LXII. THE INFLUENCE OF PHYTIN AND OF FATS ON THE PRODUCTION OF RICKETS BY A CEREAL DIET

BY N. PALMER AND J. C. MOTTRAM

From The Laboratories of Messrs Hovis Ltd.

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IN an earlier paper [1937] we showed that the tendency of cereals to produce rickets entirely disappeared when calcium lactate was added so as to make the Ca/P ratio 1.0 : 0.5.

Other explanations for the rachitogenic action of cereals have been put forward. Bruce & Callow [1934] considered that the phosphorus (approximately one-half) which is combined as phytin in cereals is not available. McCance & Widdowson [1935] estimated the amount of phytin in 64 foodstuffs and by means of feeding experiments on four human subjects, using phytin obtained from Messrs Ciba Ltd., estimated that 20–60 % of the phytin ingested was excreted unchanged in the faeces.

In the present series of experiments the authors have used similar phytin from Messrs Ciba Ltd., adding it in varying proportions to a diet consisting otherwise entirely of cereals; these diets have then been fed to white Wistar rats. At the same time for comparison other rats were fed on the same diet without added phytin but with varying amounts of calcium lactate added to it.

The experiments consisted of feeding these diets to the rats for 5 weeks, noting the increase in weight and then, after killing the rats, estimating the amount of rickets by examination of the bones stained with silver nitrate. Projection drawings of the stained bones were made and four categories were recognized, (1) severe rickets (+++), (2) rickets(++), (3) slight rickets(+), and (4) normal bones(0).

Additional information was obtained by determining the amount of bone ash in the dry, fat-extracted bones. By this means it was thought that any tendency of the phytin-P to produce rickets or prevent the proper formation of bones would be disclosed.

A summary of the results of these experiments is given in Table I.

A consideration of these results shows at once that the added phytin has no prohibitive action on bone formation. On the contrary the addition of 1.5 % greatly reduces the rickets and 5 % completely prevents it. With a gradual increase of the amount added, and consequent reduction of the disproportion of P to Ca in the diet, we have a gradual improvement in the character of the bone and a rise in the ash content.

Comparison with the results obtained by the addition of calcium lactate is best made by first considering the Ca/P ratios and then the percentage of each substance added. Thus, for equal additions of 0.75 % of calcium lactate and phytin (nos. 2 and 3), the ratio in one case is 1 : 2.5 and in the other 1 : 3.5 and we have slight and severe rickets and bone ash percentages of 40.5 and 34.6. If, however, we consider the ratio 1 : 2.5 we find that with the addition of 0.75 %

Table I

Diet	Av. initial wt. of 3 rats g.	Av. gain of 3 rats g.	Bone condi- tion at end of test	Bone ash %	Ca : P ratio calculated	Ref. no.
*Germ flour 100 %	50	17	+++ +++ +++	28.3	1:9	1
+0.75 % Ca lactate	48	57	+ + +	40.5	1:2.5	2
+0.75 % phytin	48	17	+++ ++ +++	34.6	1:3.5	3
+1.5 % Ca lactate	48	64	0 0 0	50.68	1:1.4	4
+1.5 % phytin	48	45	+ 0 0	40.4	1:3.0	5
+3.0 % Ca lactate	55	90	0 0 0	60.7	1:0.45	6
+3.0 % phytin	48	51	+ 0 0	43.0	1:2.5	7
+5.0 % phytin	58	55	0 0 0	52.8	1:2.0	8
+3.0 % Ca lactate +5 % phytin	55	71	0 0 0	56.3	1:1.3	9

* The germ flour used consisted of a mixture of 75 % white flour and 25 % sterilized wheat germ.

calcium lactate we get slight rickets and bone ash of 40.5 % and by the addition of 3 % phytin to produce a similar ratio we get slight rickets and bone ash of 43.0 % (No. 7). These amounts of calcium lactate and phytin represent in the case of lactate an addition of 0.1 g. of Ca and in the case of phytin an addition of 0.36 g. Ca. This actually tends to confirm our previous contention that it was the disproportion of P to Ca rather than the presence of any other substance which caused the rickets, especially if one considers experiment No. 9 where an addition of 3 % calcium lactate and also 5 % phytin was made. Here the additions of lactate and phytin made are equivalent to 1.0 g. Ca and 1.05 g. P to a diet containing 0.04 g. Ca and 0.36 g. P, resulting in a Ca/P ratio of 1:1.3. The results obtained were good but not equal to those of experiment No. 6 where only lactate was added, equivalent to an addition of 0.4 g. Ca and no P and resulting in a Ca/P ratio of 1:0.43, which is much closer to the optimum ratio of 1:0.5.

