
10			561	219	295	514	+47
11			488	196	257	453	+35
12	B ₁	Experimental Diet II a, including white rolls and potatoes, with 180 - 200 ml. milk daily.	440	170	235	405	+35
13			486	145	272	417	+69
14			491 ⁴⁷⁹	183 ¹⁶⁷	285 ²⁶³	468 ⁴³⁰	+23 ⁺⁴⁹
15			499	170	260	430	+69
16	B ₂	Experimental Diet II b, including about 1 lb. white bread with 180 - 200 ml. milk daily.	512	245	235	480	+32
17			503	260	230	490	+13
18			471 ⁴⁹⁶	200 ²¹⁵	225 ²³⁶	425 ⁴⁵¹	+46 ⁺⁴⁵
19			498	155	255	410	+88
20	D	Normal Diet Similar to A.	1098	240	739	979	+119
21			1087 ¹⁰⁴⁷	243 ²³³	752 ⁷¹⁸	995 ⁹⁵¹	+92 ⁺⁹⁵
22			955	215	665	880	+75

Table No. 6

The intake, Excretion, and Retention, of MAGNESIUMduring the Normal, Brown Bread, and White Bread Diets.Subject: A.W. Weight: 51 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A.	Normal Diet - including about 250 g. brown bread, and 600 ml. milk daily	495	80	405	485	+10
2			490 ⁴⁹²	81 ⁸⁰	415 ⁴¹⁰	496 ⁴⁹⁰	-6 ⁺²
3	B ₁	Experimental Diet. No. I. including about 1 lb. brown bread, and 100 ml. milk daily.	525	86	450	536	-11
4			550	79 ¹⁰⁶	620 ⁵⁴⁶	699 ⁶⁵²	-149 ⁻⁹⁶
5			575	126	570	696	-121
6			575	133	545	678	-103
7	B ₂	Ditto, same as B ₁	500	59	430	489	+11
8			585	75	560	635	-50
9			575 ⁵⁴⁷	79 ⁷⁵	485 ⁴⁷³	564 ⁵⁴⁸	+11 ⁻¹
10			580	87	475	562	+18
11			500	77	415	492	+8
12	C ₁	Experimental Diet. No. II a. including white rolls, potatoes, and 180 - 200 ml. milk daily.	310	76	225	301	+9
13			337	91 ⁸⁰	235 ²¹⁴	326 ²⁹⁴	+11 ⁺¹¹
14			300	75	210	285	+15
15			275	80	185	265	+10
16	C ₂	Experimental Diet. No. II b, including about 1 lb. white bread, and 180 - 200 ml. milk daily.	290	75	195	270	+20
17			285	80 ⁶²	195 ¹⁹⁴	275 ²⁵⁶	+10 ⁺¹²
18			250 ²⁶⁸	56	190	246	+4
19			245	36	195	231	+14
20	D.	Normal Diet.. Similar to A.	450	80	355	435	+15
21			460 ⁴⁵⁸	77 ⁸²	365 ³⁶²	442 ⁴⁴⁴	+18 ⁺¹⁴
22			465	90	365	455	+10

Table No. 7

The Intake, Excretion, and Retention, of PHOSPHORUSDuring the Normal, Brown Bread, and White Bread Diets.Subject: A.W. Weight: 51 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet - including about 250 g. brown bread and 600 ml. milk daily.	1570	960	530	1490	+80
2			1670 ¹⁴²⁰	1080 ¹⁰²⁰	555 ⁵⁴²	1635 ¹⁵⁶²	+35 ⁺⁵⁸
3	B ₁	Experimental Diet I, including about 1 lb. brown bread, and 100 ml. milk daily.	1350	850	455	1305	+45
4			1460	840	560	1400	+60
5			1520 ¹⁴¹⁵	930 ⁸⁷⁰	585 ⁵²⁹	1515 ¹³⁹⁹	+5 ⁺¹⁶
6			1330	860	515	1375	-45
7	B ₂	Ditto, same as B ₁	1250	830	410	1240	+10
8			1460	880	540	1420	+40
9			1430 ¹³⁸⁰	910 ⁸⁶²	510 ⁴⁸³	1420 ¹³⁴⁵	+10 ⁺³⁵
10			1460	860	525	1385	+75
11			1300	830	450	1260	+40
12	D ₁	Experimental Diet II a, including white rolls, potatoes, and 180 - 200 ml. milk daily	1090	750	275	1025	+65
13			1180 ¹¹⁰⁷	850 ⁷⁶⁰	315 ²⁹¹	1165 ¹⁰⁵¹	+15 ⁺⁵⁶
14			1080	750	280	1030	+50
15			1080	690	295	985	+95
16	B ₂	Experimental Diet II b, including about 1 lb. white bread and 180 - 200 ml. milk daily	970	650	220	870	+100
17			970	690	215	905	+65
18			980 ⁹⁹⁵	670 ⁶⁸⁵	240 ²³⁹	910 ⁹²⁴	+70 ⁺⁷¹
19			1060	730	280	1010	+50
20	D	Normal Diet, Similar to A.	1490	890	550	1440	+50
21			1530 ¹³⁵⁷	880 ⁹³⁵	575 ⁵⁵²	1455 ¹⁴⁸⁵	+75 ⁺⁵²
22			1590	1030	530	1560	+30

Table No. 8

The Intake, Excretion, and Retention, of PHYTIC ACID PHOSPHORUS

during the Normal, Brown Bread, and White Bread Diets.

Subject: A.W. Weight: 51 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>
1	A	Normal Diet - including about 250 g. brown bread, and 600 ml. milk daily.	170	Nil	66	66
2			187 ¹⁷⁸	Nil	64 ⁶⁵	64 ⁶⁵
3	B ₁	Experimental Diet I, including about 1 lb. brown bread, and 100 ml. milk daily.	270	Nil	35	35
4			290	Nil	32	32
5			290 ²⁸⁹	Nil	25 ³³	25 ³³
6			305	Nil	40	40
7	B ₂	Ditto, same as B ₁	290	Nil	10	10
8			280	Nil	10	10
9			280 ²⁸⁰	Nil	32 ²⁴	32 ²⁴
10			270	Nil	36	36
11			280	Nil	33	33
12	D ₁	Experimental Diet II a, including white rolls, potatoes, and 180 - 200 ml. milk daily.	72	Nil	24	24
13			90	Nil	35 ²⁶	35 ²⁶
14			100 ⁸⁴	Nil	31	31
15			75	Nil	14	14
16	E ₂	Experimental Diet II b, including about 1 lb. white bread and 180 - 200 ml. milk daily.	65	Nil	6	6
17			50	Nil	4 ⁶	4 ⁶
18			70 ⁷⁰	Nil	6	6
19			95	Nil	9	9
20	B	Normal Diet, Similar to A.	191	Nil	58	58
21			195 ¹⁹⁰	Nil	23 ⁴⁰	23 ⁴⁰
22			185	Nil	40	40

The Intake, Excretion and Retention, of IRON
during the Normal, Brown Bread, and White Bread Diets.

Table No. 9

Subject: A.W. Weight: 51 Kg.

Week	Period	Diet consumed	Intake mg. per day	Urine mg. per day	Faeces mg. per day	Total excretion mg. per day	Balance mg. per day
1	A	Normal Diet, including about	20.6	0.7	19.5	20.2	+0.4
2		250 g. brown bread, and 600 ml. milk daily.	20.5 ^{20.5}	0.3 ^{0.5}	19.9 ^{19.7}	20.2 ^{20.2}	+0.3 ^{+0.3}
3	B ₁	Experimental Diet I, including	24.7	0.5	23.8	24.3	+0.4
4		about 1 lb. brown bread,	29.0	0.4 ^{0.4}	28.0 ^{25.0}	28.4 ^{25.4}	+0.6 ^{+0.6}
5		and 100 ml. milk daily	26.5 ^{26.0}	0.3	25.7	26.0	+0.5
6			23.6	0.3	22.5	22.8	+0.8
7	B ₂	Ditto, same as B ₁ ,	23.2	0.4	22.1	22.5	+0.7
8			27.1	0.8	25.9	26.7	+0.4
9			24.0 ^{23.9}	1.0 ^{0.7}	22.6 ^{22.7}	23.6 ^{23.4}	+0.4 ^{+0.5}
10			24.4	1.0	22.8	23.8	+0.6
11			20.7	0.3	20.0	20.3	+0.4
12	B ₃	Experimental Diet IIa,	12.8	0.4	11.9	12.3	+0.5
13		including white rolls, potatoes,	16.8	0.2 ^{0.2}	15.7 ^{13.5}	15.9 ^{13.7}	+0.9 ^{+0.7}
14		and 180 - 200 ml. milk daily.	15.0 ^{14.4}	0.1	14.1	14.2	+0.8
15			13.0	0.1	12.2	12.3	+0.7
16	B ₄	Experimental Diet II b,	14.9	0.1	14.2	14.3	+0.6
17		including about 1 lb. white bread	18.3	0.3 ^{0.2}	17.0 ^{15.0}	17.3 ^{15.2}	+1.0 ^{+0.8}
18		and 180 - 200 ml. milk daily.	15.9 ^{16.0}	0.1	15.1	15.2	+0.7
19			14.9	0.1	13.9	14.0	+0.9
20	D	Normal Diet,	21.1	0.1	20.6	20.7	+0.4
21		Similar to A.	21.5 ^{22.2}	0.1 ^{0.2}	20.8 ^{21.3}	20.9 ^{21.5}	+0.6 ^{+0.7}
22			23.9	0.4	22.5	22.9	+1.0

Table No. 10

The Intake, Excretion, and Retention, of CALCIUM
during the Normal, Brown Bread, and White Bread Diets.

Subject: L.O. Body Weight 64 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day.</u>
1	A	Normal Diet	1090	139	936	1075	+15
2			927 ¹⁰⁰⁸	132 ¹³⁶	780 ⁸⁵⁸	912 ⁹⁹⁴	+15 ⁺¹⁵
3	B ₁	Experimental Diet No. I. including the consumption of about 1 lb. brown bread daily.	465	117	355	472	-7
4			485	123	375	498	-13
5			506 ⁴⁸⁵	149 ¹³⁷	394 ³⁸⁸	543 ⁵²⁵	-37 ⁻⁴⁰
6			504	155	367	522	-18
7			465	141	450	591	-126
8	B ₂	Ditto, same as B ₁	620	133	480	613	+7
9			630 ⁶³⁶	97 ¹²³	520 ⁵⁰³	617 ⁶²⁶	+13 ⁺¹⁰
10			660	140	510	650	+10
11	C	Experimental Diet No. II, including the consumption of about 1 lb. white bread daily	750	182	517	699	+51
12			720 ⁷²²	170 ¹⁵⁴	535 ⁵¹⁶	705 ⁶⁷⁰	+15 ⁺⁵²
13			695	110	495	605	+90
14	D	Normal Diet, Similar to A.	760	101	630	731	+29
15			823 ⁷⁹²	105 ¹⁰³	678 ⁶⁵⁴	783 ⁷⁵⁷	+40 ⁺³⁵

Table No. //

The Intake, Excretion, and Retention of MAGNESIUMduring the Normal, Brown Bread and White Bread Diets.Subject: L.O. Body Weight: 64 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet	512	146	360	506	+ 6
2			435 ⁴⁷³	92 ¹¹⁹	335 ³⁴⁷	427 ⁴⁶⁶	+ 8 ⁺⁷
3	B ₁	Experimental Diet No. I, including the consumption of about 1 lb. brown bread daily.	410	145	295	440	-30
4			440	190	322	512	-72
5			480 ⁴⁷⁸	138 ¹⁴¹	380 ³⁸⁷	518 ⁵²⁸	-38 ⁻⁵⁰
6			508	130	430	560	-52
7			550	101	510	611	-61
8	B ₂	Ditto, same as B ₁	551	110	440	550	+ 1
9			482 ⁵³³	70 ¹⁰⁰	425 ⁴³¹	495 ⁵³¹	-13 ⁺²
10			566	120	428	548	+18
11	D	Experimental Diet No. II, including the consumption of about 1 lb. white bread daily.	318	111	202	313	+ 5
12			278 ²⁸⁷	115 ¹⁰⁹	166 ¹⁷⁴	281 ²⁸³	- 3 ⁺⁴
13			265	102	153	255	+10
14	D	Normal Diet. Similar to A.	412	120	275	395	+17
15			385 ³⁹⁹	105 ¹¹³	271 ²⁷³	376 ³⁸⁶	+ 9 ⁺¹³

Table No. 13

The Intake, Excretion, and Retention of PHYTIC ACID PHOSPHORUSduring the Normal, Brown Bread, and White Bread Diets.Subject: L.O. Body weight: 64 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>
1	A	Normal Diet	138	Nil	50	50
2			145 ¹⁴²	Nil	56 ⁵³	56 ⁵³
3	B ₁	Experimental Diet No. I, including the consumption of about 1 lb. brown bread daily	215	Nil	16	16
4			265	Nil	26	26
5			310 ²⁸⁹	Nil	23 ²⁶	23 ²⁶
6			315	Nil	25	25
7			340	Nil	39	39
8	B ₂	Ditto, same as B ₁	325	Nil	55	55
9			350 ³⁴⁵	Nil	58 ³⁸	58 ³⁸
10			360	Nil	20	20
11	C	Experimental Diet No. II, including the consumption of about 1 lb. white bread daily	50	Nil	30	30
12			32 ⁴¹	Nil	14 ²²	14 ²²
13			40	Nil	20	20
14	D	Normal Diet Similar to A.	125	Nil	55	55
15			148 ¹³⁷	Nil	60 ⁵⁸	60 ⁵⁸

Table No. 12

The Intake, Excretion, and Retention of PHOSPHORUS
during the Normal, Brown Bread, and White Bread Diets.

