REFERENCES

Barnett, J., Henly, A. A. & Morris, C. J. O. R. (1946). *Biochem. J.* **40**, 445.
Barnett, J., Henly, A. A., Morris, C. J. O. R. & Warren, F. L. (1946). *Biochem. J.* **40**, 778.
Baumann, E. J. & Metzger, N. (1940). *Endocrinology*, **27**, 664.
Cahen, R. L. & Salter, W. T. (1944). *J. biol. Chem.* **152**, 489.
Crooke, A. C. & Callow, R. K. (1939). *Quart. J. Med.* (N.S.), **8**, 233.
Dingemanse, E., Huis in't Veld, L. G. & de Laat, B. M. (1946). *J. clin. Endocrinol.* **6**, 535.
Dobriner, K., Gordon, E., Rhoads, C. P., Lieberman, S. & Fieser, L. F. (1942). *Science*, **95**, 534.
Frame, E. G. (1944). *Endocrinology*, **34**, 175.
Freeman, W., Pincus, G. & Glover, E. D. (1944). *Endocrinology*, **35**, 215.
Pearlman, W. H. (1942). *Endocrinology*, **30**, 270.
Pincus, G. (1943*a*). *J. clin. Endocrinol.* **3**, 301.
Pincus, G. (1943*b*). *Endocrinology*, **32**, 176.
Pincus, G. (1945). *J. clin. Endocrinol.* **5**, 291.
Salter, W. T., Cahen, R. L. & Sappington, T. S. (1946). *J. clin. Endocrinol.* **6**, 52.
Scott, W. W. & Vermeulen, C. (1942). *J. clin. Endocrinol.* **2**, 450.
Talbot, N. B., Butler, A. M. & Berman, R. A. (1942). *J. clin. Invest.* **21**, 559.
Talbot, N. B., Butler, A. M. & MacLachlan, E. (1940*a*). *J. biol. Chem.* **132**, 595.
Talbot, N. B., Butler, A. M. & MacLachlan, E. (1940*b*). *New Engl. J. Med.* **223**, 369.

Studies in Human Mineral Metabolism

1. THE EFFECT OF BREAD RICH IN PHYTATE PHOSPHORUS ON THE METABOLISM OF CERTAIN MINERAL SALTS WITH SPECIAL REFERENCE TO CALCIUM

BY A. R. P. WALKER, F. W. FOX AND J. T. IRVING, *The South African National Nutrition Council, the South African Institute for Medical Research, Johannesburg, and the Department of Physiology, University of Cape Town*

(*Received* 4 *September* 1947)

The discovery that phytic acid forms relatively insoluble salts with several of the common bases, that are of importance in our diet, has led to several investigations in which the availability of these bases has been determined with varying intakes of phytic acid. The conclusions drawn from such work have been somewhat contradictory: thus, whilst all observers are agreed that the addition of a considerable amount of phytate P is invariably followed by an immediate reduction in the amount of Ca retained, some investigators have suspected, and others are emphatic, that, given time, an adaptation to such a change can occur, and that eventually the body is able to adjust itself fairly satisfactorily to the new conditions.

Obviously laboratory investigations must eventually be reconciled with what happens in everyday life, and a consideration of those human diets which are rich in cereals and hence in phytic acid, yet poor in Ca, lends a considerable amount of support to the view that some such process of adaptation must in fact take place. Otherwise it is difficult to explain how the consumers avoid the disastrous consequences which would arise if part or the whole of this Ca were rendered unavailable.

For two reasons this issue is of particular importance to a country like South Africa. In the first place the large majority of the population, including most of the non-Europeans and not a few of the poorer Europeans, live on just such a diet as has been mentioned above. Secondly, in 1941, a compulsory Standard War Bread, made from 95 to 100% extraction meal, was introduced and is still the only kind of bread allowed to be baked.

As a result of the work published by McCance & Widdowson (1942), the South African National Nutrition Council decided that it was imperative to study the problem under local conditions. For this purpose balance experiments, similar to those described by these workers, were initiated to observe the effects of breads made from high and low extraction meals upon mineral metabolism in adult male subjects.

EXPERIMENTAL

Plan of the experiments. The intake and excretion of Ca, Mg, total P, phytate P and Fe, were determined on four healthy adult European males, over periods ranging from 3 to 19 *consecutive* weeks.

The experimental periods were planned as follows: *A*, from 1 to 2 weeks on the usual everyday diet; *B*, from 4 to 9 weeks on a diet which contained 1 lb. of Standard War Bread (experimental diet no. 1); *C*, from 1 to 4 weeks in which the war bread was replaced by 1 lb. of white bread (70% extraction; experimental diet no. 2). For the first subject only the first two of these periods were observed, as this part of the experiment was of a preliminary nature carried out to gain acquaintance with the procedure.

The balance procedure and the analytical methods employed followed those described by McCance & Widdowson (1942), except in the following details: (i) Tap water was used throughout. (ii) Experience showed that the analytical procedure was facilitated by keeping the food samples separate for the following groups: milk, drinks, fatty foods, eggs, bread, and the remaining foodstuffs, i.e. soup, meat, vegetables, fruit, puddings and so forth. To obtain a thoroughly representative sample this last group was taken to dryness and finely ground. (iii) The total faeces for the week were also taken to dryness and finely ground before sampling.

Subjects. Three of the subjects were research biochemists and the fourth a reliable laboratory technician. During the investigation they lived in their homes and pursued their usual avocations. Health remained excellent in all cases throughout the investigation; no digestive or other disturbances were reported, and fluctuations in weight were insignificant. Some inconvenience was experienced in consuming the required amount of bread, but apart from the tedium inevitably associated with the daily weighing of all foods, etc., no difficulties were encountered.