If, as is suggested by McCance & Widdowson [1935], 20–60 % of the P in phytin is unavailable, the Ca/P ratios for experiments Nos. 7 and 9 might be assumed to be 1:1.5 and 1:0.7. The results, however, indicate that a much higher percentage of the phytin-P is utilized.

A recent paper by McDougall [1938] suggests that the additions of fats, olive oil or lard, prevents the occurrence of rickets on a cereal diet by creating favourable conditions for the absorption of Ca. It is inferred that an insoluble calcium phosphate is precipitated in the intestine by the action on phytin of an enzyme

which splits it into soluble sodium phosphate which then reacts with Ca to form insoluble calcium phosphate.

The above results did not confirm this suggestion and further experiments were started to see whether it would be possible to obtain results similar to McDougall's by the addition of fat to the diet. In these experiments mixtures of germ flour, casein and varying proportions of calcium lactate were made. These were fed to rats with the addition in the experiments indicated of 10% of vitamin-free hardened vegetable oil. Vitamin-free oil was chosen in order to remove any possibility of the results being affected solely by vitamin D and not by the fatty acids of the oil.

After 5 weeks the rats were killed and the bones examined for rickets as before. Table II shows the results obtained.

Table II

Diet (%)		Initial wt. of rat g.	Gain in wt. g.	Bone condi- tion at end of test	Bone ash %	Ca : P ratio calculated	Ref. no.
Germ flour	73.5	50	100	0	Not available	1 : 1.2	1
Casein	24.5	50	95	0			
Ca lactate	2.0	45	105	0			
Germ flour	66.0	50	90	0	Not available	1 : 1.5	2
Casein	22.0	50	90	0			
Ca lactate	2.0	55	80	0			
Hard. veg. oil 10.0							
Germ flour	73.7	50	90	0	54.6	1 : 1.3	3
Casein	24.5	50	60	0			
Ca lactate	1.75	55	80	+			
Germ flour	66.15	50	90	++	53.1	1 : 1.4	4
Casein	22.10	50	60	+			
Ca lactate	1.75	Found dead					
Hard. veg. oil 10.0							
Germ flour	74.25	50	90	++	46.5	1 : 1.4	5
Casein	24.25	50	50	0			
Ca lactate	1.5	55	125	+++			
Germ flour	66.5	55	80	++	47.5	1 : 1.5	6
Casein	22.0	45	50	++			
Ca lactate	1.5	45	55	+			
Hard. veg. oil 10.0							
Germ flour	74.5	60	70	+	52.3	1 : 1.8	7
Casein	24.5	55	65	+			
Ca lactate	1.0	60	50	+			
Germ flour	74.5	50	55	+++	47.2	1 : 2.5	8
Casein	25.0	50	60	+++			
Ca lactate	0.5	50	45	++			
Germ flour	67.0	50	50	++	47.5	1 : 2.5	9
Casein	22.5	50	50	+++			
Ca lactate	0.5	50	60	+			
Hard. veg. oil 10.0							
Germ flour	75.0	45	50	+	37.3	1 : 3.2	10
Casein	25.0	45	60	+			
		50	40	+			
Germ flour	67.5	50	40	+++	29.1	1 : 3.5	11
Casein	22.5	45	40	+++			
Hard. veg. oil 10.0		50	45	+			

These results show no decided prevention of rickets by the addition of oil, yet such experiments in which a diet is given to produce not severe rickets but mild or moderate rickets, ought easily to show the preventive action of oil. The

experiments show that, when such diets are given, to obtain consistently normal bones not less than 2% of calcium lactate must be added representing a calculated Ca/P ratio of 1 : 1.2.

SUMMARY

1. Our previous finding is confirmed that the tendency of cereals to produce rickets resides in their low Ca and high P contents, and that this tendency can be entirely counteracted by adding calcium lactate so as to make the Ca/P ratio 1 : 0.5.

2. The rachitogenic action of cereals does not reside in the phytin which they contain, for the reason that the addition of phytin instead of increasing this action reduces the rickets.

3. The addition of a vegetable oil free from vitamin D does not prevent the occurrence of rickets resulting from a cereal diet; here we fail to confirm the findings of McDougall.

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