Subject: L.O. Body weight: 64 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed.</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day.</u>
1	A	Normal Diet	1580	845	775	1620	-40
2			1410 ¹⁴⁹⁵	635 ⁷⁴⁰	730 ⁷⁵²	1365 ¹⁴⁹²	+45 ⁺³
3	B ₁	Experimental Diet No. I,	1370	925	485	1410	-40
4		including the consumption of	1855	1425	490	1915	-60
5		about 1 lb. brown bread daily	1630 ¹⁶⁵⁹	965 ¹⁰²²	725 ⁵⁷⁹	1690 ¹⁶⁰¹	-60 ⁻⁴²
6			1590	1080	440	1520	+70
7			1350	715	755	1470	-120
8	B ₂	Ditto, same as B ₁	1575	855	655	1510	+65
9			1625 ¹⁷¹⁶	865 ¹⁰⁰⁸	675 ⁶⁶³	1540 ¹⁶⁷¹	+85 ⁺⁴⁵
10			1950	1305	660	1965	-15
11	B	Experimental Diet no. II,	1250	835	385	1220	+30
12		including the consumption of	1140 ¹¹⁷⁸	710 ⁷⁰⁵	320 ³⁹⁵	1030 ¹¹⁰⁰	+110 ⁺⁷⁶
13		about 1 lb. white bread daily	1145	570	480	1050	+95
14	B	Normal Diet.	1205	655	515	1170	+33
15		Similar to A.	1185 ¹¹⁹⁵	625 ⁶⁴⁰	505 ⁵¹⁰	1130 ¹¹⁵⁰	+55 ⁺⁴⁵

Table No. 14

The Intake, Excretion, and Retention of IRONduring the Normal, Brown Bread, and White Bread Diets.

Subject: L.O. Body weight: 64 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet	13.0	1.1	11.6	12.7	+0.3
2			15.6 ^{14.3}	1.0	14.4 ^{12.0}	15.4 ^{14.1}	+0.2 ^{+0.2}
3	B ₁	Experimental Diet No I, including the consumption of about 1 lb. brown bread daily	19.1	1.4	17.2	18.6	+0.5
4			18.8	1.6	17.1	18.7	+0.1
5			17.2 ^{19.2}	0.9 ^{1.3}	16.0 ^{17.9}	16.9 ^{19.2}	+0.3 ⁺⁰
6			19.0	1.8	17.3	19.1	-0.1
7			22.0	0.9	22.1	23.0	-1.0
8	B ₂	Ditto. same as B ₁	22.3	1.1	21.0	22.1	+0.2
9			23.8 ^{23.1}	0.6 ^{0.9}	22.7 ^{21.8}	23.3 ^{22.7}	+0.5 ^{+0.4}
10			23.3	0.9	21.8	22.7	+0.6
11	C	Experimental Diet No. II, including the consumption of about 1 lb. white bread daily	16.6	1.0	14.7	15.7	+0.9
12			14.3 ^{15.1}	1.8 ^{1.3}	12.0 ^{13.1}	13.8 ^{14.4}	+0.5 ^{+0.7}
13			14.5	1.0	12.7	13.7	+0.8
14	D	Normal Diet. Similar to A.	16.7	1.3	15.0	16.3	+0.4
15			17.1 ^{16.9}	0.5 ^{0.9}	16.3 ^{15.6}	16.8 ^{16.5}	+0.3 ^{+0.4}

Table No. 45

The Intake, Excretion, and Retention, of CALCIUM
during the Normal, Brown Bread, and White Bread Diets.

Subject: L.G Weight 75 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet	763	193	537	730	+33
2	B	Experimental Diet No. I, including the consumption of about 1 lb. of brown bread daily	740	269	550	819	-79
3			719	220	504	724	-5
4			705 ⁷⁶¹	210 ²³³	485 ⁵³³	695 ⁷⁶⁶	+10 ⁻⁵
5			880	233	595	828	+52
6	C	Experimental Diet No. II, including the consumption of about 1 lb. of white bread daily.	890	320	535	855	+35
7	D	Normal Diet. Similar to A.	671	206	440	646	+25

Table No. 16

The Intake, Excretion, and Retention, of MAGNESIUM
during the Normal, Brown Bread, and White Bread Diets.

Subject: L.G. Weight: 75 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet	443	134	335	469	-26
2	B	Experimental Diet No. I,	790	137	584	721	+69
3		including the consumption of	677	172	528	700	-23
4		about 1 lb. of brown bread	735 ⁷³⁹	175 ¹⁶⁹	518 ⁵⁴⁷	693 ⁷¹⁶	+42 ⁺²³
5		daily.	755	190	560	750	+ 5
6	C	Experimental Diet No. II, including the consumption of about 1 lb. of white bread daily.	465	185	270	455	+10
7	D	Normal Diet Similar to A.	366	169	200	369	- 3

Table No. 17

The Intake, Excretion, and Retention, of PHOSPHORUS
During the Normal, Brown Bread, and White Bread Diets.

Subject: L.G. Weight: 75 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet	1600	1000	560	1560	+40
2	B	Experimental Diet No. I including the consumption of about 1 lb. brown bread daily.	1710	1200	670	1870	-160
3			1870	1145	770	1915	-45
4			1850 ¹⁸²⁵	1065 ¹⁰⁹⁷	780 ⁷⁶⁹	1845 ¹⁸⁶⁶	+5 ⁻⁴¹
5			1870	980	855	1835	+35
6	C	Experimental Diet No. II including the consumption of about 1 lb. of white bread daily	1540	930	500	1430	+110
7	D	Normal Diet. Similar to A.	1445	1025	370	1395	+50

Table No. 18

The Intake, Excretion, and Retention of PHYTIC ACID PHOSPHORUSduring the Normal, Brown Bread, and White Bread Diets.

Subject: L.G. Weight: 75 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day.</u>
1	A	Normal Diet	105	Nil	60	60	
2	B	Experimental Diet No. I, including the consumption of about 1 lb. of brown bread daily	325	Nil	58	58	
3			320	Nil	55	55	
4			325 ³¹⁵	Nil Nil	75 ⁶³	75 ⁶³	
5			290	Nil	64	64	
6	C	Experimental Diet No. II including the consumption of about 1 lb. of white bread daily.	90	Nil	50	50	
7	D	Normal Diet. Similar to A	86	Nil	56	56	

Table No. 19

The Intake, Excretion, and Retention, of IRONduring the Normal, Brown Bread, and White Bread Diets.

Subject: L.G. Weight: 75 Kg.

<u>Week</u>	<u>Period</u>	<u>Diet consumed</u>	<u>Intake</u> <u>mg. per day</u>	<u>Urine</u> <u>mg. per day</u>	<u>Faeces</u> <u>mg. per day</u>	<u>Total</u> <u>excretion</u> <u>mg. per day</u>	<u>Balance</u> <u>mg. per day</u>
1	A	Normal Diet	18.3	1.2	16.2	17.4	+0.9
2	B	Experimental Diet No. I, including the consumption of about 1 lb. of brown bread daily.	22.0	1.1	20.5	21.6	+0.4
3			26.5	2.0	24.6	26.6	-0.1
4			28.5 ^{25.8}	1.1 ^{1.4}	27.2 ^{24.1}	28.3 ^{25.5}	+0.2 ^{+0.3}
5			26.0	1.5	24.0	25.5	+0.5
6	C	Experimental Diet No. II including the consumption of about 1 lb. of white bread daily.	22.7	2.7	19.0	21.7	+1.0
7	D	Normal Diet Similar to A	17.5	1.3	16.0	17.3	+0.2

THE METABOLISM OF CALCIUM.

A. USUAL DIET PERIOD.

On their usual everyday diet, at the start of the investigation, all subjects save F.F. (whose calcium intake was not determined by analysis), were observed to be in positive calcium balance.

The calculated calcium intake of F.F., and the determined intakes of A.W. and L.O., were much higher than were required for maintenance needs on the 10 mg. per Kg. body weight basis; since these subjects had been consuming such diets for a long while, it was assumed that their calcium stores were well filled at the beginning of the study. The calcium intake of L.G. was just adequate for maintenance needs.

A point of interest is the somewhat large calcium retention of A.W., which averaged about 60 mg. daily over the two weeks of observation; it is doubtful whether such retentions continue indefinitely. In this connection, McCance and Widdowson(30) have suggested that there might be seasonal variations in calcium retention. This view receives some support from Nicholls (31) who is of the opinion that, "An adult may be in negative balance for a long period, such as a number of months, followed by a short period of heavy storage of calcium; or there may be day-by-day fluctuations. This suggests that in normal adult metabolism, the calcium is being alternatively withdrawn from and returned to the skeleton".

B. FIRST EXPERIMENTAL DIET.

On this diet, subjects were required to consume 1 lb of standard war bread daily, their calcium intakes being

adjusted/...

to be just sufficient to meet their maintenance requirements.

Subject F.F. As mentioned previously, the study on this subject was solely a preliminary one, designed to gain acquaintance with the collection of samples, and with the analytical and general balance techniques. The period of observation had terminated before the full significance was realized of the necessity of ingesting a calcium intake adequate for maintenance, and also of the necessity for continuing observations for a prolonged period.

The calcium intake of F.F. was too low, viz. about 510 mg., as against 650 mg. daily required for maintenance: yet in spite of this deficiency and also the shortness of the period of observation (22 days), it will be observed that the initial large negative calcium balances, -161 mg, and -246 mg., had decreased to -62 mg. by the time the experiment ended. It would therefore seem that some degree of adaptation to a reduced intake of calcium and a high intake of phytic acid, had already begun to be manifested.

Subject A.W. On this experimental diet, A.W., immediately went into negative calcium balance, and remained so for 4 weeks (Period B1). For the next 5 weeks (Period B2) his calcium balances were mainly positive, becoming wholly positive by week 8. Examination of the data for the successive weekly periods shows a fairly regular degree of accommodation made week by week.

Subject L.O. L.O., on this diet, remained in negative calcium balance for a period of 5 weeks (Period B1). Unfortunately, during this time his calcium intake essential for maintenance was too low, thus handicapping the effort of adaptation. When the deficit was made good by supplementing with additional milk (Period B2), positive calcium balance was at once reached and maintained.

Subject L.G./...

Subject L.G. On this diet, L.G. immediately went into negative calcium balance; but, he took 3 weeks only to reach positive balance. The probable reason for this early success is that the calcium intake on his usual diet was just adequate for maintenance; therefore, on changing to the Experimental diet, the intake was not reduced. Hence, L.G. was called upon to deal with one disturbance only (unlike the other 3 subjects), namely, that due to an increased ingestion of phytic acid phosphorus.

C. SECOND EXPERIMENTAL DIET.

On this diet, subjects were required to consume 1 lb. of white bread daily, the calcium intake being maintained at the previous level, i.e. sufficient to meet their maintenance needs.

Subject F.F. did not participate in this experiment.