RESULTS

Phytate-phosphorus content of meal and bread

Analysis of 12 samples of our meal showed that it contained an average of 205 mg. phytate P/100 g. as compared with 214 mg. found for the overseas 92% extraction product. In addition, when samples of bread obtained from bakeries throughout the country were analyzed, it was found that the average figure for 33 loaves was 50 mg. phytate P for 100 g. of fresh bread, as against 'about 100 mg.' found by McCance & Widdowson. Hence our meals were not only no richer in phytate P, but evidently a far greater destruction of this substance had occurred during the baking than in the case of the British 92% extraction loaf.

For the sake of brevity the terms 'brown' and 'white' breads are employed when referring to the 95–100% and the 70% extraction breads respectively.

Results of balance experiments

In view of the recent findings of Steggerda & Mitchell (1946*b*) that faecal Ca may not represent only unabsorbed Ca, attention has been focused solely upon the daily balance of the various elements.

Calcium metabolism

The results are given in Table 1.

A. *Period on the usual diet.* This period was included in order to evaluate the subject's nutritional state before the experimental diets were given. It will be seen that the subjects A.W., L.O. and L.G. were all in slight positive balance.

B. *Experimental diet no.* 1. The subjects were required to consume 1 lb. of Standard War Bread daily, but otherwise they were permitted to eat what they liked, except that the total Ca intake was regulated by adjusting the amount of milk and cheese consumed so as to comply with the requirements based on body weight (10 mg./kg. daily; see p. 458).

Subject F.F. As mentioned above, this study was of a preliminary nature. The subject immediately went into negative Ca balance, which however became less as the experiment proceeded. It was this fact, together with the gradual improvement in the amount of Ca retained, which made us realize the necessity for continuing our observations for prolonged periods. Accordingly it was decided to commence a more lengthy study, during which the degree of adjustment to each change in diet could be followed as quickly as analytical determinations of Ca would permit. In practice it was found possible to be not more than 1 week behind with these determinations. This enabled us to decide when a steady state had been reached, so that the next part of the investigation could be safely proceeded with.

Subject A.W. For no less than 4 weeks this subject remained in continuous negative balance (designated period B_1); this was followed by a further 5 weeks (designated period B_2) during which the balance was continuously positive, or almost positive. If McCance & Widdowson's (1942) suggested limits of significance of ± 25 mg./day are accepted, this subject may be regarded as having reached a state of equilibrium.

Subject L.O. This subject needed 5 weeks to adjust to the new diet (period B_1) and he then remained in equilibrium for the following 3 weeks (period B_2). It must, however, be mentioned that he experienced some difficulty in consuming the full 1 lb. of war bread daily, and hence his intake of other foods tended to suffer. In order to supply the daily requirement of Ca estimated on body weight, a small additional allowance of milk was therefore permitted after the 5th week.

Subject L.G. This subject proved of particular interest to us, since his Ca intake on his usual diet was found to be only just adequate for maintenance. With the experimental diet his Ca intake remained unchanged, and hence, unlike the other subjects, he only had to adjust to the increased ingestion of phytate P. It is presumably for this reason that he

Table 1. *Daily intake, excretion, and retention of calcium and magnesium, from the usual, brown-bread, and white-bread diets*

Period: Duration and sequence	Period: Designation	Diet consumed	(1) Calcium: Intake (mg.)	Urine (mg.)	Faeces (mg.)	Total excretion (mg.)	Balance (mg.)	(2) Magnesium: Intake (mg.)	Urine (mg.)	Faeces (mg.)	Total excretion (mg.)	Balance (mg.)
(days)		Subject F.F. Weight 65 kg. Age 50 years										
3	*A*	Usual diet	—	225	914	1139	—	—	98	300	398	—
4	*B*	Experimental diet no. 1 including 1 lb. of standard war bread daily	535	192	504	696	−161	490	108	372	480	+ 10
7	*B*	,, ,, ,,	501	235	512	747	−246	480	89	395	484	− 4
7	*B*	,, ,, ,,	490	210	350	560	− 70	535	91	440	531	+ 4
4	*B*	,, ,, ,,	533	205	390	595	− 62	560	93	450	543	+ 17
		Average figures for period *B*	510	214	437	651	−141	514	94	415	509	+ 5
(weeks)		Subject A.W. Weight 51 kg. Age 31 years										
1	*A*	Usual diet	1160	246	848	1094	+ 66	495	80	405	485	+ 10
2	*A*	,,	1175	256	865	1121	+ 54	490	81	415	496	− 6
		Average figures for period *A*	1167	251	856	1107	+ 60	492	80	410	490	+ 2
3	B_1	Experimental diet no. 1 including 1 lb. of standard war bread daily	510	194	402	596	− 86	525	86	450	536	− 11
4	B_1	,, ,, ,,	512	203	387	590	− 78	550	79	620	699	−149
5	B_1	,, ,, ,,	524	220	373	593	− 69	575	126	570	696	−121
6	B_1	,, ,, ,,	501	269	297	566	− 65	575	133	545	678	−103
		Average figures for period B_1	512	221	365	586	− 74	556	106	546	652	− 96
7	B_2	Same as in period B_1	509	225	255	480	+ 29	500	59	430	489	+ 11
8	B_2	,, ,,	515	217	320	537	− 22	585	75	560	635	− 50
9	B_2	,, ,,	509	201	321	522	− 13	575	79	485	564	+ 11
10	B_2	,, ,,	561	219	295	514	+ 47	580	87	475	562	+ 18
11	B_2	,, ,,	488	196	257	453	+ 35	500	77	415	492	+ 8
		Average figures for period B_2	516	212	289	501	+ 15	547	75	473	548	− 1
12	C_1	Experimental diet no. 2*a* including milk, white rolls and potatoes daily	440	170	235	405	+ 35	310	76	225	301	+ 9
13	C_1	,, ,, ,,	486	145	272	417	+ 69	337	91	235	326	+ 11
14	C_1	,, ,, ,,	491	183	285	468	+ 23	300	75	210	285	+ 15
15	C_1	,, ,, ,,	499	170	260	430	+ 69	275	80	185	265	+ 10
		Average figures for period C_1	479	167	263	430	+ 49	305	80	214	294	+ 11
16	C_2	Experimental diet no. 2*b* including 1 lb. of white bread daily	512	245	235	480	+ 32	290	75	195	270	+ 20
17	C_2	,, ,, ,,	503	260	230	490	+ 13	285	80	195	275	+ 10
18	C_2	,, ,, ,,	471	200	225	425	+ 46	250	56	190	246	+ 4
19	C_2	,, ,, ,,	498	155	255	410	+ 88	245	36	195	231	+ 14
		Average figures for period C_2	496	215	236	451	+ 45	268	62	194	256	+ 12

