The rickets-producing and anti-calcifying action of phytate / by Edward Mellanby.

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THE RICKETS-PRODUCING AND ANTI-CALCIFYING ACTION OF PHYTATE

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It was shown that in the absence or deficiency of a fat-soluble calcifying vitamin, cereals had a rickets-producing effect on puppies, for the greater the amount eaten the more intense the resulting disease (Mellanby, 1919, 1920, 1921). Later it was found that there was also a qualitative difference between cereals; for, although the rates of growth of the animals were approximately equal, more severe rickets developed when the diet consisted mainly of oatmeal, maize or whole wheat flour than when these substances were replaced by equal amounts of either white flour or rice, in spite of the fact that the former cereals contained more calcium and phosphorus than the latter (Mellanby, 1922, 1925). The problem was therefore not merely one of growth promotion, but also of the presence in the different cereals of varying amounts of an anti-calcifying substance which interfered in a positive way with calcium metabolism.

This evidence was strengthened by a further observation that the rickets-producing effect of oats could be reduced either by boiling them with a mineral acid or by subjecting them to a malting process (Mellanby, 1925). Later analysis by M. Mellanby (1929) of the effect of treated cereals on the calcification of teeth showed that germination of the grain, which is the first stage of the malting process, did not in itself greatly reduce the anti-calcifying effect, but that if, after germination, the grain was minced and allowed to autolyse, the teeth were less defective in structure. Thus it became more and more certain that cereals contained a chemical agent which interfered with calcium metabolism and that their anti-calcifying effect could be reduced by chemical, and possibly enzymic, action.

In the light of the recent widespread attention that the subject of anti-vitamins has received, it is of interest to note that the term was first used in the course of this work on cereals in relation to calcification, when it was stated—'As regards the mode of action of this substance, its influence is so drastically opposed to that of the anti-rachitic vitamin that the possibility of

its being an anti-vitamin suggests itself' (Mellanby, 1925). In 1926 the word anti-vitamin was replaced by the word toxamin—a substance having a toxic action which could be antagonized by a vitamin (Mellanby). In the present paper, after the experimental results have been discussed, this problem will be considered in relation to the general subject of biochemical antagonism, which has become so important in recent years.

The work was carried a stage further when Harrison & Mellanby (1939) showed that one substance in oatmeal which conferred upon it a powerful anti-calcifying action was phytic acid. Thus it appeared probable that, when oatmeal or oats were boiled with dilute HCl, the cereal phytate was hydrolysed to inositol and phosphate, and when oats and other cereals were malted, a

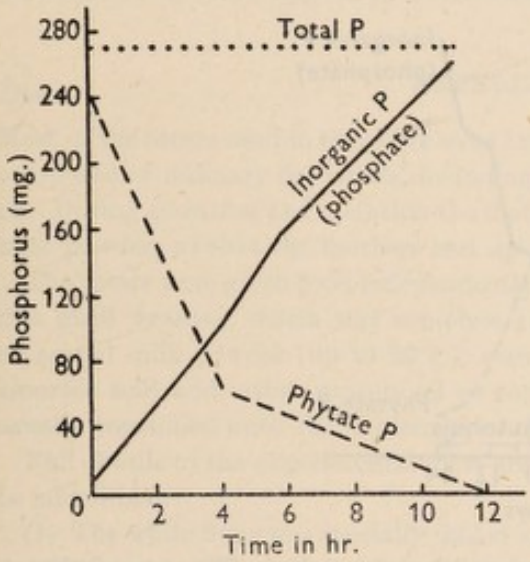


Fig. 1.

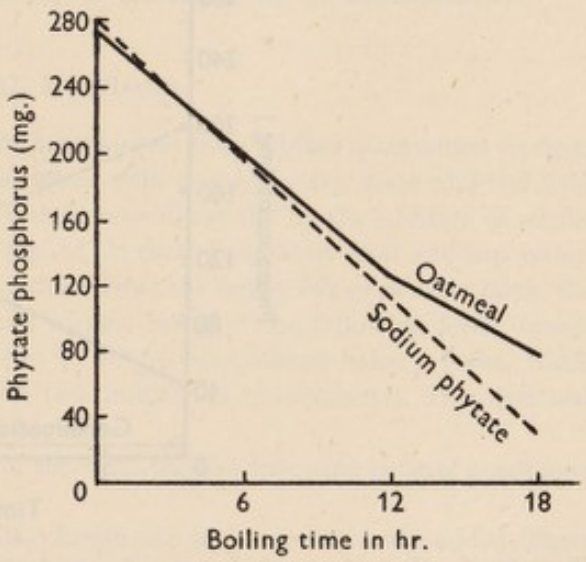


Fig. 2.

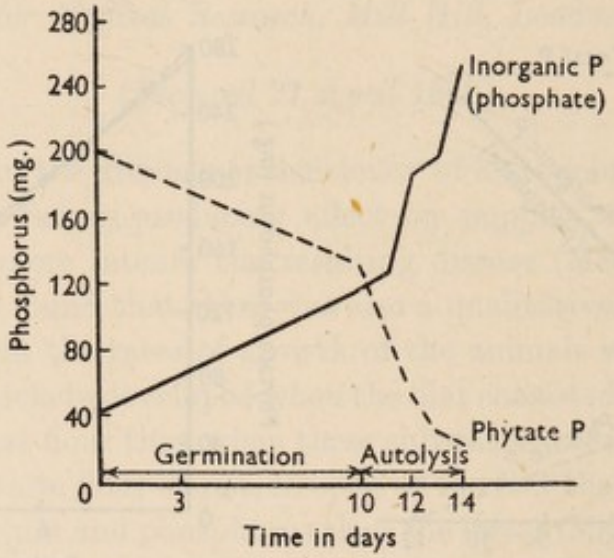
Text-fig. 1. Hydrolysis of sodium phytate to inorganic phosphate by the phytase of yeast. Na phytate containing 240 mg. of P was incubated with 5 g. of compressed baker's yeast in 240 ml. of water at pH 4·5 and 45° C.

Text-fig. 2. Hydrolysis of phytate by boiling oatmeal and sodium phytate respectively with 1% HCl. (Composite graph.)

similar change was effected by a phytase present or produced in the germinating grain; also that the reduction in the anti-calcifying action of the treated products was due to this hydrolysis of the phytate.

Early in the 1940's an investigation into the hydrolysis of phytate phosphorus during the baking of bread was made and some quantitative data were obtained which proved useful in the later animal experiments relating to the anti-calcifying effect of phytate. For instance, it was found that the yeast was a good source of phytase (Mellanby, 1944). (The effect of incubating a solution of sodium phytate with yeast at pH 4·5 and a temperature of 45° C. can be seen in Text-fig. 1.) McCance & Widdowson (1942*b*), however, used the phytase in bran to hydrolyse the phytate in flour of high extraction in their experiments on human beings.

Examples of the relative rates of hydrolysis of phytate when oatmeal and a solution of sodium phytate respectively are boiled with 1% HCl are given in Text-fig. 2. The effect on the phytate of germinating oats and then mincing and leaving them to autolyse for a few days at a temperature of about 23° C, is shown in Text-fig. 3, and it will be seen that during germination hydrolysis is slow, but that during the period of autolysis following the mincing of the germinated oats there is a rapid change of the phytate to inorganic phosphate. The changes in the phytate content of oats during germination and autolysis are in line with the effects on the calcification of the teeth brought about by diets containing oats treated in these ways and referred to above (M. Mellanby).