Subject A.W. This subject, before proceeding to the white bread diet, consumed a diet including white rolls, potatoes, and extra milk, for a period of 4 weeks, the calcium intake being maintained at the previous level, i.e. about 500 mg. daily. The reason for consuming this diet was that it was felt desirable to observe the effect on the retention of calcium when changing from a diet containing a high phytic acid content, to one of almost negligible content. Unfortunately, although the number of white rolls and scones consumed daily was small, it was not realized at the time that their phytic acid content was appreciable; for since no period of fermentation occurs prior to their being baked, the destruction of phytic acid occurring during baking is small. The change in diet did, however, lead to a considerable reduction in phytic acid intake; yet the average daily

retention/...

retention of calcium over the 4 weeks, hardly differed from the average retention over the previous 2 weeks on the standard bread diet with its high phytic acid phosphorus content.

Thus, once adaptation to the reduced calcium high phytic acid diet occurred, the large removal of the phytic acid did not favour an increased retention of calcium.

In A.W.'s white bread diet, the daily calcium intake was maintained at almost the same level as previously, i.e. about 500 mg. It will be observed that the average daily retention of calcium throughout the 4 weeks of observation showed no change.

Subject L.O. On this diet, inadvertently the calcium intake of L.O. was about 80 mg. too high for maintenance requirement. It will be seen that the retention of calcium increased from an average daily positive balance of 10 mg. during the last 3 weeks of the standard bread diet, to an average daily positive balance of 52 mg. on the 3 weeks of the white bread diet. Although the increase in retention may have been partly due to the increased calcium intake, it would seem that with this subject the white bread favoured a larger calcium retention.

Subject L.G. This subject on passing to the white bread diet, showed no improvement in calcium retention; for the retention during the last 2 weeks of the standard bread diet averaged 31 mg. daily; and the retention on the 1 week on the white bread diet week averaged 35_{mg} daily.

D. THE RETURN TO THE USUAL DIET.

Subject A.W. returned to almost exactly the same type of diet that he consumed during the first usual diet period.

Although/...

Although the calcium losses sustained on the standard bread diet had already been made good (actually by the 16th week), the retention of calcium increased by a large extent on returning to the usual diet with its high calcium content. Since equilibrium had already been established, it is difficult to understand the reason for this phenomenon.

Subject L.O. On returning to his usual diet containing a slightly increased calcium intake, L.O. showed no improvement in calcium retention.

Subject L.G. On returning to his usual diet, which had a slightly lower calcium content, L.G. showed a slight reduction in his calcium retention.

The foregoing observations on the effect of the various diets on the metabolism of calcium may be summarized as follows:-

1. Three of the four subjects, on passing from their usual diet with its high calcium content, to an Experimental diet with (a) a reduced calcium content (though adequate for maintenance), and (b) a high phytic acid phosphorus intake, showed immediate negative calcium balances. Gradually, however, adjustment to the new conditions occurred, so that given time, calcium began to be retained again and the losses sustained made good.

The fourth subject, changing from his usual diet to an Experimental diet having the same calcium content, but containing a high phytic acid intake, also went into negative calcium balance; his period of adjustment, however, was smaller than that of the other subjects, and his loss of calcium less than theirs.

2. The change from the standard bread diet to the white bread/...

bread diet with its greatly reduced phytic acid intake, scarcely affected the calcium retention of the three subjects participating.

3. Of the two factors under consideration which have been shown to disturb the metabolism of calcium, namely, a reduced calcium intake, and a high phytic acid intake, the disturbing influence of the former is clearly the more profound.

4. Finally, as a result of these observations, it may be stated that under the conditions of the investigation, the consumption of the high extraction standard bread over a long period of time had no permanently deleterious effect on the retention of calcium.

THE METABOLISM OF PHYTATE PHOSPHORUS.

Experiments by Harrison and Mellanby (7) and McCance and Widdowson (12) indicate that phytic acid, in the presence of calcium and magnesium in the proportions add at the pH value in which these bases are likely to be present in the digestive tract, forms a precipitate of calcium magnesium phytate containing roughly equal equivalents of calcium and magnesium. It is considered that this mixed phytate is far more likely to be formed than the pure calcium penta-phytate suggested by Hoff-Jorgensen (32). Assuming that the mixed phytate is precipitated, and that phytic acid is divalent, then 16.5 mg. phytic acid phosphorus will be associated with 10 mg. calcium; i.e. phytic acid phosphorus can combine with and precipitate about 60 per cent of its own weight of calcium.

Calculations from data provided by McCance and Widdowson (12) indicate that approximately half of the calcium ingested by their subjects could have been precipitated by the phytic acid present in their experimental diet. But in the author's study, only a quarter to a third of the calcium ingested in the corresponding experimental diet period could have been thus precipitated. The English subjects were therefore apparently faced with a more difficult task in attempting to achieve adaptation than the South Africans.

THE HYDROLYSIS OF PHYTIC ACID PHOSPHORUS.

The hydrolysis of phytate phosphorus has been demonstrated to occur with many animals; with the rat by Steenbock and associate workers (33,34,35), Palmer and Mottram (36) Westerlund (37,38), Marcy (39), and others; with the chick, by Lowe, Steenbock, and Krieger (40); with the hen by Common (41); and with the dog by Mellanby (7) and also Hoff-Jorgensen (42). With man, hydrolysis has also been demonstrated; by

McCance/...

McCance and Widdowson (12), Wang, Liu, Chu, Yu, and Chao (43) and by Cruikshank, Duckworth, Kosterlitz, and Warnock (44). The results of this study confirm these latter findings, and will now be considered in detail.

Examination of Tables Nos. 8, 13 & 18, reveal at once a most interesting feature, namely that a major alteration in the intake of phytic acid phosphorus results in an immediate, not gradual, maximum adjustment in the amount of this substance hydrolyzed: this behaviour stands in marked contrast to the slow step by step adjustment which occurs when the intake of calcium is restricted.

The influence of calcium intake on the hydrolysis of phytic acid phosphorus.

The following Tables Nos. 20 & 21, summarize the phytic acid phosphorus position during the usual diet, and standard bread diet periods.

Usual Diet Period. Table No. 20.

Subject	Phytic acid intake mg.P/day.	Maximum amount Ca immobilizable. mg./day.	Total Ca intake. mg. /day	Hence Ca available. mg. /day	Per cent available.	Per cent hydrolysis of phytic acid during metabolism.
A.W.	178	107	1167	1060	91%	64%
L.O.	142	85	1008	923	91%	63%
L.G.	105	63	763	700	92%	43%

Standard/...

Standard Bread Diet Period. Table No. 21.

Subject	Phytic acid intake mg. P/day.	Maximum amount Ca immobiliz- able. mg./day.	Total Ca intake. mg./day	Hence Ca available. mg./day	Per cent available.	Per cent hydrolysis of phytic acid during metabolism.
A.W.	282	169	514	345	67%	90%
L.O.	230 345	138 207	636	498 429	78% 68%	89%
L.G.	315	189	761	572	75%	80%

On the usual diet period, when the calcium intake is mainly high, and the phytic acid intake comparatively low, calculations show that 91 to 92 per cent of the calcium intake is readily available; the need therefore to hydrolyze the phytate phosphorus to make more calcium available is small, only an average figure of 57 per cent being hydrolyzed.

On the standard bread diet when the calcium intake is adequate for maintenance, and the phytic acid intake considerably increased, the proportion of readily available calcium falls to about 70 per cent; the proportion of phytate phosphorus hydrolyzed increases to about 86 per cent, to make more calcium available.

Hence as the phytic acid content of a diet increases, resulting in a decrease in the proportion of readily available calcium, an increase in the hydrolysis of phytate phosphorus occurs presumably to produce more calcium available for metabolism.

The same type of observation has been made by Lowe and Steenbock (33) for the rat, and by Common (41) for the hen. In addition, with man, Cruikshank et al (44) showed that on low calcium high phytic acid intakes, almost complete hydrolysis of phytic acid occurs; but a rise in the intake of calcium was found to depress the extent of the hydrolysis occurring.

Thus/...

Thus the level of the calcium intake, and of course the level of the phytate phosphorus intake, are the two factors that govern the extent of the hydrolysis of the phytate phosphorus.

The Agent responsible for the Hydrolysis.

Patwardhan (45) has shown that in the case of the rat, the hydrolysis of phytate phosphorus is accomplished by an enzyme phytase present in the intestine. He has also shown that the enzyme is present at birth, so that its ingestion in the food would appear to be irrelevant. This observation is in harmony with the conclusion of Boutwell, Geyer, Halverson, and Hart (46), that the presence or absence of phosphorus splitting enzymes in the diet of rats does not alter the apparent availability of phytate phosphorus.

With man, Mollgaard, Lorenzen, Hansen and Christensen (47), are emphatic that hydrolysis can only occur when the diet contains a specific enzyme which they consider as being usually destroyed during cooking. It would seem more probable, however, as Spitzer and Philips (48) have suggested for the rat, that the phosphorus splitting enzymes elaborated by the intestinal mucosa and by the flora of the gastro-intestinal tract are the critical means by which phytate phosphorus is hydrolyzed in the intestine.

The Site of the Hydrolysis.

For the hydrolysis of phytate phosphorus to be beneficial to the organism, it must occur at a site which will allow the products of hydrolysis, phosphorus, calcium and magnesium, to be absorbed. The fate of these end-products will now be considered.

Phosphorus: Steenbock and others, in the above mentioned papers, have shown that with the rat, the phosphorus

liberated/...

liberated by hydrolysis is actually absorbed: this has been shown to obtain likewise with other animals. In so far as man is concerned, McCance and Widdowson (12), Wang et al (43), and Cruikshank et al (44), consider that some at least of the phosphorus liberated is absorbed. If this did not occur, then surely phosphorus deficiency diseases would be very widespread among those peoples who ingest the bulk of their phosphorus in the form of phytate phosphorus.

Calcium and Magnesium: Harrison and Mellanby (7) working on puppies, found that calcium magnesium phytate was slightly antirachitogenic; this indicates that part of the calcium is liberated at a site which permits of its absorption.

Hoff-Jorgensen et al (21) in their experiments on the effect of phytic acid on the calcium metabolism of children, concluded that a small part of the calcium precipitated as phytate was absorbed.

Information given later (see pages 78 to 81). on peoples habituated to a very low calcium, but very high phytic acid diet, surely indicate without a doubt that a proportion of the bases liberated by hydrolysis must be utilized.

In the light of the evidence available, it is surely unjustifiable to assert that it is "obvious" that "any ion which precipitates another in the stomach or intestine will, ipso facto, prevent its absorption." (McCance and Widdowson (49)). In this connection it was once thought, for example, that tricalcium phosphate was quite unavailable to the animal body; but as Mitchell and Curzon (50) have

pointed out, the assumption is demonstrably incorrect, for under certain conditions the substance is utilized in digestion and in metabolism as well as water soluble calcium salts.

The author's study does not contribute any definite evidence on this aspect of the problem, but it is suggested that it might be clarified by the following type of experiment. Were subjects allowed to consume radioactive calcium phytate or calcium magnesium phytate, it should be possible to follow the fate of the active calcium: should part of this active element appear in the urine, it would demonstrate that some absorption of the calcium liberated by hydrolysis must have occurred.

THE RÔLE OF VITAMIN D.

The Effect of Vitamin D on the Metabolism of Calcium and Phosphorus.

Animals: It has been shown repeatedly that "when vitamin D is withheld from an animal requiring it, the processes of bone calcification are slowed down and even interrupted"(51). This has been demonstrated for the chick, poults, cats, dogs, rats and calves. On the other hand Mitchell and Curzon (50) have stated that "attempts to demonstrate a need of vitamin D by adult animals under conditions less severe than those of chronic deprivation, have generally been unsuccessful."

In regard to solar radiation, it has been shown that it is sufficient to protect hens, rats, calves, and other animals, against rickets, provided the intakes of calcium and phosphorus are adequate.

Humans: Similar to the above findings, it has been shown with infants, children and adults, that where a deficiency of vitamin D exists, leading to rickets, osteomalacia, and so forth, supplements of this vitamin, consumed in regular small doses or single massive doses, are sufficient to effect a cure.

With normal infants, Wake (52) has shown that their calcium and phosphorus retentions are constant, though differing greatly from infant to infant. The addition of vitamin D, either directly, or through the nursing mother, was found to have no effect on calcium retention, presumably because exposure to sunlight was satisfactory to ensure the maximum vitamin D requirements.

