The Influence of Phytic Acid on the Absorption of Calcium and Phosphorus

1. IN DOGS

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In 1920 Mellanby demonstrated that increasing the amount of cereals in diets deficient in vitamin D leads to increased intensity of rickets. Steenbock, Black & Thomas (1930) drew attention to the possibility that the organic phosphorus compound present in cereals may not be physiologically equivalent to inorganic phosphates added to the diet. Bruce & Callow (1934) established the fact that in rats the P of phytic acid is not so easily available as that of sodium phosphate.

Since phytic acid precipitates calcium (Starkenstein (1910)), Bruce & Callow (1934) suggested that in diets with a natural Ca/P ratio the main action of phytic acid might be that it renders Ca unavailable. In dogs the rachitogenic effect of both sodium phytate and of cereals is antagonized by addition of extra Ca to the diet (Harrison & Mellanby, 1939).

By determinations of the Ca and P balance in dogs we have attempted to investigate what part phytic acid plays in the absorption of Ca and P. The results of these experiments are now reported and the results of similar investigations on infants and children will follow shortly.

METHODS

General. The experiments were made on two male puppies (nos. 5 and 6) of the same litter, born 25 September 1944. From the 30th day of their lives each animal was given the following diet of semolina-milk gruel each day: 250 g. of whole milk; 15 g. of semolina; 15 g. of sugar; 25 g. of liver paste; 2·5 g. of sawdust; 2·5 g. of dried yeast; 0·05 g. of $FeSO_4.7H_2O$; 3000 i.u. of vitamin A; 400 i.u. of vitamin D_2; 2·860 g. of $CaHPO_4.2H_2O$; 0·668 g. of $NaH_2PO_4.H_2O$ and distilled water *ad libitum*.

This diet provides 1 g. of Ca/day (0·298 g. from the milk, 0·008 g. from the other foodstuffs and 0·694 g. from $CaHPO_4.2H_2O$) and 1 g. of P/day (0·245 g. from the milk, 0·065 g. from the other foodstuffs, 0·540 g. from $CaHPO_4.2H_2O$ and 0·150 g. from $NaH_2PO_4.H_2O$).

During the periods in which the effect of phytic acid was investigated, the diet was altered: instead of calcium phosphate 3·235 g. of pentacalcium phytate (containing 0·694 g. of Ca) and 2·905 g. of sodium phytate ($Na_{12}C_6H_6O_{24}P_6.38H_2O$) in neutral solution was mixed with the gruel. This amount of sodium phytate is exactly sufficient to precipitate the 0·306 g. of Ca in the milk and the other foodstuffs as pentacalcium phytate. The total amount of Ca in this diet is therefore also 1 g./day and the total amount of phytic acid is equivalent to the Ca content. In order that the content of non-phytic P of the diet in these periods should also total 1 g./day, 0·690 g. of P was added as a mixture of equal parts of primary and secondary sodium phosphate. As the dogs grew older the diet was supplemented with sugar (maximum 35 g./day) and rice starch (maximum 30 g./day). The increase in Ca content of the diet due to the supplementary food, less than 0·001 g./day, has not been considered. On the other hand, the increase in the P content, a maximum of 0·045 g./day, was corrected by lowering the amount of sodium phosphate. To ensure that the dogs ate their full ration every day, it was necessary to keep them slightly undernourished. They were therefore rather thin during the whole experiment.

At the age of about 140 days the dogs developed distemper and the experiment had to be broken off. Dog no. 6 recovered fairly quickly, but no. 5 was ill for about a month, and after it had otherwise recovered, it still had a slight paralysis of the hind legs. From the 142–150th day they were given a daily injection of 2 ml. of a preparation of 'vitamin B' (*Becoplex* from the firm *Ferrosan*). For a few days the dogs could not eat their ration, but even during their severest illness the administration of Ca and P was as far as possible 1 g./day. When phytate was added to the diet, carmine was also given, in order to decide which stools belonged to the phytate periods. The change in weight with age of the 2 dogs was:

Age in days ...	30	62	90	121	142	175	210	242	262
Dog no. 5: weight in g.	910	1625	2400	3125	3320	3560	4010	4490	4630
Dog no. 6: weight in g.	980	1800	2510	3440	3770	4200	4650	5100	5270

Methods of estimation. Ca was determined after dry ashing by the method of Larson & Greenberg (1938), total P after wet ashing by the method of Græsholm (1935), and phytate by the method of McCance & Widdowson (1935). To obtain complete precipitation of the ferric phytate it was necessary to boil for 30 min. and let the solutions stand for at least 12 hr.