Text-fig. 3. Hydrolysis of phytate of oats during germination and autolysis. The rate of phytate hydrolysis was slow during germination but increased when the germinated grain was minced and allowed to autolyse.

With this later knowledge at hand, some of the earlier experiments were repeated, and in most cases it was found that the cereal preparations with a high phytate content resulted in worse rickets than those in which this substance had been largely converted to inorganic phosphate either by treatment with acid or by a phytase. Sometimes, however, in the later stages the animals receiving the phosphate developed the condition even more quickly than those receiving phytate, so that at the end of the experiment the degree of rickets might differ little in the two cases.

These irregularities in what appeared to be closely comparable experiments were perplexing. With a view to clarifying the position, therefore, all the many earlier investigations made on the relative anti-calcifying properties of hydrolysed and unhydrolysed cereals were reviewed to see what possible differences there were to account for the apparent discrepancies, and it seemed that the vitamin D reserves of the puppies at the beginning of an experiment and the length of time taken to exhaust them might be important factors. For instance,

when the reserves were relatively large, owing to the ingestion of cod-liver oil or some other source of vitamin D during the pre-experimental period, then in comparatively short experiments the rickets appeared to be worse in the animals eating the untreated cereals than in those having the treated, but when the reserves were small and were presumably lost more quickly, then differentiation between the two groups did not seem to be nearly so obvious, and in longer experiments was negligible or absent.

The following experiments are representative of the many made with untreated and treated cereals. (For methods of preparation, etc., see below.) They indicate that the inconsistencies above described were related to variations in the amounts of vitamin D in the body when the animals were put on diet, and to the length of time taken for these reserves to be exhausted).

EXPERIMENTAL METHODS

Diets

Most of the litters used in this work were bred at the laboratories from bitches maintained on diets composed of ordinary foodstuffs, including cereals, whole-milk powder, scrap meat and cod-liver oil. During gestation and lactation the dietary Ca was increased, usually by the addition of whole-milk powder, so that the mothers had up to 800 mg. of Ca daily from their food and tap water.

The litters were given food independently of the mother from the 3rd or 4th week after birth, the diet until weaning, which was complete at 6 weeks of age, being of the following form: bread, separated milk powder (up to 25 g.), lean meat (up to 15 g.), compressed baker's yeast, NaCl, ascorbic acid and either peanut oil or cod-liver oil (see individual experiments). This diet was usually continued until the experimental diet was begun.

Full details of the experimental diets are given in the text, but the following general notes may be added here:

(1) The white flour was specially milled and no Ca, phosphorus or preservative was added. There was little or no Ca in the water as in most of the experiments it was either distilled or specially softened.

(2) The Ca content of the basal experimental diets was low but was sufficient, when vitamin D was added, to ensure good calcification except when the diet had a high phytate content.

(3) The daily cereal intake increased as the puppies grew and the Ca:P ratio of the diet was therefore reduced as the experiment proceeded, but was comparable for all animals within an experiment unless otherwise arranged. The cereals were cooked in a pressure steamer ($\frac{1}{2}$ lb. pressure) for $1\frac{1}{2}$ hr. and the yeast was boiled with water before being added to them.

(4) All visible fat was removed from the meat.

(5) Various sources of vitamin A were used, but in the later experiments it was given only in the form of vitamin A acetate. The source of vitamin D was calciferol (irradiated ergosterol) obtained through the generosity of British Drug Houses and the amounts administered are expressed as international units (i.u.).

Chemical treatment of some diets

Hydrolysis of phytate of oatmeal by boiling with HCl (*Exps.* 1, 2 *and* 5). 750 g. of oatmeal were mixed with dilute HCl so that the final concentration of acid was 1%, heated on a water-bath for 30 min. and then transferred to a heated sand tray. For the next 30–45 min. the mixture was stirred until it began to boil; boiling was then continued on an electric sand-bath under a reflux condenser for the requisite period. The mass was next cooled under running water, the HCl was neutralised with NaOH, and the bulk was made up to 2250 ml.

Sodium phytate and sodium phosphate (*Exps.* 4, 6, 7, 8 *and* 9). Neutral sodium phytate was prepared from commercial phytin by the method described by Harrison & Mellanby (1939). In

experiments which were made to compare the effects of sodium phytate and inorganic phosphate, the latter was prepared from sodium phytate by hydrolysis with yeast (p. 2).

A solution of sodium phytate was mixed with a suspension of baker's yeast (25 mg. of P to 1 g. of yeast), the pH was corrected to 4·5 and the mixture was incubated at 45° C. for 12 hr. with constant stirring. At the end of this period the solution was boiled for 5 min., cooled, and the HCl was neutralized with NaOH. This treatment converted all the phytate to inorganic phosphate.

As controls similar solutions and suspensions of Na phytate and yeast were incubated separately as above. The yeast was boiled and when cool was added to the phytate solution, the HCl being neutralized as before. This mixture contained the same amount of P as the previous one, but in the form of phytate, with only a trace of inorganic P.

Preparation of calcium phytate (*Exp.* 10). 4000 ml. of a solution containing 5 mg. of P per ml. as sodium phytate were brought to pH 5·5 with HCl. 1000 ml. of 6% $CaCl_2$ solution, also at pH 5·5, were added. After being stirred briskly for a few minutes the mass was filtered on a Buchner funnel, washed with a small quantity of water and then with 80 or 90% alcohol. The Ca phytate was then spread out to dry at room temperature and the Ca, total P and phytate P estimated.

Assessment of results

The methods of assessing the experimental results included: (1) X-ray examinations, (2) the estimation of the A/R ratios, and (3) a study of the mineral metabolism balances. In general these findings were supported by the appearance of the animals and the histological examination of the bones.

As regards the X-ray appearances, an attempt has been made to number the stages of rickets from 1 to 10, but it must be pointed out that these numbers, although relative, do not represent absolute degrees. During life the diagnosis was made from X-rays of the left forepaw only, but post-mortem the appearance of the ribs and other bones was considered.

The method of marking and collecting faecal and urine samples is given on p. 494. The collected faecal samples were heated to 100° C. within 9 hr. of collection, dried, weighed, ground and stored for future analysis of Ca, phytate P and total P.

The methods used in the analysis were:

Estimation of phytate phosphorus in foods, faeces and urine was carried out by the method of McCance & Widdowson (1935), with slight modifications, including an increase in the concentration of the ferric chloride solution.

Total phosphorus in foods, faeces and urine was estimated by the method of McCance & Widdowson (1935).

Inorganic phosphorus and the *total and phytate phosphorus after incineration* were estimated colorimetrically by various methods, including those of Briggs (1922), Fiske & Subarrow (1925), and Allen (1940). For the final comparisons a Spekker photoelectric absorptiometer was used.

Calcium in foods, faeces and urine was estimated by various methods, principally those used by Macy (1942), McCance & Widdowson (1942*a*), and for foodstuffs, the Imperial Bureau of Animal Nutrition (1937).