McCance and Widdowson (12), from a survey of the literature, and from the results of their own experiments, conclude that vitamin D, given in physiological doses, does

not/...

not improve the calcium metabolism of normal healthy adults. These workers do not suggest, of course, that vitamin D has no action on adults, nor that adults do not require it.

The Effect of Vitamin D on the Utilization of Phytate Phosphorus.

Animals: In studies with chicks, Lowe, Steenbeck, and Krieger (53), and McGinnis, Norris, and Heuser (54), have shown that the phytate phosphorus of cereals and legumes was poorly available in the absence of vitamin D; the latter group found that a supplement of vitamin D made phytate phosphorus nearly as available as inorganic phosphorus. The work of Krieger et al (53) provides evidence that in the absence of vitamin D, the phosphorus of isolated phytic acid is poorly available to the rat, but in the presence of adequate vitamin D, there is a marked improvement in the utilization of isolated phytic acid. Boutwell, Geyer, Halverson, and Hart, (46), have confirmed these results, i.e. that in the absence of vitamin D, the phytate phosphorus of cereals (wheat bran) was poorly utilized: an adequate intake of this vitamin increased the utilization nearly to that of inorganic phosphorus as measured by bone ash. These workers suggested moreover that the availability of phytate phosphorus is related to the amount of vitamin D in the ration, a suggestion later confirmed by Singen and Mitchell (55). In addition, Matterson, Scott, and Singen (56) showed that with poults, when the diet is relatively high in cereal phosphorus, the vitamin D of cod liver oil is less effective than that contained in animal sterols.

Humans: Analogous experiments on humans do not seem to have been made, for evidence relating to the effect of vitamin D and exposure to sunlight on the hydrolysis of phytate

phosphorus/...

phosphorus is not available. It might be imagined that the South African subjects situated in the highveld at an altitude of about 4000 ft. above sea level, consuming well irradiated food, and being themselves exposed to plentiful sunshine would be far more favourably placed in this respect than the English subjects. Yet Cruickshank's study (44) conducted in Scotland, showed that subjects living on a diet containing a calcium intake slightly deficient for maintenance purposes, and accompanied by a high phytic acid intake from oatmeal, were able to achieve an even higher degree of hydrolysis of the latter substance than the author's subjects under similar dietetic conditions.

This evidence, admittedly very scanty, would seem to show that in so far as these two studies are concerned, exposure to sunlight (within certain limits) is not a governing factor in the amount of the phytate phosphorus hydrolyzed during metabolism.

CAPACITY OF THE BODY TO ADAPT ITSELF TO LOW CALCIUM AND
HIGH PHYTATE PHOSPHORUS INTAKES.

Adaptation of the human body to a restriction in the intake of mineral salts, particularly calcium, has received considerable attention from several observers. (Nicholls and Nimalasuriya (57), Owen and Irving (58), Steggerda and Mitchell (59, 60), Kraut and Wecker (61), Mitchell (62)).

In this study it has been shown that successful adaptation can occur to a reduced calcium intake, even when accompanied by a fairly large intake of phytate phosphorus.

Probably the most important criterion of nutritional work of this character, is that conclusions reached experimentally in the laboratory should be fully in harmony with what may be observed in every day life. Now the diet in question with its comparatively low calcium and high phytic acid phosphorus contents, is by no means an artificial one, for it is typical for many millions of people in different parts of the world. Moreover, certain nations, as a result of war conditions, have been, and still are, forced to consume a diet of this type for variable periods of time. In both these types of peoples in mind, the calcium intake is very frequently lower, and the amount of phytate phosphorus consumed greater, than in the author's study. How do these people manage? A certain amount of information of their behaviour on this diet will now be given.

As examples of peoples experiencing life-long habituation, there are the Indians, the Chinese, and the South African Bantu.

The People of India: Indian diets always contain a large

amount/...

amount of cereal, but usually very little calcium. According to the observations of Wilson and Widdowson (63), "In the diets of the rice eating peoples of India, there is almost three times as much phytic acid as calcium. Hence there is a great deal more phytic acid than is sufficient to precipitate all the calcium in the diet. It is evident, however, that some calcium must be absorbed; otherwise the bodies of the adults would contain no more calcium than did their bodies at birth, which is clearly impossible." These workers, in addition, could offer no explanation for "the freedom from dental caries enjoyed by the Indian peoples living on diets exceedingly deficient in calcium and in some cases also vitamin D."

Little experimental work has been undertaken on the mineral salt metabolism of these people. Two isolated investigations will be cited. In a study by Basu, Basak, and Sircar (64), a young adult, observed for a period of 12 days on a diet containing 600g. wheat daily and including a calcium intake of 307 mg., was found to be retaining an average of 124 mg. calcium daily. Secondly, one of the Ceylonese subjects of Nicholls and Nimalasuriya (57), a child of 7 years, was observed with a retention of 169 mg. calcium daily, on an intake of 200 mg., the experimental period being 3 days, and the diet rich in phytic acid.

The Chinese: According to Wang et al (43), the diet of the Chinese is almost invariably low in calcium, and 25 to 50 per cent of their total phosphorus intake is in the form of phytate phosphorus. Habituation to this type of diet is clear, since Chu, Liu, Hsu, Chao, and Chou (65), found that a calcium intake of 7.3 mg. per Kg. body weight was adequate for the maintenance requirement of Chinese

students/...

students, a standard appreciably lower than the present one recommended by western workers.

The South African Bantu: The diet of the South African Bantu, both rural and urban dweller, is almost invariably low in calcium. Further, their staple foodstuff mealie meal, is very rich in phytic acid: due to the manner in which their porridge is customarily prepared, little destruction of phytic acid occurs during cooking. (It might be recalled that Mellanby, working on puppies, found maize to be a particularly rachitogenic cereal (10,11)).

Janisch (66) in "A study of African Income and Expenditure", in Johannesburg, found that the urban Bantu has an average calcium intake of just over 200 mg. daily. The Union Government minimum scale for Native mine labourers includes a daily calcium intake of about 340 mg. (67); the diet of the Native prisoners at the Johannesburg Fort contains a calcium intake of about 200-300 mg. daily (67). For purposes of calculation, consider the average daily calcium intake to be of the order of 250 mg. If the Bantu consumes about 1 lb. mealie meal daily (an average amount), and assuming that very little destruction of phytic acid occurs in cooking, then the calcium/phytic acid phosphorus ratio is about 1:3, i.e. almost the same as that of the rice-eating Indian (63). The phytic acid present is thus sufficient to combine with and precipitate all the calcium in the diet (see page 69). Yet the South African Bantu, although their skeletons are comparatively small, scarcely ever suffer from rickets, osteomalacia, and so forth; and the excellent state of their teeth is often the envy of the European.

No balance studies have been undertaken on the Bantu apart from the somewhat limited ones of Henderson and Kelly (68, 69). In one of their studies concerning 16 West

African boys and 8 adults, although the diet contained only about 300 mg. calcium daily and was very rich in phytate phosphorus, good retentions of calcium were observed.

These three peoples are thus habituated to a low calcium high phytic acid diet, and under these adverse circumstances are able to utilize and retain, from the meagre supply available, the mineral salts required for their growth and maintenance. Doubtless higher intakes would be of great benefit, but the major point of interest is that they do manage to live on these intakes which, judging by western standards, are grossly deficient.

Examples of peoples being forced by war conditions to consume a diet low in calcium and very rich in phytic acid, are the French and the Channel Islanders.

The French People: During the German occupation, it was stated that in 1942, the French were rationed with a calcium intake of about 180 mg. daily per person (70). In addition, their bread was almost whole grain in character. A report dated 28th November, 1945, states that "This deficiency of calcium, persisting for years, should result in complete decalcification of the skeleton, but most of the undernourished bodies adapt themselves to the deficiency." (71).

The Channel Islands People: These people fared much the same as the French during the German occupation. Their bread was stated to be very coarse and dark (72), and their supply of dairy produce was very low. Yet in a detailed survey conducted after the withdrawal of the Germans, it was stated that no evidence of widespread malnutrition was found, little rickets, and that the teeth of the children were in excellent condition (73).

It is thus clear from these cases that adaptation to the adverse circumstances was satisfactorily achieved.

How is Adaptation Accomplished?

In cases where the calcium intake is reduced to a level appreciably lower than the usual, it is only to be expected that for a variable period the change will be followed by negative calcium balances, irrespective of any simultaneous change in the state of combination of the calcium ingested. In this connection it is therefore unreasonable for McCance and Widdowson to interpret the negative calcium balances their subjects experienced on the reduced calcium high phytate phosphorus diet, almost solely in terms of interference of calcium absorption by the increased phytate phosphorus intake.

The difficulties caused by a reduced calcium intake are met firstly, by more efficient absorption. Rottensten (74) working on rats, observed a greater efficiency of absorption when the calcium stores were depleted, than when they were replenished. Secondly, an economy in excretion is exercised. Longwell (75), also working on rats reported that "When rats, whose calcium stores are nearly saturated, are changed from a high calcium intake to a low one, they conserve their calcium stores by restricting calcium excretion, eventually reaching a state of equilibrium." In the author's study, these methods of conservation ^{is} are shown by A.W. and L.O., when changing from their usual everyday diet to the standard bread diet.

To conclude, this capacity for adaptation has been adequately summarized by Steggerda and Mitchell (59) as follows:-

"It appears that in the absence of an adequate supply of any nutrient, including calcium, the body can adjust itself to the situation, either by a more economical use of what little is available, or by a lowering of its own requirements, so that eventually it comes into equilibrium with the

limited/...

limited food supply Only during this adjustment period can the body be adjudged undernourished, since only in this period is the body suffering a loss of nutrient.

.....When adaptation is complete, the body replaces from its restricted supply all losses of the nutrient, and unless some subsidiary ill effects ensue, it may reasonably be regarded as adequately supplied with food".

Comparison of the Author's Observations with those of the English Workers.

It is considered desirable to discuss very briefly the apparent disagreement between the author's conclusions and those of the English workers.

In the studies of McCance and Widdowson(12), and Krebs and Mellanby (13), the calcium intake on the Experimental diets was usually too low for requirement on the 10 mg. per Kg. body weight basis. On the high extraction bread diet, in the former study, this obtained with 8 out of 9 subjects: in the latter study, where the calcium intake was based on Leitch's estimate for minimum requirement, viz. 0.55 g. calcium daily, the calcium intake of 4 out of the 6 subjects was too low. This does not mean, of course, that these subjects were faced with an insuperable task in so far as adaptation is concerned; but it does mean that the process of adaptation would take more time than would be the case were the intakes of calcium adequate for maintenance. Clearly, the English studies were not continued long enough to allow adaptation to occur. In McCance and Widdowson's study, the experimental periods were about 3-4 weeks; adaptation was stated to be absent. In Krebs and Mellanby's study, the experimental periods were also 4 weeks; but the data given show a degree of adjustment occurring in 2 out of the 6 subjects. The salient point the author wishes to stress is that had his Experimental diet periods been limited to 3 weeks, the conclusions arrived at might well have been much the same as those of the English workers.

Conversely, had their diet periods been considerably prolonged, it is probable that the conclusions reached by them would have been much the same as the author's. The divergence in conclusions between the English and South African studies is thus more apparent than real.

THE METABOLISM OF MAGNESIUM.

The intake of magnesium, even on the white bread diet was always ample for maintenance, i.e. it exceeded 250 mg. daily, the standard put forward by Duckworth and Warnock (27).

McCance and Widdowson (12) found that all their subjects were in balance with magnesium on the white bread diet, and continued so on their high extraction bread diet. In the author's study, the initial negative calcium balances experienced on the standard bread diet were in general accompanied by negative magnesium balances. The disturbance occasioned by the increased ingestion of phytic acid phosphorus thus upset the metabolism of magnesium, though not to the same extent as it did the metabolism of calcium.

The negative calcium balances on the standard bread diet were accompanied by an increased urinary excretion of magnesium. The change to the white bread diet which had the effect of halving the intake of magnesium, rather remarkably did not affect the average daily retention of magnesium.

These results would seem to support the view that a mixed calcium magnesium phytate is produced in the digestive tract, since a large increase in the phytate phosphorus intake affects the metabolism of magnesium as well as that of calcium.

THE METABOLISM OF TOTAL PHOSPHORUS.