RESULTS

During the periods in which phytate was administered the retention of Ca decreased; the excretion of Ca in the urine, however, was not definitely

Table 1. *The influence of phytic acid on the retention of calcium and phosphorus*

Age (days)	Intake (mg.) of Ca	Intake (mg.) of Total P	Intake (mg.) of Phytate P	Excretion (mg.) of P in urine	Excretion (mg.) of P in faeces	Excretion (mg.) of Ca in urine	Excretion (mg.) of Ca in faeces	Excretion (mg.) of Phytate P in faeces	Retention (mg.) of Ca	Retention (mg.) of P
Dog no 5:										
31–36	5000*	5000	0	1770	1930	42	3860	0	1098	1300
38–43	5000†	9655	4655	2210	5800	36	4130	2840	834	1645
45–50	5000*	5000	0	1540	2255	30	3520	0	1205	1205
74–79	5000*	5000	0	1320	2500	64	3515	0	1421	1180
81–86	5000†	9655	4655	2465	5375	32	4390	2480	578	1875
88–93	5000*	5000	0	1640	2085	41	3710	0	1249	1275
119–124	5000*	5000	0	1430	2110	105	3375	0	1520	1460
126–131	5000†	9655	4655	2045	5820	56	4540	2160	404	1790
133–138	5000*	5000	0	1390	2600	52	3745	0	1203	1010
194–199	5000*	5000	0	1515	2605	80	3930	0	990	880
202–207	5000†	9655	4655	2720	5130	42	5275	2210	−317	1805
210–215	5000*	5000	0	1275	2585	65	3875	0	1060	1140
236–241	5000*	5000	0	1600	2480	38	4042	0	730	920
243–248	5000†	9655	4655	2585	5390	42	5240	2270	−282	1680
250–255	5000*	5000	0	1460	2600	65	4195	0	800	940
Dog no. 6:										
31–36	5000*	5000	0	1525	2275	20	3655	0	1325	1200
38–43	5000†	9655	4655	2205	5700	16	4275	2220	709	1750
45–50	5000*	5000	0	1430	2625	27	3775	0	1198	945
74–79	5000*	5000	0	1360	2125	30	3350	0	1620	1515
81–86	5000†	9655	4655	2080	5750	30	4240	2865	730	1825
88–93	5000*	5000	0	1640	2100	38	3445	0	1517	1260
119–124	5000*	5000	0	1460	2115	15	3720	0	1265	1425
126–131	5000†	9655	4655	2195	5545	16	4475	3020	509	1915
133–138	5000*	5000	0	1230	2545	42	3490	0	1468	1225
154–159	5000*	5000	0	1065	3175	46	4135	0	819	760
161–166	5000†	9655	4655	2385	5090	12	4530	2125	458	2180
168–173	5000*	5000	0	1745	2170	28	3645	0	1327	1085
194–199	5000*	5000	0	1260	2860	60	4000	0	940	880
203–207	5000†	9655	4655	2420	5355	32	5180	2265	−212	1880
209–214	5000*	5000	0	1120	3035	28	4140	0	832	845
236–241	5000*	5000	0	1535	2280	43	4090	0	867	1185
243–248	5000†	9655	4655	2415	5520	25	5355	3420	−360	1720
250–255	5000*	5000	0	1605	2570	28	4030	0	942	825

* 1·490 g. Ca derived from milk, 0·040 g. Ca derived from other foodstuffs and 3·470 g. Ca derived from $CaHPO_4.2H_2O$.
† 1·490 g. Ca derived from milk, 0·040 g. Ca derived from other foodstuffs and 3·470 g. Ca derived from calcium phytate ($C_6H_8O_{24}P_6Ca_5$).

Table 2. *The influence of oxalic acid on the retention of calcium and phosphorus*

Dog no.	Age (days)	Intake (mg.) of Ca	Intake (mg.) of P	Intake (mg.) of Calcium oxalate	Intake (mg.) of Sodium oxalate	Excretion (mg.) of P in urine	Excretion (mg.) of P in faeces	Excretion (mg.) of Ca in urine	Excretion (mg.) of Ca in faeces	Retention (mg.) of Ca	Retention (mg.) of P
5	257–262	5000	5000	11,120	5121	2240	1330	20	5860	−880	1430
6	257–262	5000	5000	11,120	5121	2435	945	36	5975	−1011	1620

Table 3. *The influence of oxalic acid and phytic acid on the absorption of phosphorus*