		Subject L.O. Weight 64 kg. Age 28 years										
1	*A*	Usual diet	1090	139	936	1075	+ 15	512	146	360	506	+ 6
2	*A*	,,	927	132	780	912	+ 15	435	92	335	427	+ 8
		Average figures for period *A*	1008	135	858	993	+ 15	473	119	347	466	+ 7
3	B_1	Experimental diet no. 1 including 1 lb. of standard war bread daily	465	117	355	472	− 7	410	145	295	440	− 30
4	B_1	,, ,, ,,	485	123	375	498	− 13	440	190	322	512	− 72
5	B_1	,, ,, ,,	506	149	394	543	− 37	480	138	380	518	− 38
6	B_1	,, ,, ,,	504	155	367	522	− 18	508	130	430	560	− 52
7	B_1	,, ,, ,,	465	141	450	591	−126	550	101	510	611	− 61
		Average figures for period B_1	485	137	388	525	− 40	478	141	387	528	− 50
8	B_2	Same as in period B_1	620	133	480	613	+ 7	551	110	440	550	+ 1
9	B_2	,, ,,	630	97	520	617	+ 13	482	70	425	495	− 13
10	B_2	,, ,,	660	140	510	650	+ 10	566	120	428	548	+ 18
		Average figures for period B_2	636	123	503	626	+ 10	533	100	431	531	+ 2
11	*C*	Experimental diet no. 2 including 1 lb. of white bread daily	750	182	517	699	+ 51	318	111	202	313	+ 5
12	*C*	,, ,, ,,	720	170	535	705	+ 15	278	115	166	281	− 3
13	*C*	,, ,, ,,	695	110	495	605	+ 90	265	102	153	255	+ 10
		Average figures for period *C*	722	154	516	670	+ 52	287	109	174	283	+ 4
		Subject L.G. Weight 75 kg. Age 35 years										
1	*A*	Usual diet	763	193	537	730	+ 33	443	134	335	469	− 26
2	*B*	Experimental diet no. 1 including 1 lb. of standard war bread daily	740	269	550	819	− 79	790	137	584	721	+ 69
3	*B*	,, ,, ,,	719	220	504	724	− 5	677	172	528	700	− 23
4	*B*	,, ,, ,,	705	210	485	695	+ 10	735	175	518	693	+ 42
5	*B*	,, ,, ,,	880	233	595	828	+ 52	755	190	560	750	+ 5
		Average figures for period *B*	761	233	533	766	− 5	739	169	547	716	+ 23
6	*C*	Experimental diet no. 2 including 1 lb. of white bread daily	890	320	535	855	+ 35	465	185	270	455	+ 10

required only 3 weeks to reach equilibrium. After 2 weeks of positive Ca balance it was considered permissible to proceed with the next experimental diet.

C. *Experimental diet no.* 2. *Subject A.W.* Before passing on to the white-bread diet, this subject for a period of 4 weeks was given a diet in which the Ca supplied by the war bread was replaced mainly from milk. The supply of calories was maintained by means of white rolls and extra potatoes (period C_1). The amount of phytate P was thus very considerably reduced, although not as much as might be expected, since white rolls contain more of this substance than white bread made from the same flour. The Ca retention of the first balance period on this diet was the same as that of the last of the previous series. Thereafter the balances became more positive.

After this period of 4 weeks, the subject was placed on a diet in which the rolls and extra potatoes were replaced by 1 lb. white bread (period C_2). It will be seen that the retention of Ca was hardly affected. This period was continued for 4 weeks, and the consistency of the results showed that the subject was well adjusted to the change.

Subjects L.O. and L.G. changed over directly to the white-bread diet without the intervening period C_1. (It is unfortunate that, for reasons beyond our control, both L.O. and L.G. inadvertently consumed slightly more Ca than had been intended.) L.O. showed a slight improvement in Ca retention, while L.G. was virtually in equilibrium in the single balance period in which he was studied.

Magnesium metabolism

The intake of Mg, as will be seen from Table 1, even on the white-bread diet, was always ample for maintenance, i.e. it exceeded 250 mg. daily, the standard suggested by Duckworth & Warnock (1942).

McCance & Widdowson's (1942) subjects were in equilibrium throughout their experiments, but we found, on passing from the usual to the brown-bread diet, that two of our subjects began to lose more Mg than they ingested. As with Ca, however, Mg retention gradually improved. The urinary excretion of Mg was markedly increased during part of the brown-bread-diet period. During the white-bread period, the same total amount was retained as in the previous period, although the intake was only half of that on the brown-bread diet. The possible effect of variations in the Mg intake upon the utilization of Ca is referred to in a later section.