The metabolism of this element was least affected of the elements studied by the changes in diet. In general there was a slightly better retention on the white bread diet, in spite of the fact that during this dietetic period the total phosphorus intake was slightly lower than with the brown bread diet.

The metabolism of phytate phosphorus has already been discussed.

THE/...

THE METABOLISM OF IRON.

The retention of iron, especially by male subjects, is so small that the intake very closely approximates to the output: the determination of iron balances in metabolism experiments is therefore of great value in checking the accuracy of the marking off of the weekly periods.

The white bread favoured the retention of iron more than the standard bread. This phenomenon, however, does not appear to have resulted in any deleterious effect. Observations in England, Ireland, and Switzerland, have indicated that during the war-time period of consumption of high extraction bread, reductions have been reported in the incidence of anaemia, and improvements in the haemoglobin levels of both children and adults.

It has been observed that with an almost constant calcium intake, the retention of this element was scarcely affected by the change from the standard to the white bread diet; with magnesium the corresponding change-over, while almost halving the intake, hardly affected the retention.

With phosphorus and iron, the change-over occasioned an appreciable reduction in their intakes, yet their retentions improved. Thus, under the circumstances of the investigation, it may be stated that the standard bread regimen had a slightly depressing effect on the retention of phosphorus and iron, when compared with the white bread regimen. Whether such an effect has any physiological or nutritional significance or not, it is difficult to say, in the absence of further experiments of longer duration than the author's; but it is a phenomenon which must be borne in mind when the nutritional merits of high and low extraction breads are considered.

N.B. Preliminary notice of the results and conclusions reached in this investigation have been embodied in a letter to NATURE. 157: 769(1946).

PART II A.

THE PHYTIC ACID PHOSPHORUS CONTENT
OF SOUTH AFRICAN STANDARD BREAD.

PART II A.

THE PHYTIC ACID PHOSPHORUS CONTENT
OF SOUTH AFRICAN STANDARD WAR BREAD,
MADE FROM 95 TO 100 PER CENT. EXTRACTION MEAL.

Object of the Investigation.

In Part I, it was concluded that the ingestion of a fairly large amount of phytic acid phosphorus, contained in the standard war bread, does not have any permanently deleterious effect on the utilization of the calcium of the diet. But, since a slight improvement in calcium absorption resulted when the ingestion of the phytic acid was largely eliminated, it is surely desirable that the content of this substance in the bread should be kept as low as possible.

The object of this section of the work is, therefore, to obtain information on the phytic acid content of the war bread: it is desired to learn of the extent of the destruction of the phytic acid that occurs when the bread is baked normally, and to determine whether any slight workable modification of the baking procedure will increase the destruction.

The Phytic Acid Content of the Standard War Bread
when baked normally.

Hammersma ¹⁶ (51) obtained the following results for the phytic acid content of the standard bread.

Table No. ²²₂₆.

<u>Source.</u>	<u>Number of Analysis.</u>	<u>Phytic acid P. (as mg. P. per 100 g. fresh bread.)</u>
Pretoria.1st set.	10	50
Pretoria.2nd set.	13	47
Cape Town.2nd set.	12	48
Johannesburg.2nd set.	8	50

The author determined the phytic acid content of samples of the standard war bread used during the balance experiments. For each of the 4 subjects, a composite sample was made of the brown bread used during the weekly periods. ²³ Table No ₂₇

<u>Subject.</u>	<u>Phytic acid P. (as mg. P. per 100 g. fresh bread.)</u>
F.A.	49
A.W.	52
L.O.	48
L.G.	59

It will be observed that the standard bread (of 95 to 100 per cent. extraction rate) contains an average of about 50 mg. phytic acid phosphorus per 100 g. fresh bread. ~~Turning to Table No. 2~~
 It will be observed that this figure is very much less than the "about 100 mg." found by McCance and Widdowson for the English 92 per cent. extraction bread, and it is not very much more than the figure of 40 mg. found by Pringle and Moran ¹⁷ (56) for the 85 per cent. National Wheatmeal loaf. The reason why the South African high extraction bread has this comparatively low phytic acid content is not clear.

Upon what factors does the destruction of the phytic acid that occurs during baking depend?

The Destruction of Phytic Acid Phosphorus that occurs
during the Baking of Bread.

It was Widdowson (24)⁹ who first showed that part of the phytic acid present in the meal or flour from which bread is made, is destroyed during the doughing and baking process.

Pringle and Moran (50)¹⁷ carried out further work on this subject and arrived at several important conclusions:-

1. The time of fermentation is important, i.e. the longer the time, the greater is the amount of phytic acid destroyed.
2. The amount of yeast used is relatively unimportant.
3. If the pH value of the dough is artificially reduced by an addition of acetic acid, to bring the value down to 5.2 - 5.4, the hydrolysis of phytic acid is almost complete.
4. The greater the phytic acid content of the meal, the greater is the destruction effected.
5. The addition of calcium carbonate as creta preparata has little effect on the pH value, and on the amount of destruction normally occurring.
6. If the pH value of the dough is reduced artificially by an addition of acetic acid, the degree of destruction of phytic acid is increased, even in the presence of creta preparata, though not so much as in its absence.

Conclusions 5 and 6 will be discussed in Part II B.

Pringle and Moran ascribed the increased destruction that accompanies the reduction of pH value, to the increased solubility of the phytates in the bread medium, thus presenting a more favourable target for the action of the enzyme phytase.

The next point that arises is, ^{can the} destruction of phytic acid be increased by any slight modification of the baking procedure?

The Effect of different baking procedures on the destruction of phytic acid phosphorus.

In South Africa, apart from the commercial baking of bread, a large proportion of rural housewives, and a by no means insignificant proportion of urban housewives, bake their own bread. It was therefore decided to determine whether the various baking procedures employed have any appreciable effect on the extent of the destruction of phytic acid occurring during baking.

A representative South African wheat mixture was selected, and through the courtesy of Mr. E.C. Everett of The Premier Milling Co. Ltd., Johannesburg, was milled to yield the following fractions: firstly, unsifted meal, of approximately 100 per cent. extraction rate; and secondly, sifted meal, of about 80 per cent. extraction rate, together with the bran etc., fraction separated from it.

(N.B. The unsifted meal is virtually of the same extraction as the standard bread meal.)

The moisture, ash, calcium, magnesium, iron, phosphorus and phytic acid phosphorus, were determined on the wheat mixture and the three milling products. The results are given in Table No. 28 .

The Unsifted Meal Bread. (Almost 100 per cent. extraction rate.)

The unsifted meal was made into bread by a) two unskilled rural housewives, b) two skilled urban housewives; c) also in a commercial bakery, and d) in a laboratory bakery attached to The Premier Milling Co. Ltd., Johannesburg. In all cases, there was no departure from the usual baking procedure employed. In addition, a soda bread loaf made from the unsifted meal, using a baking powder containing bicarbonate of soda, instead of yeast, was also baked in the labora-

tory bakery. This type of loaf is seldom made in South Africa; it was made however to make the investigation more complete.

Data on the different baking procedures employed.

Rural Housewife. (Zeerust, Transvaal.)

The mixture of dough, yeast, and salt, was kneaded for 5 minutes, warmed in a bread pan, covered with a bread cloth, and left for 1 hour : the bread was then baked in a stone oven for 1 hour.

Rural Housewife. (Uppington, North Cape Province.)

The method followed was that used in the average poor home. The "rising" of the meal by the yeast was commenced on the previous night, using only salt and boiling water; the pot was covered with a bread blanket. Early next morning the dough was kneaded and allowed to rise for 2 hours, covering again with bread blanket. The mass was then put in pans, allowed to rise for 1 hour more, and then baked in^a fire heated oven.

Urban Housewife No. 1. (Johannesburg.)

The meal, with yeast, salt, and warm water, after mixing, was allowed to rise for 3 hours on the rack of an electric stove; by the end of this period, the dough had doubled in volume. The dough was then re-kneaded, placed in warm tins and allowed to rise for 1 hour further, keeping it warm meanwhile. It was then baked in an electric oven for 1 hour at a steady temperature of 250°C.

Urban Housewife No.2. (Johannesburg.)

A "sponge" composed of a portion of the meal, yeast, a little sugar and fat, and warm water, was allowed to rise on top of a warm oven for 1 to 1½ hours, "until bubbly." The remainder of the meal was then incorporated and the mass re-kneaded, placed in tins, and allowed to rise for 1 hour in a warm place. The baking was ultimately carried out at 400°F, later reduced to 350°F., for a period of 1½ hours.

Commercial Bakery. (Johannesburg.)

The details of the baking method were not divulged.

Laboratory Bakery. (Premier Milling Co. Ltd., Johannesburg.)

The first proofing period was 75 minutes; the second period lasted for 50 minutes; the yeast bread was baked for 50 minutes at a temperature of 450°F; the soda bread loaves were baked for 75 minutes at the same temperature.

The phytic acid content of the various loaves is given in Table No. ²⁵29. (Page ⁹⁶146)

In spite of the difference in the baking methods employed, which yielded loaves of different appearance and texture, it will be seen that the variation in the amount of phytic acid destroyed was so small as to be of little or no practical importance. An increased destruction of phytic acid cannot therefore be achieved by any workable alteration in the baking technique.

In the case of the soda bread, it will be seen that only very little destruction of phytic acid took place. The soda apparently reduced the acidity of the bread medium, and, according to Pringle and Moran, a decrease in the solubility of the phytates resulted, so that the phytates became less easily attacked by the phytase.

Although this investigation mainly concerns the high extraction meal and bread, it was considered advisable, since the opportunity presented itself, to carry out corresponding experiments on the sifted meal and bread; for when the wheat position becomes more favourable, this type of bread might well become the staple, and the information thus obtained might be of value.

The Sifted Meal Bread. (80 per cent. extraction rate.)

The sifted meal was made into bread in a) a commercial bakery, and b) the laboratory bakery. A sifted meal loaf, using a baking

powder containing bicarbonate of soda, was also made in the latter bakery.

The phytic acid content of the three loaves was determined; the results are given in Table No. ²⁶~~30~~. (See Page ⁹⁶~~116~~).

It will be observed that during the baking of the first two sifted meal loaves, almost complete destruction of the phytic acid occurred. This is most remarkable, for the extraction rate of the bread meal is not much less than that of the British National Wheatmeal loaf, which contains quite an appreciable amount of phytic acid. Thus, when the wheat position becomes more favourable and permits South Africa to adopt a whiter loaf, made, say, from this 80 per cent. extraction sifted meal, the phytic acid present will be insufficient to cause any disturbing affect on mineral metabolism.

It will further be noticed that in the baking of the sifted meal loaf using soda, almost half of the phytic acid was destroyed. This is also remarkable, for it was imagined that the reduced acidity of the bread medium would not favour the destruction of phytic acid to the extent that actually occurred.

What is the reason for the greater destruction of
phytic acid that occurs during baking in

South Africa?

The reason for the greater destruction with both the unsifted and sifted meal loaves cannot be explained at present. It is true that the phytic acid contents of the two meals are slightly less than the contents of the corresponding meals used overseas, but the differences however are not significant. Again, it can hardly be due to any unusual method of baking being employed in South Africa,

Table No. ²⁴~~23~~ .

Analysis of the sample of wheat and its milling products.

	<u>Wheat.</u>	<u>Bran.</u>	<u>Unsifted Meal.</u>	<u>Sifted Meal.</u>
Moisture. per cent.	11.8	10.1	11.7	11.5
<u>Results on moisture-free basis.</u>				
Ash. per cent.	1.49	4.41	1.47	0.74
Calcium. mg. per 100 g.	55	125	53	33
Magnesium. mg. per 100 g.	110	330	105	58
Iron. mg. per 100 g.	4.1	11.0	3.9	2.6
Phosphorus. mg. per 100 g.	275	655	265	160
Phytate Phosphorus. mg. per 100 g.	155	420	145	86
<u>Phytate Phosphorus</u> per cent.	56%	64%	55%	54%
Total Phosphorus.				

25
Table No. 29.

The destruction of phytic acid phosphorus during the baking
of unsifted meal bread.

Phytic acid phosphorus content of unsifted meal

= 145 mg.P. per 100 g. dry meal.