RESULTS

The relative effect of phytate and phosphate with varying bodily vitamin D reserves

The litters used in these experiments received different amounts of vitamin D during the pre-experimental period. When good supplies were given to the mothers during pregnancy and lactation and to the puppies individually during and after weaning, the body reserves of the vitamin at the beginning of the experimental period would be high. When, however, the puppies had no supplies of the vitamin in this period, their reserves would be lower.

A description is given of the effects on the growing bones of various diets largely composed of cereals. When oatmeal or oats, which are rich in phytate (i.e. about 250 and 180 mg. respectively per 100 g.), were used, they were given to some animals with the phytate intact and to others after much of it had been hydrolysed to inorganic phosphate. In other experiments the effects of phytate and phosphate were tested by giving them as additions to diets whose cereal moiety was white flour which, after cooking, contained little phytate P. In each experiment now to be quoted the basal diet was the same for all members of a litter, so that comparable animals received the same amounts and ratio of calcium and phosphorus.

Untreated oatmeal (phosphorus mainly phytate) compared with oatmeal after boiling with 1% HCl *(phytate hydrolysed to phosphate)*

Exps. 1 *and* 2

Puppies 1 and 2 (Exp. 1) each received 5 ml. of cod-liver oil daily for 3½ weeks before being given the experimental diet at the age of 7 weeks and should, therefore, have had good reserves of vitamin D. The second pair, 3 and 4, which were 6 weeks old at the beginning (Exp. 2), had no cod-liver oil or other source of the vitamin in the pre-experimental period and their reserves of this sub-

TABLE 1 (Exp. 1)

		Dietary conditions					Bone results			
			Phosphorus content of oatmeal eaten (mg./day)					Rickets as judged by X-rays†		
No. of puppy	Vitamin D reserves at beginning of exp.	Oatmeal boiled with 1% HCl (hr.)	Phytate Min.	Phytate Max.	Phosphate* Min.	Phosphate* Max.	*A/R* ratio of femur shaft	After 6 weeks on diet	After 8 weeks on diet	After 11 weeks on diet (P.M.)‡
1	High	0	222	415	118	222	1·00	3	6	8
2	High	18	54	100	286	537	1·37	0	0	1

* Included any organic phosphorus which did not react as phytic acid phosphorus.
† Rickets graded 1–10, the number increasing with the severity of the disease.
‡ P.M. = post-mortem.

Table 2 (Exp. 2)

		Dietary conditions					Bone results			
			Phosphorus content of oatmeal eaten (mg./day)					Rickets as judged by X-rays†		
No. of puppy	Vitamin D reserves at beginning of exp.	Oatmeal boiled with 1% HCl (hr.)	Phytate Min.	Phytate Max.	Phosphate* Min.	Phosphate* Max.	*A/R* ratio of femur shaft	After 6 weeks on diet	After 8 weeks on diet	After 10 weeks on diet (P.M.)
3	Low	0	63	167	50	133	0·97	6	7	8
4	Low	18	18	48	95	252	1·15	1	5	6

* Included any organic phosphorus which did not react as phytic acid phosphorus.
† Rickets graded 1–10, the number increasing with the severity of the disease.

stance would therefore be lower than those of 1 and 2. All had a similar basal diet which was deficient in vitamin D, but one of each pair (1 and 3) had untreated oatmeal whilst the other (2 and 4) received oatmeal which had been boiled with 1% HCl for 18 hr. to hydrolyse its phytate to inorganic phosphate. (About 70% of the phytate was hydrolysed; see Text-fig. 2.)

After 6 weeks each of the animals getting untreated oatmeal (1 and 3) had developed worse rickets than its litter-mate receiving treated oatmeal (2 and 4). This differentiation was maintained between animals 1 and 2 (high vitamin D reserves) for the remainder of the 11-week experimental period (Pl. 1*a*,*b*). In puppies 3 and 4 (relatively low vitamin reserves), however, the differentiation was disappearing at 8 weeks (Pl. 1 (cf. *c* and *d* with *e* and *f*).

These results, typical of the older experiments, supported the suggestion that the differences between the rickets-producing effects of untreated oatmeal (phosphorus present as phytate) and treated oatmeal (phytate hydrolysed to phosphate) were modified by differences in the bodily stores of vitamin D at the beginning of the experiment.

In these early experiments attention was focused on the condition of the bones, as judged by X-ray examination at the beginning and during the course of the experiments and by determining the mineral ash content and the A/R ratio of one or more bones at the end of the period. As will be seen in discussing Exp. 9, it was later found that, although the A/R ratio was a good indication of bone quality, it did not necessarily reflect the relative amounts of Ca absorbed from the intestines of the different animals. Although this was not realised at the time of these earlier experiments, it nevertheless seemed essential at this stage to extend the investigation to the study of the absorption of Ca and P from the intestines and the excretion of these substances in both the faeces and the urine under the varying conditions, for it was likely that any difference there might be between the effects of phytate and phosphate on calcium and phosphorus metabolism would be at least partially, and possibly wholly, shown in the alimentary canal. Thus in all the experiments to be described both the end results as regards bone calcification and the course of Ca and P balances during the experimental period were investigated. In the case of P metabolism, both phytate and total phosphorus were determined. The animals were put at intervals of 2–4 weeks in metabolism cages for periods of 2 and later 3 days, the faecal excretion of the period being marked at the beginning and the end by giving carmine in the food and so colouring the stools. The total urine passed during a timed 2- or 3-day period was also collected. Any dogs suspected of eating excreta were muzzled when in the metabolism cages and care was taken to ensure as prompt removal as possible of faeces from the cages. This was usually carried out without difficulty, but in some cases, due to loose stools, shaggy coats, or excessive activity of the dogs, there must have been some loss. When severe rickets occurred it was sometimes impossible to obtain true

samples towards the end of the experiment, partly because the animals did not always eat their food readily and partly because of their immobility. In most of these cases the metabolic tests were perforce stopped, although they might be continued for other members of the litter. For these reasons the curves representing Ca and P balances must be considered as a whole and too much emphasis ought not to be placed on individual estimation.

Untreated oats (phytate) compared with germinated and autolysed oats (phosphate)

The relative effects of untreated (high phytate), as compared with germinated and autolysed oats (high phosphate), also varied with the body reserves of vitamin D and were similar to the effects found in the HCl-treated and untreated oatmeal experiments.

Exp. 3

Pre-experimental diet. The first pair of puppies (5 and 6) had a daily addition of 2·5 ml. of cod-liver oil for 4 weeks prior to the experiment and would have good reserves of vitamin D at the beginning. The second pair (7 and 8) received no additional vitamin D or cod-liver oil and would therefore have lower reserves of the vitamin.

Basal experimental diet. Oats, 20–200 g.; separated milk powder, 30 g.; lean meat, 15 g.; peanut oil, 10 ml.; salt, 1 g.; baker's yeast, 5% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1000 i.u. (Ca content: initial, 407 mg.; final, 565 mg. P content: initial, 326 mg.; final, 904 mg.)