Total phosphorus metabolism

For the sake of brevity the metabolism data for total P and phytate P have been given in Tables 2 and 3 as averages for the different dietetic periods. This course was adopted because the weekly figures during those periods differed little from one another.

The metabolism of total P showed least disturbance of all the elements studied with the changes in diet. Although the amount consumed on the white-bread diet was less than that on the brown-bread diet, the retention rose somewhat. McCance & Widdowson (1942) found the same balance with either type of bread.

Table 2. *The daily intake, excretion, and retention of total phosphorus from the usual, brown-bread, and white-bread diets*

Period: Duration	Period: Designation	Diet consumed*	Intake (mg.)	Urine (mg.)	Faeces (mg.)	Total excretion (mg.)	Balance (mg.)
(days)	Subject F.F.*						
3	*A*	Usual diet	—	1520	650	2170	—
22	*B*	Brown-bread diet	885	1435	454	1889	− 4
(weeks)	Subject A.W.*						
2	*A*	Usual diet	1620	1020	542	1562	+ 58
4	B_1	Brown-bread diet	1415	870	529	1399	+ 16
5	B_2	Brown-bread diet	1380	862	483	1345	+ 35
4	C_1	Diet of milk, white rolls and potatoes	1107	760	291	1051	+ 56
4	C_2	White-bread diet	995	685	239	924	+ 71
	Subject L.O.*						
2	*A*	Usual diet	1495	740	752	1492	+ 3
5	B_1	Brown-bread diet	1559	1022	579	1601	− 42
3	B_2	Brown-bread diet	1716	1008	663	1671	+ 45
3	*C*	White-bread diet	1178	705	395	1100	+ 78
	Subject L.G.*						
1	*A*	Usual diet	1600	1000	560	1560	+ 40
4	*B*	Brown-bread diet	1825	1097	769	1866	− 41
1	*C*	White-bread diet	1540	930	500	1430	+110

* For fuller details see Table 1.

Table 3. *The daily intake, excretion, and retention of phytate phosphorus from usual, brown-bread, and white-bread diets*

Period: Duration	Period: Designation	Diet consumed*	Intake (mg.)	Faeces (mg.)	Phytate hydrolyzed (%)
(days)	Subject F.F.*				
3	*A*	Usual diet	—	135	—
22	*B*	Brown-bread diet	425	56	87
(weeks)	Subject A.W.*				
2	*A*	Usual diet	178	65	64
4	B_1	Brown-bread diet	289	33	88
5	B_2	Brown-bread diet	280	24	91
4	C_1	Diet of milk, white rolls and potatoes	84	26	69
4	C_2	White-bread diet	70	6	91
	Subject L.O.*				
2	*A*	Usual diet	142	53	63
5	B_1	Brown-bread diet	289	26	90
3	B_2	Brown-bread diet	345	38	89
3	*C*	White-bread diet	41	22	46
	Subject L.G.*				
1	*A*	Usual diet	105	60	43
4	*B*	Brown-bread diet	315	63	80
1	*C*	White-bread diet	90	50	44

* For fuller details see Table 1.

Table 4. *The daily intake, excretion, and retention of iron, from usual, brown-bread, and white-bread diets*

Period: Duration	Period: Designation	Diet consumed*	Intake (mg.)	Urine (mg.)	Faeces (mg.)	Total excretion (mg.)	Balance (mg.)
(days)	Subject F.F.*						
3	*A*	Usual diet	—	1·0	20·5	21·5	—
22	*B*	Brown-bread diet	26·1	0·4	24·6	25·0	+1·1
(weeks)	Subject A.W.*						
2	*A*	Usual diet	20·5	0·5	19·7	20·2	+0·3
4	B_1	Brown-bread diet	26·0	0·4	25·0	25·4	+0·6
5	B_2	Brown-bread diet	23·9	0·7	22·7	23·4	+0·5
4	C_1	Diet of milk, white rolls and potatoes	14·4	0·2	13·5	13·7	+0·7
4	C_2	White-bread diet	16·0	0·2	15·0	15·2	+0·8
	Subject L.O.*						
2	*A*	Usual diet	14·3	1·1	13·0	14·1	+0·2
5	B_1	Brown-bread diet	19·2	1·3	17·9	19·2	±0·0
3	B_2	Brown-bread diet	23·1	0·9	21·8	22·7	+0·4
3	*C*	White-bread diet	15·1	1·3	13·1	14·4	+0·7
	Subject L.G.*						
1	*A*	Usual diet	18·3	1·2	16·2	17·4	+0·9
4	*B*	Brown-bread diet	25·8	1·4	24·1	25·5	+0·3
1	*C*	White-bread diet	22·7	2·7	19·0	21·7	+1·0

* For fuller details see Table 1.

Phytate-phosphorus metabolism

When the Ca intake was high and that of phytate P comparatively low (i.e. on the usual diet), an average of 59 % of the phytate P was hydrolyzed, whereas when the Ca intake was just adequate for maintenance and phytate-P intake high, an average of 84 % was hydrolyzed. Judging by the conclusions reached by Harrison & Mellanby (1939), one might argue that in the absence of an abundance of Ca, the phytate P forms more readily hydrolyzable salts with bases such as Na and K. The condensed arrangement of the tables, however, masks the interesting fact that, after the change from the usual to the brown-bread diet, the amount of phytate P hydrolyzed reached its maximum extent *immediately*, without the incremental steps of adjustment found for Ca and Mg.

Iron metabolism

Following the suggestion made by McCance & Widdowson (1942), we carried out Fe balances for each subject, mainly because they afforded a simple means of checking the accuracy of the experimental procedure, more especially the marking off of the different faecal periods. The results (Table 4) show that the consumption of brown bread did not affect the amount of Fe retained. Such a conclusion would tend to support the work of several investigators who have found that the consumption of higher extraction breads had no deleterious effect upon the retention of this metal.