<u>Type and Name of baker.</u>	<u>Phytic acid phosphorus.</u> mg. P. per 100 g. <u>fresh bread.</u>	<u>Percentage of phytic</u> <u>acid phosphorus</u> <u>destroyed during</u> <u>baking.</u>
Unskilled housewife. (Zeerust).	49	51%
Unskilled housewife. (Uppington).	47	52%
Skilled housewife. (Johannesburg).	49	48%
Skilled housewife. (Johannesburg).	49	50%
Commercial baker. (Messrs. Gains, Johannesburg).	58	41%
Laboratory baker. (Miss Parrish).	56	43%
Soda bread. (Baked by Miss Parrish).	92	5%

(The laboratory loaves were baked by Miss Parrish in the
laboratory attached to The Premier Milling Co. Ltd., Johannesburg).

26
Table No. 30.

The destruction of phytic acid phosphorus during the
baking of sifted meal bread.

Phytic acid phosphorus content of sifted meal

= 86 mg. P. per 100 g. dry meal.

<u>Type of bread and</u> <u>name of baker.</u>	<u>Phytic acid Phosphorus.</u> mg. P. per 100 g. <u>fresh bread.</u>	<u>Percentage of phytic</u> <u>acid phosphorus</u> <u>destroyed during</u> <u>baking.</u>
Commercial loaf. (Messrs. Gains, Johannesburg).	7.0	87%
Laboratory loaf. (Miss Parrish).	6.5	89%
Soda bread. (Miss Parrish).	34	49%

for the procedure followed differs little from overseas practice. It may be due to the fact the South African bread meals are richer in phytase, or perhaps on the other hand, for some unknown cause, the substrate is more favourable for the action of the phytase. It is hoped to carry out further experimental work on this problem at a later date.

Summary.

1. Due to the large measure of destruction of phytic that occurs during the baking of the South African high extraction war bread, the phytic acid content is much lower than might have been anticipated, and is little more than that of the British National Wheatmeal loaf which is made from meal of a much lower extraction level.

2. This degree of destruction is almost unaffected by the baking procedure employed.

3. Almost complete destruction of phytic acid occurs during the baking of the 80 per cent. extraction sifted meal bread; hence should this type of bread be nationally adopted at a later date, the amount of phytic acid ingested from it will be of little or no metabolic significance.

4. With the high extraction soda bread, little destruction of phytic acid occurs during baking; in the case of the sifted meal soda bread, almost half the phytic acid is destroyed during this process.

This study has been published in the Journal of the South African Chemical Institute, May issue, page 9, 1947.

PART II B.

THE FORTIFICATION OF SOUTH AFRICAN

STANDARD WAR BREAD

WITH CALCIUM CARBONATE.

The Object of the Investigation.

In Part I, it was concluded that the ingestion of a large amount of phytic acid phosphorus contained in the Standard War Bread, did not have a permanently deleterious effect on the utilization of the calcium of the diet. Therefore, the need to add a calcium salt to the bread, to guard against this danger, does not arise.

There is, however, considerable evidence, on the one hand, that an increase in the calcium intake of the population as a whole would be undoubtedly beneficial; and on the other hand, that the calcium intake of the bulk of the population of South Africa is very much on the low side.

In this section of the work, the fortification of the standard war bread with calcium carbonate will be investigated, so that in the event of a decision being reached to proceed with the addition, the relevant information will be available.

The desirability of increasing the amount of calcium ingested by the
general public, particularly by the poorer section
of the population.

Although the animal body is able to adapt itself successfully to a comparatively low calcium intake without any very apparent ill effects ensuing, there is considerable evidence to show that beneficial effects follow the ingestion of a higher intake of dietary calcium.

Lanford and Sherman (⁷⁷115) have stated that,

"Animal experimentation has yielded a large amount of evidence, some as yet unpublished and some published only in brief, that liberal calcium allowances are good for adults of both sexes at all times - not merely for pregnant and lactating mothers. The effects of different liberal levels of calcium intake have been studied through the entire life histories of hundreds of rats, and in a large proportion of cases, through successive generations." The chief conclusions from these experiments are as follows. "When the calcium content of a diet which originally contained an amount slightly above that of minimal adequacy was doubled, there resulted better development, a higher level and longer period of adult capacity and vitality, and longer life, without increased incidence of calcification of arteries, but a better store of calcium in the skeletal system."..."Scientific probability strongly supports the view that these general relationships of calcium intake to nutritional well-being throughout the life span are equally true for the human species. A liberal margin of calcium intake above the literal requirement for maintenance thus appears clearly desirable for all."

The Medical Research Council, in their First Memorandum (^{77a}7) stated that,

"An insufficiency of calcium is an important defect in human diets in Great Britain, but it would not be adequately counteracted by increasing the extraction of the flour for bread. Bread is in any case a poor source of calcium." The third Recommendation of the Report reads that "The public would be benefited by the addition of calcium salts to the flour from which bread is made - in order to secure an adequate supply of calcium."

The Council on Foods and Nutrition of the American Medical Association is in agreement with this view (^{77b}34). The Council recognizes that even whole wheat is a minor source of calcium.

"It was therefore decided that, if calcium is to be added to white flour, it would be well not to stop at the level of the whole grain, but to add an amount which is really significant from the nutritional point of view."

Opinion is therefore strongly in favour of adding a calcium salt to the peoples' bread as a general measure of improving their nutrition.

The background of the South African position in relation to the addition of a calcium salt, e.g., calcium carbonate, to the standard bread.

The addition of calcium carbonate to the standard war bread affects the public, the miller and baker, and the nutritionist.

From the point of view of the public, it is of the utmost importance that the addition should have no prejudicial effect on the appearance and palatability of the loaf. The loaf is viewed with considerable disfavour as it is; any intensification of that disfavour must therefore be vigilently guarded against. Since, however, the addition has been made successfully overseas, there would appear to be no reason for apprehension in South African in that regard.

Upon the miller will rest the responsibility of making the addition of the calcium salt to the meal. In South Africa, where there are a small number of very large mills, and a large number of small rural mills, the difficulties attending the addition, and the effective supervision that would be required, will be considerable, and may even rule out the feasibility of the project.

The baker, also, is likely to be confronted with difficulties. The effect of the addition of a calcium salt, e.g. calcium carbonate, is to make the bread medium less acid, i.e. to raise its pH value. The major effect of this change in pH value, in so far as the baker is concerned, is to make the bread more liable to the development of "rope." In the summer rainfall areas in the Union

this is a matter of very great importance; it is a danger which has always to be guarded against. Voster (⁷⁸116) recommended that bakers experiencing this trouble, in the ordinary course of events, may remedy matters by adding vinegar to the bread at the rate of 5 pints (4 per cent. acetic acid) to every 200 lb. bag of meal, thereby making the bread medium more acid. Now the addition of calcium carbonate may lead to the necessity of having to increase the rate of addition of vinegar to counter "rope" development; and may indeed necessitate the universal addition of vinegar to the bread meal as a matter of normal procedure. The magnitude of the difficulties likely to be encountered can of course be solved only by experimental means.

Other factors complicate the issue, namely, the type of calcium carbonate employed, its availability and cost, and also the cost of the additions, both of calcium carbonate and of vinegar. In the first instance, Halton (⁷⁹117) has shown that some forms of calcium carbonate have a much greater effect than others on the pH value of bread. Obviously the form of calcium carbonate that leads to the least change in pH value is the most desirable to employ. The form chosen must be available in quantity, and be inexpensive. Moreover the cost of the addition of both calcium carbonate and vinegar must be insignificant, so that the price of the bread to the consumer remains unchanged.

In so far as the nutritionist is concerned, it is desirable, firstly that a worth while addition of calcium to the diet of the general public be made; and secondly, that the phytic acid content of the bread ingested be as low as possible. Now as has been referred to already, the hydrolysis of the phytic acid occurring during baking, is promoted by an acid medium, the optimum pH value lying between 5.2 to 5.4. It would seem likely that the addition of

calcium carbonate will reduce the acidity of the bread medium, and so reduce the extent of the hydrolysis occurring. The extent of this reduction is a matter of importance, and can of course be solved only by experimental means.

How much calcium carbonate is it desirable to add?

In the United States, the Council on Foods and Nutrition of the American Medical Association (⁸⁰47) has recommended that the equivalent of 500 to 2,000 mg. of calcium be added per lb. of white flour. This addition is optional at present.

In Canada (⁸¹36), calcium is added to the bread in the form of non-fat milk solids, 4 per cent. of this product being added to the bread flour.

McCance and Widdowson (¹²23) recommended the following additions:-

To white flour, 65 mg. Ca. as CaCO_3 per 100 g.
To 85 per cent. National Wheatmeal, 120 mg. Ca. as CaCO_3 per 100 g.
to 92 per cent. Wheatmeal, 200 mg. Ca. as CaCO_3 per 100 g.

The British Medical Research Council in their second Memorandum (⁶9), recommended that calcium carbonate as creta preparata should be added to the National Wheatmeal flour to the extent of 14 ozs. per 280 lb. bag. The British Government eventually decided to sanction the addition of half this amount, namely, 7 ozs. creta preparata per 280 lbs. bag. The sanctioning of only half of the addition recommended by McCance and Widdowson, and the Medical Research Council, means that in the view of these authorities, the phytic acid danger is still present, and that the substance is still capable of depressing the absorption of calcium.

In the following table a comparison of the above figures is made: the South African recommendation (see page ¹¹⁴134) is also included. The figures are given as milligrams calcium per lb. of flour.

27
Table No. 3+.

<u>Authority.</u>	<u>Extraction rate of flour.</u>				
	<u>95 - 100%</u>	<u>92%</u>	<u>85%</u>	<u>75 - 80%</u>	<u>70%</u>
U.S.A. Recommendation.	-	-	-	-	500-2000 mg.
Canada Recommendation.	-	-	-	254 mg.	-
McCance and Widdowson Recommendation.	-	912 mg.	550 mg.	-	295 mg.
Medical Research Council Recommendation.	-	-	580mg.	-	-
Figure adopted by British Ministry of Food.	-	-	290mg.	-	-
South Africa Recommen- dation.	908 mg.	-	-	-	-

The Experimental Investigation.

The experimental work about to be described was carried out by the author in collaboration with Dr.P.W. Voster of the College of Agriculture, Stellenbosch.

Dr. Vorster's part in the investigation was concerned mainly with the baking quality of the bread, and with the pH value and hence liability to "rope" development, occasioned by the various additions of calcium carbonate and vinegar to the bread.

The author's part in the work was to determine the effect of these various additions on the amount of phytic acid phosphorus destroyed during baking.

The report of this work formed the basis of the Second Interim Report of the Mineral Research (Bread) Sub-Committee of the South African Nutrition Council.

A brief account of Dr. Vorster's work will now be given.

Dr. P. W. Vorster's Investigation.

The effect of the addition of several forms of calcium carbonate, and of vinegar, in varying quantities, on the pH value and the development of "rope", in the standard war bread.

Materials.

All tests were carried out on standard meal (No.1 unsifted) obtained from a large commercial mill at Paarl. The meal was milled from Western Province Wheats, and may be regarded as being typical of the standard meal sold in that province.

The different forms of calcium carbonate used were:-

1. Creta Preparata (97 per cent. CaCO_3).
2. Precipitated chalk (98 per cent. CaCO_3).
3. Standard whiting (98 per cent. CaCO_3).
4. Ground Limestone. (93 per cent. CaCO_3). No.1.
5. Ground Limestone. (95 per cent. CaCO_3). No.4.

Four quantities of calcium carbonate, at the rate of 10 ozs., 12 ozs., 14 ozs., and 16 ozs., per 200 lb. bag of meal, were used.

Ordinary brown commercial vinegar (4 per cent. acetic acid), was used as a rope "inhibiter," in quantities at the rate of 5, 6, 7, and 8 pints, per 200 lb. bag of meal.

The additions of vinegar were made alone, and also together with the different forms and quantities of calcium carbonate.

Experimental.

The standard baking technique used in the Stellenbosch laboratory was followed. This baking formula is:-

Meal 330 g. (12.4 per cent. moisture.)
 Sugar 15 g. (cane sugar.)
 Table salt 5 g.
 Yeast 10 g. (compressed.)
 Distilled water - sufficient to produce a dough of correct consistency.

In all cases, the dough was fermented for 2 hours and proofed in the pan for 30 minutes; it was then baked for 40 minutes at a temperature of 425°F.

The quality factors taken into consideration were loaf volume, external appearance, crumb texture, and taste.

The pH value of the loaves was determined the day after baking, on the crumb, according to the method used by Halton (⁷⁹117).