Variations in treatment of oats

No. of puppy	
5	Ground and boiled (phytate intact)
6	Germinated, minced and autolysed (phytate hydrolysed to phosphate)
7	Ground and boiled (phytate intact)
8	Germinated, minced and autolysed (phytate hydrolysed to phosphate)

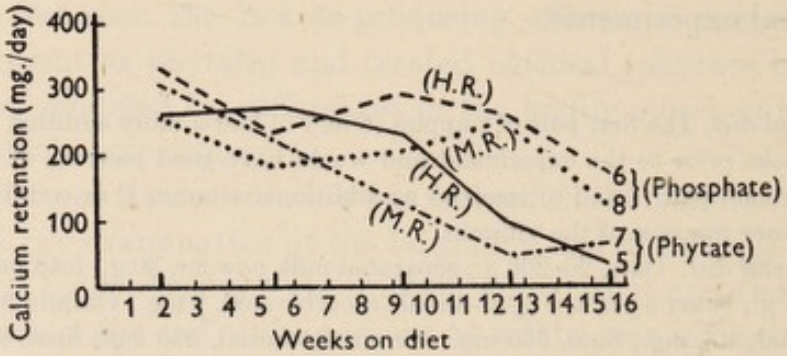
Age at beginning of experiment: 6 weeks; duration of experiment: 17 weeks

Radiographs (Pl. 2*a–d*) indicate that when the body had good reserves of vitamin D at the beginning of the experiment, as in puppies 5 and 6 (*a* and *b*), the differentiation between the rickets-producing effects of phytate and phosphate was rather greater than when the reserves were lower, as in puppies 7 and 8 (*c* and *d*). Text-fig. 4, representing the daily calcium retentions of the four puppies, shows that, when they were compared according to their vitamin D reserves, there was but little difference between the phytate and the phosphate animal of each pair up to the sixth week of the experimental feeding, when they were eating only relatively little oats and the amount of phytate consumed was still small. From this point, however, as the phytate of the diet increased there was a division into two groups, the first including puppies 5 and 7 (phytate of oats intact) and the second puppies 6 and 8 (phytate of oats converted to phosphate), the amount of calcium retained by the phosphate pair remaining at a higher level than that retained by the phytate pair. In each pair the puppy which originally had high reserves of vitamin D retained slightly more Ca than that with the medium reserves.

TABLE 3 (Exp. 3)

Dietary conditions							
			Phosphorus content of oats eaten (mg./day)				Bone results. Rickets as judged by X-rays at P.M.†
			Phytate		Phosphate*		
No. of puppy	Vitamin D reserves	Treatment of oats	Min.	Max.	Min.	Max.	
5	High	Ground and boiled	28	280	33	330	5
6	High	Germinated, minced and autolysed	0	0	61	610	2
7	Medium	Ground and boiled	28	280	33	330	6
8	Medium	Germinated, minced and autolysed	0	0	61	610	4

* Included any organic phosphorus which did not react as phytic acid phosphorus.
† Rickets graded 1–10, the number increasing with the severity of the disease.



Balance tests on dates marked ⊥.

Text-fig. 4 (Exp. 3). Relative effects of untreated and of germinated and autolysed oats on calcium retention in the presence of high and medium reserves of vitamin D. *Note.* In the presence of both high (H.R.) and medium (M.R.) vitamin D reserves the Ca retention of puppies 5 and 7, having untreated oats (phytate), was lower than that of puppies 6 and 8, receiving germinated, minced and autolysed oats (phytate converted to phosphate).

In view of the above results, from which it appeared that phytate in oatmeal and oats lost some of its rachitogenic effect after hydrolysis to inorganic phosphate and that the relative effects of these two forms of P in the diet were in some way determined by the presence in or absence from the body of vitamin D, it was decided to make a more complete study of this problem. Instead of giving cereals containing phytate, this substance was prepared as the neutral sodium salt and given to puppies either as such or, after hydrolysis by yeast, as inorganic phosphate. It thus became possible to get a clearer view of the relative action of these substances on calcification uncomplicated by other unknown dietetic factors.

Sodium phytate prepared from commercial phytin compared with sodium phosphate

The object of Exp. 4 was to see in the first place whether the results obtained in Exps. 1–3, in which treated and untreated oatmeal and oats were the variable factors, could be repeated with phytate and inorganic phosphate. It was also

desired to determine whether there was a difference in the amounts of Ca and P absorbed from the gut when these two salts were used and, if so, whether this difference persisted when the vitamin D reserves were exhausted. The experimental diets did not contain vitamin D and the pre-experimental diets were so arranged that two of the four animals would have high and two low D reserves. Substantial differences in calcium retention would thus be expected, the animals having good initial reserves of the vitamin being likely to retain more calcium than the corresponding low-reserve animals.

Exp. 4

Pre-experimental diet. From the age of 4–7 weeks puppies 9 and 10 were each given 500 i.u. vitamin D_2 daily. It was certain, therefore, that both had substantial stores of the vitamin at the beginning of the experiment. Puppies 11 and 12, on the other hand, although receiving the same low D basal diet, had no vitamin D_2 supplement during the same period, so that their stores were smaller.

Basal experimental diet. White flour, 35–145 g.; separated milk powder, 30 g.; lean meat, 15 g.; peanut oil, 10 ml.; NaCl, 1% of cereal; baker's yeast, 10% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1000 i.u. (Ca content: initial, 406 mg.; final, 421 mg. P content: initial, 301 mg.; final, 393 mg.)

Daily additions to basal diet

No. of puppy	Mg. phytate *P*/100 g. flour	Mg. phosphate *P*/100 g, flour
9	250	10
10	0	260
11	250	10
12	0	260

Age at beginning of experiment: 7 weeks; duration of experiment: 23–24 weeks

All four puppies received the same total amount of phosphorus daily, but the quantity given to 10 and 12 in excess of that present in the basal diet was in the inorganic form, whereas the extra given to 9 and 11 was mainly phytate phosphorus. It will be seen from Text-fig. 5 that the calcium retention of 9 and 10, both of which had a comparatively large store of vitamin D at the beginning of the experiment, remained high for the first 9 weeks. There was, however, during this period a real difference between the amount retained by the two animals, 10 (receiving P as phosphate) having a consistently higher retention than 9 (phytate). After 11 weeks of the diet it would appear that the vitamin D stores of these two puppies were greatly reduced and the power to retain calcium was rapidly lost. In puppies 11 and 12, whose vitamin D reserves were known to be smaller at the beginning of the experimental period, the Ca retention soon began to fall and there was little difference between the absorption of Ca in the phytate and phosphate animals after 4–6 weeks.