DISCUSSION

Environment

When a comparison is made between the experiments of McCance & Widdowson (1942) and our own, it should be pointed out that the present study extended over the different seasons. Owing to our climatic conditions, the subjects not only consumed food which had been fully irradiated by the sun, but were themselves continuously exposed to bright light at an altitude of approximately 6000 feet. There was thus this difference between the two studies, but, as shown by the very full review given by McCance & Widdowson, there is little evidence to suggest that the addition of vitamin D to the diet of healthy adults has any appreciable effect upon the absorption of Ca.

Calcium balances

At the outset we desire to draw attention to certain sources of error inherent in balance studies of this particular kind, which do not always appear to be sufficiently appreciated.

In recent years evidence has been accumulating that, when an individual who is habituated to one level of Ca intake is suddenly placed on a lower level, a period of adjustment is inevitable and may even be somewhat prolonged (Nicholls & Nimalasuriya, 1939; Cathcart, 1940; Steggerda & Mitchell, 1941; Kraut & Wecker, 1943). According to these workers the time taken to reach a steady state at the new level of Ca intake depends upon the magnitude of the difference between the two levels. It must also depend upon the extent to which the new supplies of Ca are available. Thus, if the reduction of the Ca intake is accompanied by an increase in the phytate-P intake, the negative balances following such changes cannot be interpreted as being solely due to the interfering effects of the phytate P.

Also, when passing from a high to a lower Ca intake it is necessary to insure that at least enough Ca is provided to satisfy the minimum requirements of the individual concerned. Otherwise the negative balances that will be observed are likely to be due to what may be termed an 'excretion momentum', in addition to any effects arising from a change in the availability of the supply. The difficult question of what constitutes a minimum daily requirement of Ca has recently been the subject of intensive study by Leitch (1937), Holmes (1945) and more especially Mitchell and his associates (Mitchell & Curzon, 1939; Steggerda & Mitchell, 1939, 1941, 1946*a*). These workers have reached substantial agreement with a figure of about 10 mg./kg. body weight, and we accordingly adopted this standard when planning our experiments.

Furthermore, in the reports given by some investigators the experimental periods are interrupted by days or weeks during which the food consumed is not specified. Unless the same level of Ca intake is maintained it seems inevitable that such interruptions will affect the subsequent observations. Lastly, there are other workers who employ such short experimental periods, and change the diets so frequently, that it is questionable whether the body has time to adjust itself to one diet before the next is taken. An extreme example of this is to be seen in the study reported by Wang, Liu, Chu, Yu, Chao & Hsu (1944), where, within the space of 38 days the diet was changed no less than twelve times.

The assimilation of calcium in the presence of phytic acid

Although our main experiments were carried out on only three subjects, the results obtained confirmed one another.

It is not easy to compare our findings with those reported by other observers because of the comparatively short experimental periods usually employed by them. Thus, whilst the experiments reported by McCance & Widdowson (1942) lasted intermittently over 9 months, the actual periods of continuous observation were usually 3–4 weeks. These workers were emphatic that there was no indication of any adaptation occurring. Similarly, the experimental periods employed by Krebs & Mellanby (1943) lasted for 4 weeks; but it is worth noting that before the experiments on the 85% extraction bread terminated, one of their six volunteers (no. 3) was already showing improvement in the retention of Ca and finally reached equilibrium. In their recent experiments with children, Hoff-Jørgensen, Andersen & Nielsen (1946) suspected that the retention of Ca was gradually improving with time. (We presume, although this is not stated, that the same diet, rich in phytic acid, was continued during the short intervals that occurred between their 5-day periods.) Indeed, had we discontinued our observations after 3–4 weeks on the high-extraction bread diet, our findings would have been very similar to those obtained by these three groups of investigators.

Combining our own evidence with that already available, we suggest the following: subjects passing abruptly from their usual diet to one containing less Ca and much more phytate P show an immediate negative Ca balance. As the body becomes accustomed to such a diet, the retention of Ca improves, so that, *given time*, equilibrium again occurs and the losses of Ca are slowly made good. The period of adjustment is shorter, and the loss of Ca is less, if the disparity between the usual Ca intake and that of the experimental diet is small.

Evidence available from studies made on individuals habituated to diets high in phytate phosphorus and low in calcium.

There is, however, another method of approach which throws valuable light on the whole problem. Instead of placing a few selected individuals on prescribed diets and then observing how they adjust themselves to the new conditions over shorter or longer periods (which for obvious practical reasons can never be really very prolonged relative to the life span), it ought to be possible to gain more reliable information by studying people who, through force of circumstances, are compelled to subsist for long periods on diets which are very rich in phytate P and very low in Ca. Some of the few available Ca balance studies that are on record for such people have been assembled in Table 5.

Although we have deliberately selected examples where the Ca balances were positive, it may be stated that this obtained in all the cases reported by Nicholls & Nimalasuriya (1939); in two out of the five cases reported by Henderson & Kelly (1929–30) for boys on prison diet; in two out of four adult cases on the same type of diet (Kelly & Henderson, 1929–30); and in two out of the three subjects studied by Basu, Basak, & Sircar (1939). This must presumably indicate that such positive balances occur fairly frequently. Even the survival of these individuals on such diets is remarkable. That they are somewhat shorter and lighter than well nourished Europeans is admitted, but with a diet lacking in so many other respects it is hardly fair to attribute this solely to a lack of available Ca. As far as our rural South African Bantu are concerned there is not much evidence of poor skeletal calcification, whilst the teeth are often well formed and sometimes remarkably free from caries. Thus in a recent careful dental survey carried out on some 1000 Bechuanaland children, whose diet consists mainly of Kaffir corn, no less than 70% were found to be free from all signs of caries. Equally striking results have been reported by Wilson & Widdowson (1942) for Indian children.