Age (days)	Intake (mg.) of Ca	Intake (mg.) of Total P	Intake (mg.) of Calcium oxalate	Intake (mg.) of Sodium oxalate	Intake (mg.) of Phytic acid P	Absorption* of P (mg.) Dog no. 5	Absorption* of P (mg.) Dog no. 6
236–241	5000	5000	0	0	0	2520	2720
250–255	5000	5000	0	0	0	2400	2430
257–262	5000	5000	11,120	5121	0	3670	4065
243–248	5000	9655	0	0	4655	4265	4135

* Difference between intake and faecal output.

influenced, not even in periods with a negative Ca balance (Table 1). The decrease in the absorption of Ca was far more pronounced when the dogs received phytate (Fig. 1), and their ability to absorb Ca when in a low concentration in the intestine seems to be very much reduced as they grow older.

Table 1 also shows that both absorption and retention of P is greater when phytate is administered than in the phytate-free periods. This fact may either be due to the phytate being hydrolyzed in the intestine and the split phosphate then being absorbed, or to the Ca ions being bound to the phytate, which would cause an increased concentration of P ions. The testing of these possibilities might be made with phytate with labelled P atoms. At present this is hardly possible. Another way of

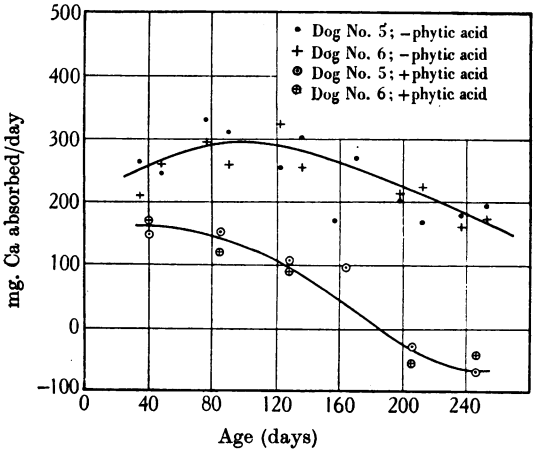

Fig. 1. Changes in Ca absorption in dogs. Daily intake of Ca: 0·298 g. from milk, 0·008 g. from other foodstuffs and 0·694 g. from $CaHPO_4.2H_2O$ or pentacalcium phytate. In the phytate periods sodium phytate sufficient to precipitate the Ca content in the milk and the other foodstuffs as pentacalcium phytate was added to the diet.

solving the problem was attempted. In experiments similar to the above sodium oxalate and calcium oxalate were added to the diet instead of sodium phytate and calcium phytate. The feeding of sodium oxalate and calcium oxalate to the dogs led to a decreased absorption of Ca—but an increased absorption of P (Table 3). Calculation of the absorption of P for the four last experimental periods (Table 3) shows that the increase in phosphate absorption is of the same order of magnitude whether the Ca of the food is present as oxalate or as phytate. It may, therefore, be supposed that in spite of the fact that about half the ingested phytate is hydrolyzed, the phosphate split off will only be absorbed to a small degree, probably because the hydrolysis is due to bacteria and therefore mainly takes place in the colon.

DISCUSSION

In accordance with the result of Harrison & Mellanby (1939) that the rachitogenic action of phytic acid can be antagonized by adding extra Ca to the diet, we have found that the addition of phytic acid to the diet of dogs entails a diminution both in absorption as well as in retention of Ca. The decrease is, however, moderate when the puppies are less than 2 months old, but becomes much more pronounced when the animals grow older. On a diet containing equivalent amounts of Ca and phytic acid (i.e. when Ca/phytic acid P = 5/6) the Ca balance becomes negative at about the time of maturity, that is, when the animals are about 6 months old, and while they are still growing rapidly. In 1944, Hoff-Jørgensen demonstrated that pentacalcium phytate ($C_6H_8O_{24}P_6Ca_5$) is precipitated in the pH range of 5–7. He also showed that the solubility of pentacalcium phytate is greater than that of hydroxy-apatite ($Ca_5(PO_4)_3.OH$), but less than that of secondary calcium phosphate. The Ca salt of phytic acid is precipitated instantly, whilst secondary calcium phosphate precipitates slowly. The difference in the solubility of the secondary phosphate and the phytate is not great enough to explain why Ca absorption is so much impaired when Ca is administered together with phytate. The difference in speed of precipitation, however, might explain the difference in the absorption of Ca present as phosphate and as phytate. In accord with McCance & Widdowson (1942) we found that about 50 % of the phytic acid in the diet is hydrolyzed in the intestine, and that the addition of phytic acid to the diet increases the absorption of phosphate. McCance & Widdowson (1942) think that the increased phosphate-absorption is due to the absorption of a great part of the hydrolyzed phytic acid P. However, our experiments show that the phosphate absorption is almost equally great when oxalate is added to the diet instead of phytic acid. It is therefore more likely that the increased phosphate absorption is due to the fact that phytic acid, like oxalate, precipitates the Ca, the concentration of phosphate ions being thereby increased, since $[Ca^{++}].[HPO_4^{--}] = K_s$. It is furthermore probable that the hydrolysis of phytic acid is mainly due to bacteria and takes place in the colon, from which no phosphate is absorbed.