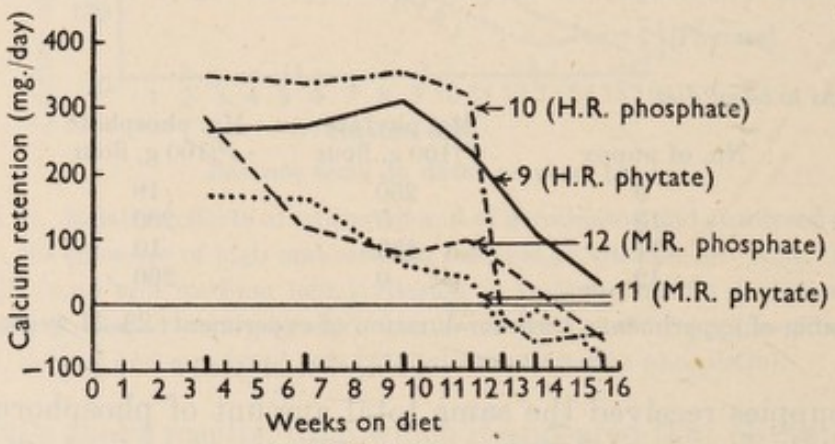
The radiographs of these animals showed that the high vitamin D reserves of puppies 9 and 10 were sufficient to protect them against rickets until the thirteenth week of the experiment, at which time 11 and 12, which had smaller

reserves, both had severe rickets. At the end of the experimental period (i.e. after approximately 24 weeks) the disease had developed in the previously rickets-free pair but to a less severe extent. In both pairs the puppy receiving phytate was more rachitic than that receiving phosphate, but in the pair with only moderate vitamin D reserves the early definite differentiation was gradually reduced.

TABLE 4 (Exp. 4)

		Dietary conditions. Phosphorus added to basal diet (mg./day)				Bone results. Rickets as judged by X-rays†		
		Na phytate		Inorganic phosphate*				
No. of puppy	Vitamin D reserves	Min.	Max.	Min.	Max.	After 10 weeks on diet	After 13 weeks on diet	At P.M.
9	High	88	363	3	14	0	0	3
10	High	0	0	91	377	0	0	2
11	Medium	88	363	3	14	8	9	10
12	Medium	0	0	91	377	4	6	8

* Na phytate hydrolysed by phytase of yeast.
† Rickets graded 1–10, the number increasing with the severity of the disease.



Text-fig. 5 (Exp. 4). Relative effects of phytate and phosphate on calcium retention in the presence of high and medium reserves of vitamin D. *Note.* (1) when the vitamin D reserves were high (H.R.) the Ca retention of puppy 9 (phytate) was lower than that of puppy 10 (phosphate prepared from phytate); (2) when the vitamin D reserves were lower (M.R.), there was but little difference in the Ca retentions of puppies 11 (phytate) and 12 (phosphate prepared from phytate).

The results of this experiment, therefore, lent support to the hypothesis upon the basis of which it was carried out. It suggested that: (1) when vitamin D was present in the body, even when there was none in the food, phytate prevented the absorption and retention of calcium to a greater extent than phosphate of equal phosphorus content; and (2) when the body had no reserves of the vitamin and there was none in the food, calcium absorption was very small in any case, and phosphate was probably as potent as phytate in further reducing its absorption (Mellanby, 1937).

It was next decided to see whether phytate and phosphate, both in oatmeal and as the Na salts prepared from commercial phytin, had the same relative

effects on the absorption and utilization of Ca when animals received no vitamin D and 20 i.u. daily respectively.

The relative effect of phytate and phosphate in the presence and absence of dietary vitamin D_2

Untreated oatmeal (phytate) compared with oatmeal hydrolysed by HCl *(phosphate)*

Exp. 5

Pre-experimental diet. When fed independently of the mother, this series of puppies received no cod-liver oil or other source of vitamin D. Thus at the beginning of the experiment there should have been only moderate reserves of the vitamin in all the animals.

Basal experimental diet. Oatmeal, 35–140 g.; separated milk powder, 30 g.; lean meat, 15 g.; peanut oil, 10 ml.; NaCl, 4·8% of cereal; baker's yeast, 5% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1000 i.u. (Ca content: initial, 362 mg.; final, 414 mg. P content: initial, 436 mg.; final, 883 mg.)

Variations in treatment of oatmeal

No. of puppy	
13	Untreated (phytate intact)
14	Boiled with HCl for 18 hr. (most of phytate hydrolysed to phosphate)
15	Untreated (phytate intact)
16	Boiled with HCl for 18 hr. (most of phytate hydrolysed to phosphate)

Daily additions to basal diet

No. of puppy	
13	20 i.u. vitamin D_2
14	20 i.u. vitamin D_2
15	None
16	None

Age at beginning of experiment: 7 weeks; duration of experiment: 15 and 16, 17½ weeks; 13 and 14, 19½ weeks.

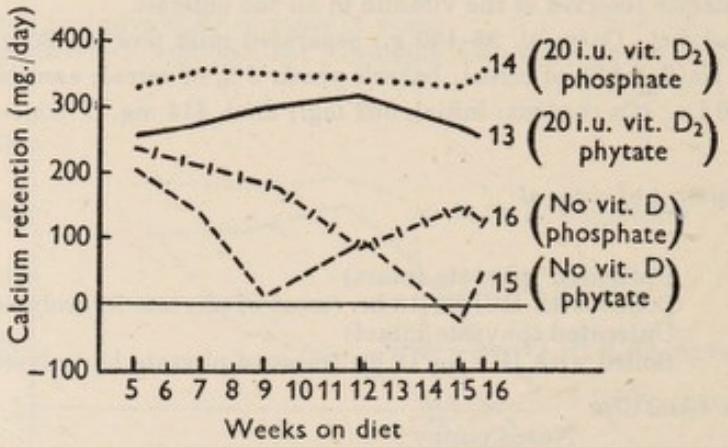
In this experiment oatmeal was the source of phytate and phosphate. Although all the animals in the litter received the same total amount of P in the food, it will be seen from Table 5 that the P of 14 and 16 (acid-treated oatmeal) was mostly in the inorganic form, whereas that of 13 and 15 contained much phytate. The Ca balance results are given in Text-fig. 6, which shows that in 15 and 16, neither of which received vitamin D and both of which developed severe rickets (as judged by X-rays), the Ca retention fell throughout the experiment and the phytate reduced the Ca balance to a greater extent than the phosphate. Vitamin D protected puppies 13 and 14 against rickets, but although both maintained a good positive calcium balance, puppy 13, receiving phytate P, had a lower retention than 14, receiving phosphate.

This experiment, therefore, indicated that, even in the presence of sufficient dietary vitamin D_2 to protect the animals against rickets, phytate exerted a greater inhibiting effect on Ca absorption than phosphate, thereby lowering retention, whereas in the absence of the vitamin the absorption of Ca was small and irregular whether phytate or phosphate was given.

TABLE 5 (Exp. 5)

No. of puppy	Oatmeal boiled with 1% HCl (hr.)	Dietary conditions: Phosphorus in oatmeal eaten (mg./day) Phytate Min.	Phytate Max.	Phosphate* Min.	Phosphate* Max.	Vitamin D_2 (i.u.)	Bone results. Rickets as judged by X-rays at P.M.†
13	0	80	380	47	220	20	0
14	18	23	113	104	487	20	0
15	0	80	380	47	220	0	10
16	18	23	113	104	487	0	9

* Included any organic phosphorus which did not react as phytic acid phosphorus.
† Rickets graded 1–10, the number increasing with the severity of the disease.



Text-fig. 6 (Exp. 5). Relative effects of untreated and HCl treated oatmeal on calcium retention in the absence of vitamin D and in the presence of 20 i.u. daily. Untreated oatmeal (phytate) (13 and 15) exerted a greater inhibiting action on Ca absorption than treated oatmeal (phosphate) (14 and 16) both in the presence of dietary vitamin D_2 (13 and 14) and in its absence (15 and 16).