When the influence of calcium carbonate and vinegar on the development of "rope" was investigated, all doughs were heavily infected with rope organisms (*Bacillus mesentericus*). The inoculated loaves were stored in an incubation cabinet at 84°F., under conditions of high humidity. They were examined each day for a period of 6 - 7 days for signs of "rope".

The inclusion of the detailed results is unnecessary.

Conclusions.

1. The various additions of calcium carbonate had no significant effect on the baking quality of the bread.
2. The various additions of vinegar whether added alone, or together with the different amounts and forms of calcium carbonate, had no effect on the baking quality of the bread.
3. Precipitated chalk affected the pH value of the bread to a much greater degree than did the other forms of calcium carbonate; the other forms differed little in their effect on this factor.
4. In all cases where no vinegar was added, irrespective of whether calcium carbonate was added or not, the loaves became badly ropy by the 2nd and 3rd day after baking, when stored at 84°F.
5. 5 pints of vinegar per bag of meal inhibited the development of "rope" in standard bread for at least 6 days after baking.
6. The addition of 6 pints of vinegar, instead of the previous 5 pints per bag, inhibited "rope" development for 6 days after baking, with all the forms of calcium carbonate tried out, save precipitated chalk.
7. In this latter case, "rope" development was observed in the loaves when treated with 8 pints of vinegar and 16 ozs. of chalk per bag, 5 days after baking.

The Effect of the Addition of Vinegar and
Calcium Carbonate, either alone or together, on the
destruction of Phytic Acid that occurs during the
baking of Standard Bread.

Objects of experiments.

(a) To determine whether the calcium content of bread can be raised by the addition of calcium carbonate without an appreciable decrease in the amount of phytic acid destroyed ~~occurring~~ during baking. (b) To compare the effect of different types of calcium carbonate when used for this purpose. (c) To ascertain whether the destruction of phytic acid during baking can be promoted either with or without the addition of calcium carbonate, when the degree of acidity is increased by the addition of vinegar.

Method.

The baking of the different loaves and other details such as the different types of calcium carbonate used, have been fully described in Dr. Vorster's Report.

Two representative samples of each loaf were taken on the day of baking for the determination of moisture and phytic acid; 5 grams were used for the former determination and about 100 grams for the latter.

The samples were dried in an air oven at 70°C, until moisture free. The phytic acid content of each sample was then determined.

The results obtained are recorded in Tables No. ²⁹~~35~~ and No. ³⁰~~34~~.
(fuller details being given in the Appendix Tables No. ³⁵~~40~~ and No. ³⁶~~41~~.)

Conclusions.

- (1) The addition of calcium carbonate alone. (Table No. ²⁹~~33~~)

The addition of calcium carbonate in amounts ranging from 10 to 16 ounces per 200 lb. bag, brought about a barely significant decrease in the amount of phytic acid destroyed during baking.

The decrease was much greater in the case of precipitated chalk (Table No.²⁹33); but the other types gave lower reductions.

Hence, if the use of precipitated chalk is excluded, the addition of as much as 1 lb. of calcium carbonate per bag of flour can be employed, without there being any appreciable reduction in the amount of phytic acid destroyed during baking.

(2) The addition of vinegar alone. (Table No.²⁹33)

It is a common practice in South Africa to add vinegar to meal before bread is made, in order to prevent the formation of "rope". In fact the Wheat Industry Control Board, ^{following Voster's recommendation} has advised the addition of 5 pints per bag for this purpose. Since Pringle and Moran had already shown that an increase in the acidity of the medium led to increased destruction of phytic acid during the baking of National Wheat meal, it was anticipated that a similar effect would be found with the Standard Meal. That this proved to be the case will be seen from the tables. Thus, whilst bread baked from this meal in the normal manner showed a destruction of about 36 per cent. of the phytic acid, the addition of vinegar at the rate of 5 and 8 pints per bag led to destruction of 53 and 69 per cent. respectively.

Hence, the practice of adding vinegar to meal, although primarily employed for inhibiting "rope" formation, has the additional advantage of considerably increasing the destruction of phytic acid.

(3) The addition of both calcium carbonate and vinegar. (Table No.²⁹33).

It would be expected that when calcium carbonate was added to a meal containing vinegar, the neutralising effect of the former on the latter, by reducing the acidity, would decrease the destruction of phytic acid during baking. It will be seen, however,

that although the amount destroyed is less than with vinegar alone, the decrease is not very marked. Precipitated chalk again behaved in a far less satisfactory manner than did the other forms of calcium carbonate employed. These differences in the behaviour of different forms of calcium carbonate have previously been observed by Halton (⁷⁹117) when working with National Wheatmeal.

Hence, it is concluded that the increased destruction of phytic acid brought about by the addition of vinegar is not seriously interfered with by the addition of calcium carbonate in the desired amounts, provided precipitated chalk is not employed.

(4) The effect of the amount of yeast used. (Table No. ³⁰~~34~~).

The observation of Pringle and Moran that an increase in the amount of yeast used (from 1.5 to 3.0 per cent.) had an insignificant effect on the amount of phytic acid destroyed, is also confirmed.

(5) The effect of increasing the time of fermentation.
(Table No. ³⁴~~38~~).

Similarly, the results agree with those of Pringle and Moran that the destruction of phytic acid increases as the time of fermentation is prolonged.

In the investigation in Part II A, it was observed that during the normal baking of the standard war bread, a destruction of phytic acid took place of the order of 50 per cent: the destruction was greatest with the housewives' baking and least with the commercial and laboratory baking. The work was carried out on a meal having a comparatively low phytic acid content. In this present investigation, it will be observed that the degree of destruction taking place with the control loaves varied somewhat from loaf to loaf, and averaged 34 per cent. The reason for this lower destruction is not clear; it may be due to the fact that the work was carried out on a meal of exceptionally high phytic acid content. It would therefore seem

that, in contrast to the finding of Pringle and Moran, the greater the phytic acid content of the bread meal, the smaller is the destruction that takes place during baking.

The cost of fortifying bread with calcium carbonate.

In order to ascertain whether the addition of 1 lb. of a suitable type of calcium carbonate would add appreciably to the cost of the fortified loaf, the following particulars are included. Whilst exhaustive inquiries have not been made, these figures indicate that the actual cost of the ingredient would be insignificant.

Table No. ²⁸ 32.

<u>Type of calcium carbonate.</u>	<u>Cost of adding 1 lb. per 200 lb. bag of meal.</u>	<u>Cost per 1 lb. loaf, assuming 280 loaves per bag.</u>
Creta preparata	15.0 pence	0.01 pence
Limestone flour - 50 mesh	0.3 pence	0.001 pence
Limestone flour - Standard whiting.	0.9 pence	0.003 pence
Limestone flour - No.1.(another source)	0.8 pence	0.003 pence
Limestone flour - No.4.(same source as No. 1.)	1.1 pence	0.004 pence

All these types of calcium carbonate were found to be suitable in practice with the exception of the 50 mesh Limestone flour, which was too coarse. It is therefore considered that there should be no real difficulty in devising a means for incorporating the calcium carbonate uniformly throughout the meal, at least in the larger mills.

The cost of treating the meal with vinegar.

With the wholesale cost price of vinegar at 1/3 per gallon the cost of the added vinegar per 1 lb. loaf would be about 0.04 pence. Here again, the cost of the addition of vinegar is not significant.

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Table 33.

Comparison of the amounts of phytic acid destroyed during the baking of bread, when varying amounts of a) calcium carbonate: b) vinegar (4 per cent acetic acid); and c) calcium carbonate plus vinegar; are added to the meal. *

A. Type of calcium carbonate used:- Precipitated chalk.

Amount of Calcium carbonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.				
	Nil	5 pints	6 pints	7 pints	8 pints.
Nil	23%	53%	58%	63%	69%
10 ounces	19%	23%	25%	31%	31%
12 ounces	14%	19%	29%	29%	27%
14 ounces	11%	19%	25%	25%	27%
16 ounces	14%	20%	23%	25%	25%

* The amount of phytic acid destroyed during baking is expressed as percentage of that originally present in the meal.

Note. By determining the total phosphorus in the untreated meal and in the bread made from it, due allowance was made in the above calculations for the presence of the other ingredients of the bread, viz. sugar, salt and yeast.

B. Type of calcium carbonate used:- Creta Preparata.

Amount of calcium carbonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.				
	Nil	5 pints	6 pints	7 pints	8 pints
Nil	36%	53%	58%	63%	69%
10 ounces	33%	48%	50%	57%	62%
12 ounces	33%	50%	50%	58%	61%
14 ounces	29%	48%	48%	56%	58%
16 ounces	27%	46%	48%	54%	56%

C. Type of calcium carbonate:- Ground limestone.50 mesh.

Amount of calcium carbonate per 200 lbs.bag.	Amount of vinegar per 200 lbs. bag.				
	Nil	5 pints	6 pints	7 pints	8 pints.
Nil	37%	53%	58%	63%	69%
10 ounces	36%	52%	57%	61%	67%
12 ounces	37%	52%	55%	60%	67%
14 ounces	36%	51%	55%	58%	65%
16 ounces	33%	50%	54%	57%	63%

D. Type of calcium carbonate:- Ground limestone.

(Standard whiting)

Amount of calcium carbonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.				
	Nil	5 pints	6 pints	7 pints	8 pints
Nil	31%	53%	58%	63%	69%
10 ounces	29%	48%	52%	58%	60%
12 ounces	29%	48%	54%	55%	61%
14 ounces	32%	46%	51%	55%	58%
16 ounces	29%	46%	50%	54%	54%

E. Type of calcium carbonate used: Ground Limestone No.1

Amount of calcium carbonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.				
	Nil	5 pints	6 pints	7 pints	8 pints
Nil	36%	53%		63%	69%
10 ounces	33%	52%		58%	65%
14 ounces	33%	50%		58%	65%
16 ounces	31%	48%		57%	60%

F. Type of calcium carbonate used:- Ground Limestone No.4.

Amount of calcium carbonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.			
	Nil	5 pints	7 pints	8 pints.
Nil	36%	53%	63%	69%
10 ounces	34%	52%	59%	65%
14 ounces	33%	51%	58%	63%
16 ounces	32%	52%	57%	60%

Table ³⁰₃₄.

The effect of the time of fermentation and the amount of yeast used on the percentage destruction of phytic acid during the baking of the bread.

Time of fermentation	Proportion of yeast.	
	1.5%	3.0%
1 hour.	21%	23%
2 hours.	25%	27%
3 hours.	40%	42%
4 hours.	48%	52%

Percentage destruction with untreated normally baked loaf:
42 per cent.

Table _____.

~~Comparison of the effect on the phytic acid content of standard bread when a) calcium carbonate, b) vinegar (4 per cent acetic acid), and c) calcium carbonate plus vinegar, are added in varying amounts to the meal.~~

Recommendation.

Should a decision be reached by the responsible authorities that an addition of calcium carbonate be made to the nation's bread, it is recommended that calcium carbonate as standard whiting, be added at the rate of 1 lb. per 200 lbs. bag of meal. This addition corresponds to 908 mg. calcium per 1 lb. of meal: much the same amount, namely 912 mg. calcium per 1 lb. of meal, was recommended by McCance and Widdowson for inclusion in the English 92 per cent. extraction meal. The addition also corresponds to about 650 mg. calcium per 1 lb. of fresh standard bread.

The official recommendation in regard to the use of vinegar as "rope" inhibitor, would have to be altered from 5 to 6 pints vinegar per 200 lbs. bag of meal, were the above recommendation put into effect.

Summary.

1. Although on the one hand the need to fortify the standard bread with a calcium salt, specifically on account of the adverse effect caused by the phytic acid ingested, has been shown to be unnecessary, - on the other hand, evidence of a different nature has been presented to indicate that such an addition would be nutritionally beneficial.

2. A comparison has been made of the amount of calcium recommended by the various authorities for addition to the bread.

3. An investigation on the addition of calcium carbonate (in varying forms and quantities), with and without the addition of vinegar, (also in varying amounts), to the standard war bread, has been carried out by Dr. P.W. Vorster and the author.

Briefly, the conclusions are as follows:-

- a) Additions to the bread of calcium carbonate, up to 1 lb. per 200 lbs. bag of meal, alone or with vinegar, up to 8 pints per 200 lbs. bag of meal, do not affect the baking quality of the bread.