It must be admitted that such observations cannot easily be reconciled with orthodox opinions; however, we do not consider it satisfactory to dispose of them as being due to racial differences, but think rather that they reflect an adaptation which any human being may be capable of making under similar circumstances. That somewhat similar adaptation can be achieved by Europeans is suggested by the experience of the French people during the occupation. They consumed a diet extremely low in Ca and including high-extraction bread (Paris

Table 5. *Examples of positive calcium balances observed with non-Europeans of different races when consuming their typical cereal diets, rich in phytate phosphorus and low in calcium*

Diet and authority	Sex and age (yr.)	Experimental period (days)	Intake (mg./day)	Urine (mg./day)	Faeces (mg./day)	Total excretion (mg./day)	Balance (mg./day)
(1) Nicholls & Nimalasuriya (1939). Sinhgalese children consuming usual diet of cereals, legumes, roots and vegetables	*M* 4	3	205	25	55	80	+125
	M 4	3	183	24	96	120	+ 63
	F 7	3	245	29	135	164	+ 81
	M 7	3	70	4	15	19	+ 51
	M 7	3	223	7	47	54	+169
	M 7	3	220	5	45	50	+170
(2) Henderson & Kelly (1929–30). Bantu boys consuming long term prison diet including 1·5 lb. maize, beans, potatoes, fat and meat. Average of two 4-day periods taken during a 21-day study	*M* 16	8	300	23	271	294	+ 6
	M 16	8	300	19	242	261	+ 39
(3) Kelly & Henderson (1929–30). Bantu adults consuming diet almost the same as in (2). Average of a 4-day period taken once during a 42-day study. Prison diets (2) and (3) both resembled diets commonly consumed	*M*	4	300	23	151	174	+126
	M	4	300	10	172	182	+118
(4) Basu *et al.* (1939). Indian adults. Diet (*a*) included 400 g. rice; average of 6-day period. Diet (*b*) included 600 g. whole wheat: average of 12 consecutive days	(*a*) *M*	6	280	70	162	232	+ 48
	(*b*) *M*	12	310	19	167	186	+124

letter, 1942), but it is claimed that the decalcification which might have been expected did not take place (Paris letter, 1946).

It is possible that some similar process of adaptation had occurred, though to a lesser extent, in our subjects, since for several years the inhabitants of South Africa have been restricted solely to the use of high extraction bread, and hence some degree of habituation to it may have occurred. This capacity for adjustment may well have been lacking in the subjects studied by McCance & Widdowson (1942).

The mechanism of adaptation to diets with a high phytic acid content

This adaptation can be explained in two ways: one based on our knowledge of the chemical changes which phytates may undergo in the intestine, the other on the body's known ability to adapt itself to a lower intake of Ca.

(*a*) *The digestion of phytate phosphorus.* Most workers seem to agree that phytate P and Ca combine to form an insoluble salt in the intestine. Thus Harrison & Mellanby (1939) from their work on puppies remark: 'It is not possible to say whether such actual precipitation occurs under the conditions present in the gut, but it seems not unlikely.' McCance (1946), discussing the position as far as humans are concerned, concludes that phytic acid 'precipitates Ca in the intestine and by so doing prevents its absorption'. Assuming that the pH values in the intestinal tract of man resemble those observed in the pig, Møllgaard, Lorenzen, Hansen & Christensen (1946) conclude that 'phytic acid may precipitate Ca even in the first part of the small intestine thus causing a serious fall in its absorption'. Hence the consensus of opinion is definitely that the precipitation of Ca by phytic acid occurs, and the fact must therefore be accounted for that much of this Ca is eventually absorbed.

There is general agreement that some degree of hydrolysis of phytate P occurs in the digestive tract. This has been demonstrated for pigs, rats, dogs and hens. Similar results for man have been reported by McCance & Widdowson (1935), Wang *et al.* (1944), Cruickshank, Duckworth, Kosterlitz & Warnock (1945), and recently for babies and children by Hoff-Jørgensen, Andersen, Begtrup & Nielsen (1946) and Hoff-Jørgensen, Andersen & Nielsen (1946).

As far as animals are concerned, the extent to which hydrolysis occurs appears to depend on the level of the Ca intake, e.g. for rats, hens and dogs it has been found that the lower the Ca intake the greater the amount of phytate P hydrolyzed. Likewise for man Cruickshank *et al.* (1945), in a study in which the cereal employed was oatmeal, found that the amount of phytate P hydrolyzed was inversely proportional to the Ca intake. Similar results are reported in the present paper.

At present there are two views as to how this hydrolysis is brought about in man. Some maintain that it is due to the action of the intestinal flora in the digestive tract, whilst Møllgaard *et al.* (1946) insist that hydrolysis can only occur when the diet contains a specific enzyme, which they regard as being normally destroyed when food is cooked.

Both in the case of animals and man there seems to be no difference of opinion that at least a proportion of the P liberated by hydrolysis is absorbed. The question at issue is whether hydrolysis occurs at such a level as will also permit of the liberated Ca being absorbed.

It has been shown that under certain circumstances this does take place in the case of pigs, rats, hens and dogs, and it is of interest to note that Hoff-Jørgensen, Andersen, Begtrup & Nielsen (1946) conclude from their experiments on children 'that a small part of the Ca which was precipitated as Ca phytate was absorbed'. Similarly, Wilson & Widdowson (1942) reviewing their Indian observations, where they were dealing with diets which contained a great deal more phytic acid P than was sufficient to precipitate all the Ca present, came to the same conclusion.