Sodium phytate compared with sodium phosphate

In this experiment, unlike the last, sodium phytate and phosphate respectively were added to the diets, instead of oatmeal being used as a source of phytate and acid-treated oatmeal as a source of phosphate.

Exp. 6

Pre-experimental diet. The mother of the litter received cod-liver oil during pregnancy and the first 4 weeks of lactation, but the diet of the puppies after weaning contained no cod-liver oil or other form of vitamin D.

Basal experimental diet. White flour, 25–160 g.; separated milk powder, 30 g.; lean meat, 15 g.; peanut oil, 10 ml.; NaCl, 1% of cereal; baker's yeast, 10% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1000 i.u. (Ca content: initial, 390 mg.; final, 434 mg. P content: initial, 296 mg.; final 450 mg.)

Daily additions to basal diet

No. of puppy	Mg. phytate P/100 g. flour	Mg. phosphate P/100 g. flour	Vitamin D_2 (i.u.)
17	250	10	20
18	0	260	20
19	250	10	0
20	0	260	0

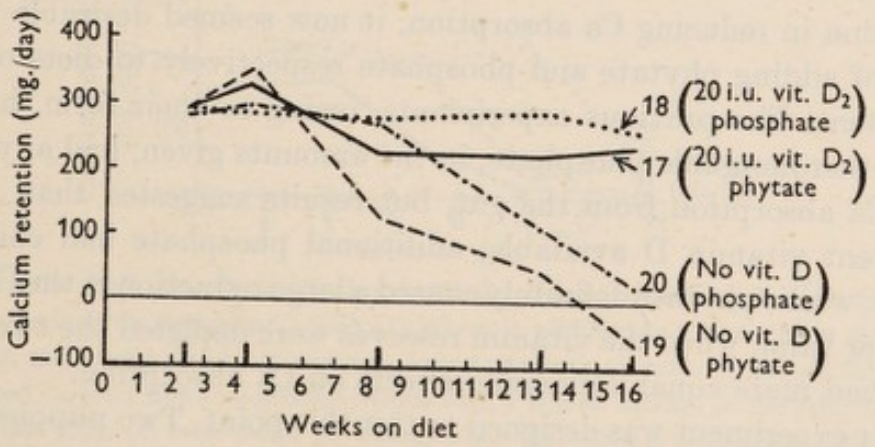
Age at beginning of experiment: 6½ weeks; duration of experiment: 23 weeks.

These animals were periodically tested for calcium and phosphorus retention and the results are given in Text-fig. 7. It will be seen that the addition of vitamin D_2 to the diet improved the calcium retention of both phytate and phosphate animals. Phytate, however, still exerted an inhibiting effect on Ca retention and, as the figure shows, both with and without the addition of vitamin D to the diet phosphate allowed a higher Ca retention than phytate.

TABLE 6 (Exp. 6)

	Dietary conditions					Bone results.
	Phosphorus added to basal diet (mg./day)					
	Na phytate		Inorganic phosphate*			
No. of puppy	Min.	Max.	Min.	Max.	Vitamin D_2 (i.u.)	Rickets as judged by X-rays at P.M.†
17	62	400	3	16	20	0
18	0	0	65	416	20	0
19	62	400	3	16	0	9
20	0	0	65	416	0	9

* Na phytate hydrolysed by phytase of yeast.
† Rickets graded 1–10, the number increasing with the severity of the disease.



Text-fig. 7 (Exp. 6). Relative effects of phytate and phosphate on Ca retention in the absence of vitamin D and in the presence of 20 i.u. daily. *Note.* (1) In the presence of dietary vitamin D_2 retention of Ca was lower with phytate (17) than with phosphate (18); (2) in the absence of dietary vitamin D_2 the power to retain Ca was soon reduced in both animals irrespective of the form of P ingested [phytate animal (19) about 2 weeks before phosphate (20)].

In all the foregoing experiments the dietary Ca : P ratio was low at the beginning and fell during the course of the feeding period as the consumption of cereal increased. Although the amounts and ratios of Ca and P varied from experiment to experiment they did not vary within an experiment, being the same for all the puppies of a litter at any given time. The form of the phosphorus, however, varied; sometimes it was present as inorganic phosphate and sometimes partly as phytate.

The results of Exps. 4–6 showed that:

(1) In the presence of vitamin D, either in the diet or in the body as reserves, phytate reduced Ca absorption from the gut more than did inorganic phosphate of equal P content.

(2) As the vitamin D stores of the body were used up, Ca absorption was always greatly diminished in the presence of either phytate or phosphate, phytate usually, though not in all cases, proving the more powerful in this respect.

(3) So far as was indicated by Ca retention and radiographs of the bones, the effects of giving a high phytate-containing cereal such as oatmeal, before and after treatment which converted much of its phytate to inorganic phosphate, could be repeated by adding sodium phytate and sodium phosphate respectively to diets of low phytate content.

The time and rate at which the Ca retention of D-deficient animals fell varied from experiment to experiment. This irregularity was largely due to differences in and rates of loss of body stores of the vitamin and could be roughly controlled by regulating the vitamin D intake in the pre-experimental period.

Having demonstrated that, in the presence of vitamin D, phytate had a specific action in reducing Ca absorption, it now seemed desirable to compare the effect of adding phytate and phosphate respectively to diets of relatively low P content. The previous experiments, owing to their form, had not disclosed whether inorganic phosphate, in the amounts given, had any depressing effect on Ca absorption from the gut, but results suggested that, when there was sufficient vitamin D available, additional phosphate had comparatively little effect, whilst phytate definitely caused a large reduction in the Ca absorbed; on the other hand, when the vitamin reserves were depleted the two substances probably had more equal depressing effects on Ca absorption.

The next experiment was designed to test this point. Two puppies were given diets of a lower Ca : P ratio than the control, one receiving an addition of P as phytate and the other an equal amount as phosphate. All three animals had a trace of dietary vitamin D_2 to supplement the body reserves but not sufficient to prevent rickets.

Effect of increasing the dietary P by additions of phytate and phosphate on calcium retention

Exp. 7

Pre-experimental diet. When the puppies were fed separately from the mother they received no cod-liver oil or other source of vitamin D.

Basal experimental diet. White flour, 20–160 g.; separated milk powder, 20 g.; lean meat, 15 g.; peanut oil, 10 ml.; NaCl, 2 g.; baker's yeast, 10% of cereal; ascorbic acid, 5 mg.; vitamin A acetate 1500 i.u.

(Ca content: initial, 267 mg.; final, 292 mg. P content: initial, 230 mg.; final, 410 mg.)