- b) The addition of calcium carbonate, in all the forms and amounts tried out, except precipitated chalk, has very little adverse effect on the amount of phytic acid destroyed during baking.
- c) The addition of vinegar significantly promotes the destruction of phytic acid.
- d) The increased destruction resulting from (c) is not seriously interfered with by the addition of calcium carbonate (all forms save precipitated chalk) in the amounts tried out.
- e) The destruction of phytic acid is unaffected by the amount of yeast used, but is promoted by increasing the period of fermentation before baking.
- f) Should the addition of calcium carbonate to the standard meal, at the rate of 1 lb. per 200 lbs. bag, be sanctioned, then the quantity of vinegar recommended for remedying "rope" development would have to be raised from 5 to 6 pints per 200 lbs. bag of meal.

4. The cost of the calcium carbonate to be added, and if necessary, of the vinegar also, is insignificant. Furthermore, the cost of actually adding these ingredients, at least for the larger mills, may likewise be insignificant. Nevertheless it must be stated that in South Africa with its very large number of small mills, the difficulties attending the effective supervision and control of the addition may well be insuperable.

S U M M A R Y A N D C O N C L U S I O N S .

S U M M A R Y A N D C O N C L U S I O N S .

INTRODUCTION.

1. The war compelled South Africa to adopt a standard loaf made from 95-100 per cent extraction meal. Conclusions from work carried out overseas indicated that as the extraction rate rises, the absorption of mineral salts, particularly of calcium, is adversely affected, owing to the interfering action of the increased amount of phytic acid phosphorus ingested. Since certain sections of the South African population are large consumers of bread, the National Nutrition Council considered that it was imperative to investigate the question under local conditions, using the procedure and methods fully described by McCance and Widdowson in 1942.

PART I.

2. In the investigation, the mineral metabolism of 4 healthy European adults, in respect of calcium, magnesium, iron, phosphorus, and phytic acid phosphorus, was studied continuously over periods ranging from 3 to 22 weeks. The periods of observation included usual diet, standard bread, and white bread diets (when sufficient bread was ingested to provide 40 to 50 per cent of the caloric requirement), and a return to the usual diet. In the two experimental diets, the calcium intake was maintained at a level of 10 mg. per Kg. body weight.
3. 3 of the 4 subjects, on changing from their usual diet with its high calcium content, to the standard bread diet with its adequate calcium but greatly increased phytic acid contents, showed immediate negative calcium balances. Gradually, adjustment occurred, and in time calcium began to be retained again and the losses sustained made good.

The 4th subject, whose calcium intake on his usual

diet/...

diet was the same as that on the standard bread diet, became adjusted sooner than did the other subjects, and his calcium loss was less than theirs.

4. The change in diet, caused the phytic acid ingested to be almost completely hydrolyzed; on returning to the usual diet, with its lower phytic acid intake, the proportion hydrolyzed fell to that previously observed.
5. The change from the standard bread to the white bread diet scarcely affected the subjects' retention of calcium.
6. The change to the standard bread diet occasioned temporary negative magnesium balances; it is considered that some precipitation of magnesium as phytate probably occurred during this period.
7. The latter dietetic change favoured the retention of iron and total phosphorus; but throughout the investigation, their metabolism was comparatively unaffected.
8. Summing up, the consumption of South African standard bread over long periods of observation had no deleterious effect on the metabolism of calcium.

PART II A.

9. Due to the remarkably high destruction of phytic acid that occurs during baking of standard bread, its phytic acid content is only slightly higher than that of the British National Wheatmeal loaf made from 85 per cent extraction meal. Thus, the disturbance in metabolism associated with the ingestion of the standard bread will be less than what was originally anticipated.

Experiments showed that no workable modification of the baking procedure could lower still further the phytic acid content.

PART IIB.

10. Although the addition of calcium carbonate to the standard bread, specifically on account of the phytic acid factor, has been shown to be unnecessary, its addition is favoured by general nutritional considerations.
11. Investigation showed that additions of calcium carbonate (except in the form of precipitated chalk) up to 1 lb. per 200 lbs. bag of meal, had no adverse effect on the baking quality of the bread; the effect on the pH value and hence on the liability to "rope" development was found to be insignificant; in addition the extent of the destruction of the phytic acid during baking was scarcely affected.
12. Additions of vinegar (frequently necessary to employ to counter "rope" development in hot humid areas) in amounts up to 8 pints per 200 lbs. bag of meal, had no adverse effect on the baking quality of the bread; the destruction of phytic acid during baking was significantly increased, even in the presence of calcium carbonate (except precipitated chalk), though not so much in its absence.

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APPENDIX.

Additional Data on Balance Experiments.

Table No. ³¹~~35~~.

<u>Subject : F.F.,</u>		<u>Preliminary Data.</u>				
<u>Period and Diet.</u>	<u>Body weight.</u>	<u>Urine.ml. per day.</u>	<u>Wet Faeces. g.per day.</u>	<u>Dry Faeces. g.per day.</u>	<u>Moisture in faeces per cent.</u>	<u>Mo-tions.</u>
A. 3 days. Normal Diet.	142 lbs.	1377 ml.	151 g.	44.5	70%	7
B. 22 days. Brown Bread Diet.	142 lbs.	1121 ml.	169 g.	45.1	73%	7

Table No. ³²~~36~~.

<u>Subject : A.W.,</u>		<u>Preliminary Data.</u>				
<u>Period and Diet.</u>	<u>Body weight.</u>	<u>Urine.ml. per day.</u>	<u>Wet Faeces. g.per day.</u>	<u>Dry Faeces g.per day.</u>	<u>Moisture in faeces per cent.</u>	<u>Mo-tions per week.</u>
A. 2 weeks. Normal Diet.	114 lbs.	1250 ml.	140 g.	41.9 g.	70%	14
B. 4 weeks. Brown Bread Diet.	110 lbs.	1039 ml.	156 g.	51.3 g	67%	10
C. 5 weeks. Brown Bread Diet.	110 lbs.	1031 ml.	142 g.	41.1 g.	71%	9
D. 4 weeks. White Rolls, etc., Diet.	111 lbs.	959 ml.	66g.	19.9 g.	70%	6.5
E. 4 weeks. White Bread Diet.	110 lbs.	706 ml.	68 g.	19.3 g.	72%	8
F. 3 weeks. Normal Diet.	112 lbs.	1003 ml.	138 g.	35.8 g.	74%	10

Table No. ³³₃₇.

<u>Subject : L.O.</u>		<u>Preliminary Data.</u>				
<u>Period and Diet.</u>	<u>Body weight.</u>	<u>Urine.ml. per day.</u>	<u>Wet Faeces. g.per day.</u>	<u>Dry Faeces. g.per day.</u>	<u>Moisture in faeces per cent.</u>	<u>Mo- tions per week.</u>
A. 2 weeks. Normal Diet.	140 lbs.	1062 ml.	111 g.	35.4 g.	68.5%	5
B. 5 weeks. Brown Bread Diet.	139 lbs.	856 ml.	144 g.	38.6 g.	73%	8
C. 3 weeks. Brown Bread Diet.	140 lbs.	983 ml.	149 g.	42.1 g.	70%	8
D. 3 weeks. White Bread Diet.	138 lbs.	1109 ml.	69 g.	24.2 g.	64%	5
E. 2 weeks. Normal Diet.	139 lbs.	1095 ml.	103.5 g.	30.4 g.	71%	5.5

Table No. ³⁴₃₈.

<u>Subject : L.G.</u>		<u>Preliminary Data.</u>				
<u>Period and Diet.</u>	<u>Body weight.</u>	<u>Urine.ml. per day.</u>	<u>Wet Faeces. g.per day.</u>	<u>Dry Faeces. g.per day.</u>	<u>Moisture in faeces per cent.</u>	<u>Mo- tions per week.</u>
A. 1 week Normal Diet.	164 lbs.	1130 ml.	200 g.	35.5 g.	82%	9
B. 4 weeks. Brown Bread Diet.	163 lbs.	1291 ml.	219 g.	54.0 g.	75%	13
C. 1 week. White Bread Diet.	164 lbs.	1643 ml.	166 g.	43.7 g.	74%	6
D. 1 week. Normal Diet.	165 lbs.	1314 ml.	90 g.	27.6 g.	69%	8

Additional Data. The effect on the phytic acid content of Standard Bread
when calcium carbonate and vinegar are added in varying
amounts to the meal.

All results expressed as milligrams phytic acid
phosphorus per 100 grams of bread.

Table No. ³⁵ 40.

A. Type of calcium carbonate:- Precipitated chalk.

Amount of calcium car- bonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.									
	Nil		5 pints		6 pints		7 pints		8 pints	
	<u>Bread.</u>									
	Dry Fresh.		Dry Fresh.		Dry Fresh.		Dry Fresh.		Dry Fresh.	
Nil	185	113	112	69	100	61	88	54	75	46
10 ounces	195	126	185	118	180	112	165	101	165	101
12 ounces	205	129	185	120	170	105	170	105	175	109
14 ounces	215	134	195	127	180	112	180	112	175	109
16 ounces	205	129	195	126	185	119	180	114	180	113

B. Type of calcium carbonate:- Creta Preparata.

Amount of calcium car- bonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.									
	nil		5 pints		6 pints		7 pints		8 pints	
	Bread.									
	Dry Fresh.		Dry Fresh.		Dry Fresh.		Dry Fresh.		Dry Fresh.	
Nil	155	95	112	69	100	61	88	54	75	46
10 ounces	160	98	125	77	120	73	104	64	90	56
12 ounces	160	98	121	74	120	73	100	61	95	58
14 ounces	170	103	125	77	124	77	105	65	100	61
16 ounces	175	108	130	79	125	77	110	68	104	63

C. Type of calcium carbonate:- Ground limestone 50 mesh.

Amount of calcium car- bonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.									
	Nil		5 pints		6 pints		7 pints		8 pints	
					Bread.					
	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.
Nil	150	91	112	69	100	61	88	54	75	46
10 ounces	155	94	115	70	104	63	95	58	80	49
12 ounces	150	91	115	70	105	64	96	59	80	50
14 ounces	155	95	118	71	105	64	100	61	84	51
16 ounces	160	98	120	73	110	68	102	63	90	56

D. Type of calcium carbonate: Ground Limestone.
Standard whiting.

Amount of calcium car- bonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.									
	Nil		5 pints		6 pints		7 pints		8 pints	
					Bread.					
	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.
Nil	165	100	112	69	100	61	88	54	75	46
10 ounces	170	103	125	79	115	71	99	60	95	57
12 ounces	170	104	124	80	110	68	105	64	94	58
14 ounces	164	99	130	79	118	72	105	63	100	62
16 ounces	170	103	130	79	120	74	108	66	108	66

E. Type of calcium carbonate:- Ground Limestone No.1.

Amount of calcium car- bonate per 200 lbs. bag.	Amount of vinegar per 200 lbs. bag.									
	Nil		5 pints		6 pints		7 pints		8 pints	
					Bread.					
	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.	Dry	Fresh.
Nil	155	94	112	69			88	54	75	46
10 ounces	160	97	115	68			100	61	85	52
14 ounces	160	96	120	72			100	61	85	52
16 ounces	165	100	124	76			103	63	95	58

F. Type of calcium carbonate:- Ground Limestone.No.4.

Amount of calcium car- bonate per 200 lbs. bag.	Amount of vinegar per 200 lbs.bag.							
	Nil		5 pints		7 pints		8 pints.	
	<u>Bread.</u>							
	Dry		Fresh.		Dry		Fresh.	
Nil	155	94	112	69	88	54	75	46
10 ounces	158	95	115	69	98	59	85	52
14 ounces	160	97	118	72	100	62	88	54
16 ounces	163	98	115	69	102	62	97	58

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Table 41 .

The effect of time of fermentation and the amount of yeast used on the phytic acid content of bread.

Results expressed as milligrams phytic acid phosphorus per 100 grams bread.

Time of fermentation	Proportion of yeast.			
	1.5 per cent.		3.0 per cent.	
	Dry loaf	Fresh loaf.	Dry loaf	Fresh loaf.
1 hour	189	113	184	109
2 hours	179	109	175	104
3 hours	144	88	140	84
4 hours	125	76	115	73

Phytic acid content of untreated normally baked loaf =
140 mg. per 100 grams dry bread.

Phytic acid content of untreated normally baked loaf =
83 mg. per 100 grams fresh bread.