SUMMARY

1. In puppies replacement of the calcium phosphate of the diet by pentacalcium phytate and the addition of sodium phytate sufficient to precipitate dietary Ca of foodstuffs led to decreased retention of Ca and increased retention of P.

2. A decline in Ca retention occurred with age.

3. Administration of phytate and oxalate increased the absorption of P in nearly the same degree.

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Studies on Cholinesterase

5. THE SELECTIVE INHIBITION OF PSEUDO-CHOLINESTERASE *IN VIVO*

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Mendel & Rudney (1943*a*) showed that two cholinesterases exist in the animal body: a specific cholinesterase acting exclusively on choline esters, and a non-specific enzyme capable of hydrolyzing not only esters of choline but a variety of non-choline esters as well. Mendel & Rudney named the non-specific cholinesterase pseudo-cholinesterase because they doubted the possibility that this enzyme could play an essential part in the hydrolysis of acetylcholine *in vivo*. This assumption has gained further support from the results of recent experiments: (*a*) the non-specific cholinesterase is absent from brain tissue throughout the animal kingdom (Mendel & Rudney, 1943*b*); (*b*) it is not present in the blood and tissues of ox and sheep (Mendel, Mundell & Rudney, 1943; Gunter, unpublished); (*c*) it persists in the superior cervical ganglion of the cat when total degeneration of the preganglionic nerve endings has been effected, whereas the specific cholinesterase disappears completely under these conditions (Sawyer & Hollinshead, 1945).

In spite of the above-mentioned indications, definite proof that this enzyme is not essential to the process of nerve impulse transmission is still lacking. The experiments to be reported here were designed to study this problem.

In the course of investigations on the inhibitory action of certain prostigmin analogues on the specific and on the non-specific cholinesterase *in vitro*, the dimethylcarbamate of (2-hydroxy-5-phenylbenzyl) trimethylammonium bromide (Hoffmann-LaRoche Nu-683) was examined. This compound, in concentrations which had little or no effect on the activity of the specific enzyme, was found to inhibit the non-specific enzyme almost completely. It seemed, therefore, that if a dose of this inhibitor which would suppress the activity of the non-specific cholinesterase *in vivo* elicited no symptoms indicative of acetylcholine poisoning, proof that this enzyme is not essential to the hydrolysis of acetylcholine *in vivo* would be established.

MATERIALS AND METHODS

Measurement of enzymatic activity. In all experiments to be reported here the activity of the specific and of the non-specific enzyme was determined manometrically by Warburg's method at 38°. *In vitro*, the activity was measured in a 0·025 M-$NaHCO_3$ solution saturated with 5% Co_2 (pH 7·4), while for experiments *in vivo* the activity was assayed in undiluted plasma, saturated with 5% CO_2. In this latter series a correction for retention (Warburg, 1925) was necessary and all records for enzymatic activity in this section represent corrected values.

Since some of the tissues examined contained a mixture of the specific cholinesterase and the non-specific enzyme, a means of estimating the activity of each enzyme separately was required, if changes in the level of both were to be followed. Such a method was first provided by Mendel *et al.* (1943) when they showed that acetyl-β-methyl choline is hydrolyzed by the specific cholinesterase but not by the non-specific enzyme and that benzoylcholine is hydrolyzed by the non-specific enzyme but not by the specific cholinesterase. Accordingly, acetyl-β-methyl choline (0·03 M) (Mecholyl-Merck) was used in the present investigation to estimate the activity of the specific cholinesterase, and benzoylcholine (0·006 M) to measure the activity of the non-specific enzyme.

In the determinations where benzoylcholine was used as substrate, proof had to be obtained that the hydrolysis of this compound was due entirely to the non-specific