A further interesting point arises from a consideration of the part Mg may play in this connexion. It is more than likely that the phytate P precipitates in the intestinal tract a compound which is not solely the Ca salt, as seems to be assumed by Hoff-Jørgensen (1946), Hoff-Jørgensen, Andersen, Begtrup & Nielsen (1946) and Hoff-Jørgensen, Andersen & Nielsen (1946). Both Harrison & Mellanby (1939) and McCance & Widdowson (1942) have shown that when precipitation occurs *in vitro* in the presence of both bases, a mixed phytate is formed containing approximately equal equivalents of Ca and Mg. The latter workers, who attempted to simulate the conditions which occur in the intestinal tract in regard to pH value, are of the opinion that a precipitate of this nature is likely to be formed. Obviously this is an important point, for it would decide the amount of Ca made less available by a given amount of phytate P. Our own results tend to support the proposition that phytic acid reacted with Mg as well as with Ca.

(*b*) *The ability of the body to adapt itself to a lowered calcium intake.* Irrespective of the composition of the diet, it is now well known that the body can adapt itself to a Ca intake often well below 10 mg./kg. body weight daily. This has been stressed by Nicholls & Nimalasuriya (1939), Owen, Irving & Lyall (1940), Steggerda & Mitchell (1941, 1946*a*) and Kraut & Wecker (1943). This adaptation, in the present experiments, was not due only to increased hydrolysis of phytate, since the increased hydrolysis took place immediately the brown-bread regime was instituted, whereas the adaptation was gradual.

The ability of the body in this respect has been well summarized by Steggerda & Mitchell (1941) as follows: 'It appears that in the presence of an inadequate supply of any nutrient, including Ca, 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 food supply.' These writers (Steggerda & Mitchell, 1946*a*) consider that no harm can come from such an adaptation, provided the Ca intake is 10 mg./kg. body weight daily.

This work was originally undertaken to find out if the results of McCance & Widdowson (1942) applied in South Africa. It is evident that our results confirm those of these workers in so far as we have shown that a high-brown-bread regime renders a certain amount of the dietary Ca less available. However, further evidence shows beyond doubt that the body can adjust to this lessened availability, and that the consumption of such a diet over long periods has no deleterious effect upon Ca metabolism.

SUMMARY

1. The metabolism of calcium, magnesium, phosphorus, phytate phosphorus, and iron has been followed in three healthy adult European males for periods lasting from 7 to 19 *consecutive* weeks. During this time the subjects consumed (*a*) their usual diet, (*b*) a diet including 1 lb. of bread made from 95 to 100% extraction meal, and (*c*) a diet including 1 lb. of bread made from 70% extraction meal. During dietary periods (*b*) and (*c*) the daily calcium intake was restricted to a level of approximately 10 mg./kg. of the subject's body weight.

2. When the subjects passed abruptly from their usual diet to one containing much more phytate phosphorus and, in two cases, less calcium, they showed an immediate negative calcium balance.

3. As they became accustomed to such a change, the retention of calcium improved, so that, *given time*, equilibrium was again reached and the losses of calcium were slowly made good.

4. The period of adjustment was shorter, and the loss of calcium was less, if the disparity between the usual calcium intake and that from the experimental diet was small.

5. The lower the calcium content of the diet, the greater was the amount of phytate phosphorus hydrolyzed.

6. The results are tentatively explained as being due to hydrolysis of the calcium and magnesium phytate at such a level in the digestive tract as to permit of the calcium being subsequently absorbed, and to a gradual adaptation of the body to a lowered intake of available calcium.

7. In two of the subjects, the retention of magnesium was at first lowered by the diet containing more phytate phosphorus. It was considered that this might be due to the precipitation of magnesium by the phytic acid. Later, while the subjects were still on this regime, magnesium retention improved, and changed but slightly when the phytate phosphorus of the diet was lowered.

8. The retention of total phosphorus was at first lowered in two of the subjects by the addition of phytate phosphorus to the diet, but subsequently improved. It improved still further in all subjects when the phytate phosphorus of the diet was reduced.

9. The retention of iron was virtually the same with high and low phytate-phosphorus diets.

10. Cereal diets rich in phytate phosphorus and low in calcium are commonly consumed in various parts of the world; consideration of what must occur under these conditions, together with an examination of the small amount of relevant experimental evidence available, lends a considerable amount of support to the conclusions reached in this paper.

The authors wish to express their gratitude to Prof. H. H. Mitchell, Department of Animal Nutrition, University of Illinois College of Agriculture, for reading the manuscript and for many valuable suggestions. They are indebted to the South African Union Health Department for bearing the major portion of the expenses of the investigation. One of them (A.R.P.W.) desires to express his thanks to the Medical Officer of Health, and the City Council of Johannesburg, for granting his secondment to the National Nutrition Council.

REFERENCES

Basu, K. P., Basak, M. N. & Sircar, B. C. R. (1939). *Indian J. med. Res.* **27**, 471.

Cathcart, E. P. (1940). *Lancet*, **238**, 586.

Cruickshank, E. W. H., Duckworth, J., Kosterlitz, H. W. & Warnock, G. M. (1945). *J. Physiol.* **104**, 41.

Duckworth, J. & Warnock, G. M. (1942). *Nutr. Abstr. Rev.* **12**, 167.

Harrison, D. C. & Mellanby, E. (1939). *Biochem. J.* **33**, 1660.

Henderson, J. M. & Kelly, F. C. (1929–30). *J. Hyg., Camb.*, **29**, 429.

Hoff-Jørgensen, E. (1946). *Biochem. J.* **40**, 189.

Hoff-Jørgensen, E., Andersen, O., Begtrup, H. & Nielsen, G. (1946). *Biochem. J.* **40**, 453.