Daily additions to basal diet

No. of puppy	Mg. P as Na phytate/100 g. flour	Mg. P as Na phosphate/100 g. flour	Vitamin D_2 (i.u.)
21	250	10	2
22	0	260	2
23	0	0	2

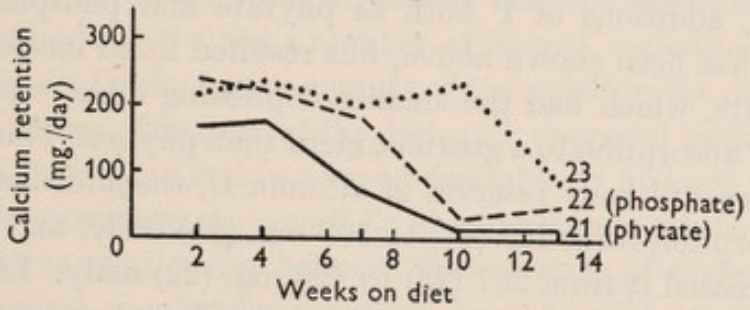
Age at beginning of experiment: 6½ weeks; duration of experiment: 15½ weeks.

Text-fig. 8 shows that during the early part of the experiment, when all three animals had some reserves of vitamin D, the addition to the diet of phosphate (22), and incidentally the reduction of the Ca:P ratio, had but little effect on

TABLE 7 (Exp. 7)

	Dietary conditions					Bone results.		
	Phosphorus added to basal diet (mg./day)					Rickets as judged by X-rays at†		
	Na phytate		Na phosphate*					
No. of puppy	Min.	Max.	Min.	Max.	Vitamin D_2 (i.u.)	7 weeks	11 weeks	P.M.
21	50	400	2	16	2	2	6	8
22	0	0	52	416	2	1	3	5
23	0	0	0	0	2	0	1	3

* Na phytate hydrolysed by phytase of yeast.
† Rickets graded 1–10, the number increasing with the severity of the disease.



Text-fig. 8 (Exp. 7). Relative effects of reducing the Ca:P ratio of the diet by additions of phytate or phosphate P on Ca retention. *Note.* (1) When, in addition to a trace (2 i.u. daily) of dietary vitamin D_2 there were also body reserves, lowering the Ca:P ratio by the addition of phytate (21) reduced Ca retention to a greater extent than a similar change in the ratio brought about by phosphate (22); (2) later (after 10 weeks) when the vitamin D reserves were depleted the effects of the two forms of P were more alike; (3) the addition of P in either form appeared to hasten the loss of vitamin D reserves, as indicated by diminution of Ca absorption, and phytate probably did this to a greater extent than phosphate.

Ca retention, whereas the addition of the same amount of P as phytate (21) and the same alteration in the Ca:P ratio had a definite effect. After 10 weeks of the experiment the differentiation between puppies 21 and 22 was disappearing, phytate and phosphate then behaving similarly in that both reduced the Ca retention. After a further 3 weeks, when the last metabolic test was made, all the animals had greatly reduced powers of Ca absorption, but the two receiving diets with the lower Ca:P ratios were still retaining less than the control animal (23).

Text-fig. 8 also shows that the 2 i.u. of vitamin D given daily to each of the animals were not sufficient either to maintain good calcium absorption or even to conserve the body reserves of the vitamin. The Ca retention

curves of the three animals fell sharply at different times of the experimental feeding. Thus the control animal (23) began to lose its power to retain Ca after 10 weeks, the phosphate animal (22) after 7 weeks and the phytate animal (21) after 4 weeks. It would appear that the addition of either type of P hastened the removal of stored vitamin D and that phytate did so more rapidly than phosphate.

X-ray examination of the bones showed that after 7 weeks of the experimental feeding there was definite rickets in puppy 21 (phytate), whilst 22 (phosphate) was still nearly normal and 23 was without doubt normal from this point of view. From this time onwards the disease developed in the three animals, the two with additional P developing more severe rickets than the control (23), while puppy 21 (phytate) had worse rickets than 22 (inorganic phosphate).

Most of the criticism of phytate as an anti-calcifying agent has been based on rat experiments in which diets with a high-Ca, low-P ratio were used and in which P was without doubt the limiting factor as regards calcification. In such experiments the addition of P to the diet improved both Ca and P absorption and bone calcification. In the present experiment, however, where Ca was the limiting factor, additions of P both as phytate and phosphate reduced Ca absorption, as has been shown above, but resulted in an increased absorption of P. Phosphate, which had the smaller depressing effect on Ca absorption, increased the P absorption to a greater extent than phytate. Thus after 4 weeks, when there were still body reserves of vitamin D, the addition of phytate increased P absorption from 267 (23) to 393 mg. (21) daily, and the addition of phosphate increased it from 267 (23) to 495 mg. (22) daily. Later (13 weeks), when the vitamin reserves were very low, the P absorptions were 191 mg. (23, no extra P), 385 mg. (21, phytate), and 589 mg. (22, phosphate). Most of the additional P absorbed from the high P diets was excreted via the urine so that the amounts of P retained by the three animals were approximately the same.

This experiment therefore indicates that:

(1) Since 2 i.u. of vitamin D daily do not prevent the depletion of this vitamin, and all three animals so fed lost the power to maintain Ca absorption.

(2) Adding equal amounts of phytate and phosphate, respectively, to the diets of litter-mate puppies, and thereby lowering the Ca:P ratio of their food to the same extent, has widely different effects on their Ca retention as long as sufficient vitamin D is available.

(3) The addition of phytate to the diet hastens the onset of rickets and increases its severity to a greater degree than the addition of an equal amount of P as phosphate.

(4) The addition of either type of P appears to hasten the exhaustion of body reserves of vitamin D, but phytate P probably acts more strongly than an equal amount of P as phosphate.

(5) Increasing the dietary P by additions of phytate or phosphate increases P absorption under these conditions, and even when the vitamin D supply is insufficient, the absorption of P is increased to a greater extent by phosphate than by phytate.

Exp. 7 shows quite clearly that the Ca:P ratio is not a reliable indication of the calcifying qualities of a diet. It is true that, when the dietary supply of vitamin D was very low and the body reserves were depleted, lowering the Ca:P ratio by additions of P reduced Ca absorption. On the other hand, during the early part of the experiment, when there was a sufficiency of vitamin D, there was a distinct difference in the results produced by altering the ratio to the same extent by the addition of phytate and phosphate respectively. This difference was not due to unavailable P of the phytate reducing the need for Ca, for puppy 21 (phytate) absorbed more P, some of which must have come from the phytate, than 23 (no addition), although the Ca absorption was much reduced. Thus some of the P fed as phytate was available for absorption and it seems probable that it was only the remainder which, combining with Ca in the gut and thereby removing it from the influence of absorptive processes, reduced Ca absorption (Harrison & Mellanby, 1939).

These experiments on dogs demonstrated that the calcifying qualities of a diet could only be judged when a number of factors, including the presence or absence of vitamin D, the total amounts of Ca and P in the diet and the form of the P, were known. The form of the dietary P appeared in these experiments to be a crucial factor and it now seemed desirable to find out how phytate, as compared with phosphate, decreased Ca absorption and whether, in fact, there was a quantitative relationship between the excretion of these two substances, so that an increase in phytate excretion brought with it an increase in Ca excretion. In the next experiment, therefore, whilst keeping the total dietary P and calcium equal, three different levels of phytate P were given in order to test the possibility of this relationship.

Exp. 8 The effect of increasing the dietary phytate but not the total phosphorus on calcium and phytate excretion

The pre-experimental diet of the puppies contained no cod-liver oil or other source of vitamin D.