Hoff-Jørgensen, E., Andersen, O. & Nielsen, G. (1946). *Biochem. J.* **40**, 555.

Holmes, J. O. (1945). *Nutr. Abstr. Rev.* **14**, 597.

Kelly, F. C. & Henderson, J. M. (1929–30). *J. Hyg., Camb.*, **29**, 418.

Kraut, H. & Wecker, H. (1943). *Biochem. Z.* **315**, 329.

Krebs, H. A. & Mellanby, K. (1943). *Biochem. J.* **37**, 466.

Leitch, I. (1937). *Nutr. Abstr. Rev.* **6**, 353.

McCance, R. A. (1946). *Lancet*, **250**, 77.
McCance, R. A. & Widdowson, E. M. (1935). *Biochem. J.* **29**, 2694.
McCance, R. A. & Widdowson, E. M. (1942). *J. Physiol.* **101**, 44.
Mitchell, H. H. & Curzon, E. G. (1939). *Actualités scientifiques et industrielles*, no. 771. Nutrition, 18.
Møllgaard, H., Lorenzen, K., Hansen, I. G. & Christensen, P. E. (1946). *Biochem. J.* **40**, 589.
Nicholls, L. & Nimalasuriya, A. (1939). *J. Nutrit.* **18**, 563.
Owen, E. C., Irving, J. T. & Lyall, A. (1940). *Acta med. Scand.* **103**, 235.
Paris letter (1942). *J. Amer. med. Ass.* **118**, 475.
Paris letter (1946). *J. Amer. med. Ass.* **130**, 101.
Steggerda, F. R. & Mitchell, H. H. (1939). *J. Nutrit.* **17**, 253.
Steggerda, F. R. & Mitchell, H. H. (1941). *J. Nutrit.* **21**, 577.
Steggerda, F. R. & Mitchell, H. H. (1946*a*). *J. Nutrit.* **31**, 407.
Steggerda, F. R. & Mitchell, H. H. (1946*b*). *J. Nutrit.* **31**, 423.
Wang, K., Liu, S. H., Chu, H. I., Yu, T. F., Chao, H. C. & Hsu, H. C. (1944). *Chin. med. J.* **62**, 1.
Wilson, D. C. & Widdowson, E. M. (1942). *Indian med. Res. Mem.* no. 34.

β-Glucuronidase and Cell Proliferation*

By G. A. LEVVY (Imperial Chemical Industries Research Fellow), LYNDA M. H. KERR AND J. G. CAMPBELL, *Department of Biochemistry, University of Edinburgh and Royal (Dick) Veterinary College, Edinburgh*

(*Received* 22 *January* 1948)

After repeated feeding of menthol to mice, Fishman (1940) obtained results which, on statistical examination, showed an increase in β-glucuronidase activity in liver, spleen and kidney, as compared with organs from untreated animals. Similar results were obtained in dogs fed with borneol. Glucuronidase in uterus and other sex organs was unaffected by menthol and borneol. In Fishman's own interpretation of these important experiments, β-glucuronidase is assumed to be responsible for glucuronide synthesis in the body. A synthetic role for the enzyme has, however, still to be demonstrated, its physical properties and distribution in the body having been studied solely by means of its hydrolytic action on conjugated glucuronides. Since menthol and borneol have been proved to be excreted as the glucuronides in, e.g. the dog, and may conceivably behave in the same way in the mouse, Fishman suggested that in his experiments he was measuring adaptation by glucuronidase in response to the presence of excess substrate for its hypothetical synthetic action. Later this theory was extended to explain the elevation in uterine glucuronidase observed after administration of oestrogens to ovariectomized mice (Fishman & Fishman, 1944; Fishman, 1947). Oestrogens did not affect the enzyme in liver, spleen and kidney, and the additional assumption was required, and made, that the enzyme is specific in its synthetic action, according to its source, for different groups of substrate. No such specificity was, however, observed in its hydrolytic action *in vitro*, menthol glucuronide being used throughout in the assay of uterine glucuronidase under conditions found to be optimal for hydrolysis by spleen preparations.

Fishman determined the activity of his enzyme extracts by measuring, by means of its reducing power, glucuronic acid liberated from menthol glucuronide (Fishman, 1939). Sources of error in this procedure, arising largely from its lack of specificity, have been pointed out by other authors (Graham, 1946; Levvy, 1946, 1948), and have led to the development of more satisfactory methods of assay (Talalay, Fishman & Huggins, 1946; Kerr, Graham & Levvy, 1948).

Using phenol glucuronide as substrate in the assay of glucuronidase (Kerr *et al.* 1948), an attempt was made to confirm Fishman's findings (1940) with menthol. Within 24 hr. of a single intraperitoneal injection of L-menthol into mice, there was a marked rise in glucuronidase activity in liver, but not in spleen and kidney. Liver damage was observed and confirmed histologically, and it was subsequently shown that a rise in β-glucuronidase in liver or kidney, depending upon the organ or organs attacked, followed administration of a variety of toxic agents to mice. A more extensive examination of the action of menthol revealed, in addition to the effect on liver, delayed damage to kidney, followed by an increase in glucuronidase activity in this organ also. An increase in the glucuronidase activity of an organ was found, in general, to be associated with active cell proliferation provoked by injury, rather than with the injury itself, and high values were seen in the livers of adult mice after sub-total hepatectomy, and in the liver, spleen and kidneys of infant mice.

* Preliminary accounts of parts of this work have been published elsewhere (Kerr & Levvy, 1947; Kerr, Levvy & Campbell, 1947), and the principal findings were described in a paper read to the Biochemical Society on 27 September 1947 (Levvy, Kerr & Campbell, 1948).