Basal experimental diet. White flour, 20–160 g.; separated milk powder, 20 g.; lean meat, 15 g.; peanut oil, 10 ml.; NaCl, 1% of cereal; baker's yeast, 12% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1500 i.u. (Ca content: initial, 265 mg.; final, 297 mg. P content: initial, 209 mg.; final 377 mg.)

Daily additions to basal diet No. of puppy	Mg. phytate P/100 g. flour	Mg. phosphate P*/100 g. flour	Vitamin D_2 (i.u.)
24	300	18	20
25	150	168	20
26	0	318	20

* Na phytate hydrolysed by phytase of yeast.

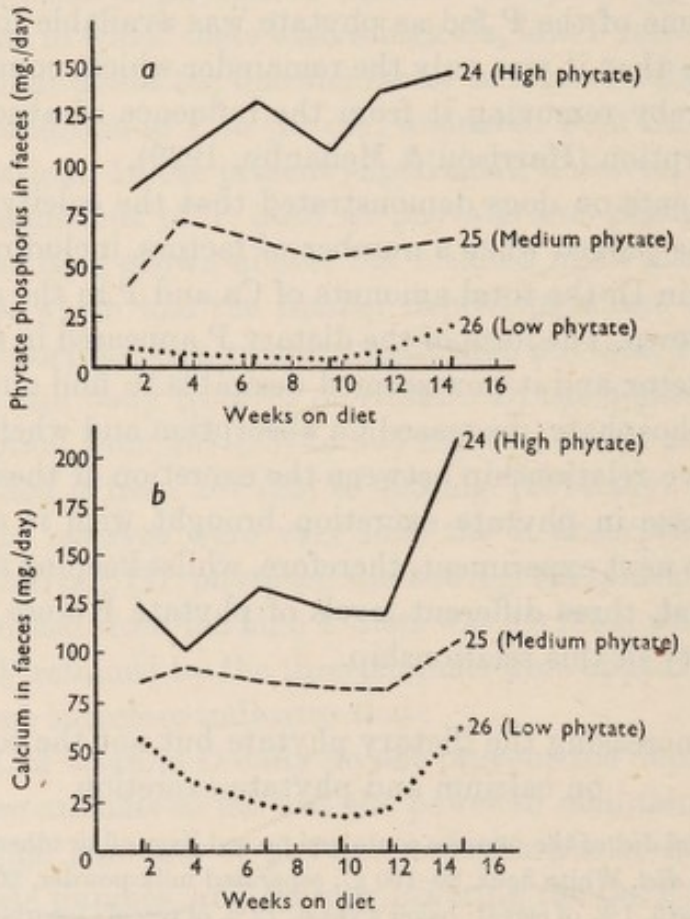
Age at beginning of experiment: 6½ weeks; duration of experiment: 16½ weeks.

The effect of the different diets tested in this experiment on the phytate and Ca in the faeces is shown in Text-fig. 9*a*, *b*. The amount of dietary phytate varied greatly (high in puppy 24, low in 26 and intermediate in 25), and it was found that the unabsorbed phytate was related to the intake (Text-fig. 9*a*).

TABLE 8 (Exp. 8)

	Dietary conditions				
	Phosphorus added to basal diet (mg./day)				
	Na phytate		Na phosphate*		
No. of puppy	Min.	Max.	Min.	Max.	Vitamin D_2 (i.u.)
24	60	480	4	29	20
25	30	240	34	269	20
26	0	0	64	509	20

* Na phytate hydrolysed by phytase of yeast.



Text-fig. 9 (Exp. 8). Effect of increasing the phytate P, but not the total P of the diet, on the faecal excretion of phytate and Ca. (*a*) In the presence of vitamin D, increasing the phytate of the diet when the total P intake was kept constant raised the faecal phytate; (*b*) in the presence of vitamin D increasing the phytate of the diet when the total P intake was kept constant raised the faecal calcium.

Although the Ca, P and vitamin D content of the food of these animals was identical, a high-phytate low-phosphate intake was accompanied not only by a high phytate but also by a high Ca excretion (Text-fig. 9*b*), a low-phytate

high-phosphate intake by both a low phytate and low Ca excretion, whilst an intermediate phytate intake resulted in an intermediate Ca excretion. It is evident, therefore, that under the conditions of this experiment the amount of phytate in the diet assumed a position of great importance. Text-fig. 9*a* leaves no doubt as to the Ca-depriving action of phytate as compared with phosphate of the same P content, when vitamin D was available to the animal.

Comparison of the Ca excretions of puppies 24, 25 and 26 after only 2 weeks of the experimental period, when the total daily intake of phosphorus was 451 mg., shows that an increase in the phytate P from 21 mg. in the diet of puppy 26 to 111 mg. in that of 25, and from 111 mg. in the diet of 25 to 201 mg. in that of 24, produced in both instances about a 50% increase in Ca excretion.

Only three animals have been described in this experiment, although tests were carried out on six. The group not mentioned above received the same amounts and forms of P and Ca, but the vitamin D intake was 100 i.u. instead of 20 i.u. daily. This increase in vitamin D supplies did not appear to modify the Ca-depriving effect of phytate in this instance. In the next experiment, therefore, the effect of giving 20, 100 and 1000 i.u. respectively of vitamin D daily at two levels of phytate intake was determined.

The relative effect of phytate and phosphate on calcium and phytate excretion with varying quantities of dietary vitamin D_2

Exp. 9 *Sodium phytate and sodium phosphate*

Pre-experimental diet. From the age of 3 weeks each puppy received 2·5 ml. of cod-liver oil daily for 23 days, after which period the oil was discontinued.

Basal experimental diet. White flour, 50–160 g.; separated milk powder, 30–20 g. (reduced after 3 weeks on diet); lean meat, 15 g.; peanut oil, 11 ml.; NaCl, 1% of cereal; baker's yeast, 12% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1500 i.u. (Ca content: initial, 367 mg.; final 293 mg. P content: initial, 305 mg.; final, 372 mg.)

Daily additions to basal diet

No. of puppy	Mg. phytate P/100 g. flour	Mg. phosphate P*/100 g. flour	Vitamin D_2 (i.u.)
27	0	318	20
28	300	18	20
29	0	318	100
30	300	18	100
31	0	318	1000
32	300	18	1000

* Na phytate hydrolysed by phytase of yeast.

Age at beginning of experiment: 7 weeks; duration of experiment: 15½ weeks.

Text-fig. 10*a*, *b* shows that, as in the previous experiment, a high phytate intake was accompanied by both a high phytate and a high Ca excretion. The point to be noted from these figures is that increasing the vitamin D intake from 20 i.u. in the case of puppies 27 and 28 to 100 i.u. in 29 and 30 and to 1000 i.u. in 31 and 32 did not greatly increase the amount of Ca absorbed (Text-fig. 11) and did not significantly alter the depressing effect of phytate on Ca absorption.

This result on Ca absorption was in marked contrast to the difference observed in Exp. 6 (Text-fig. 7) between an animal having no vitamin D in the food or body and one to which a relatively small amount, 20 i.u. daily, was given. In such a case the presence of vitamin D was the determining factor as regards Ca absorption from the gut. On the other hand, Text-fig. 10*b* shows that under

TABLE 9 (Exp. 9)

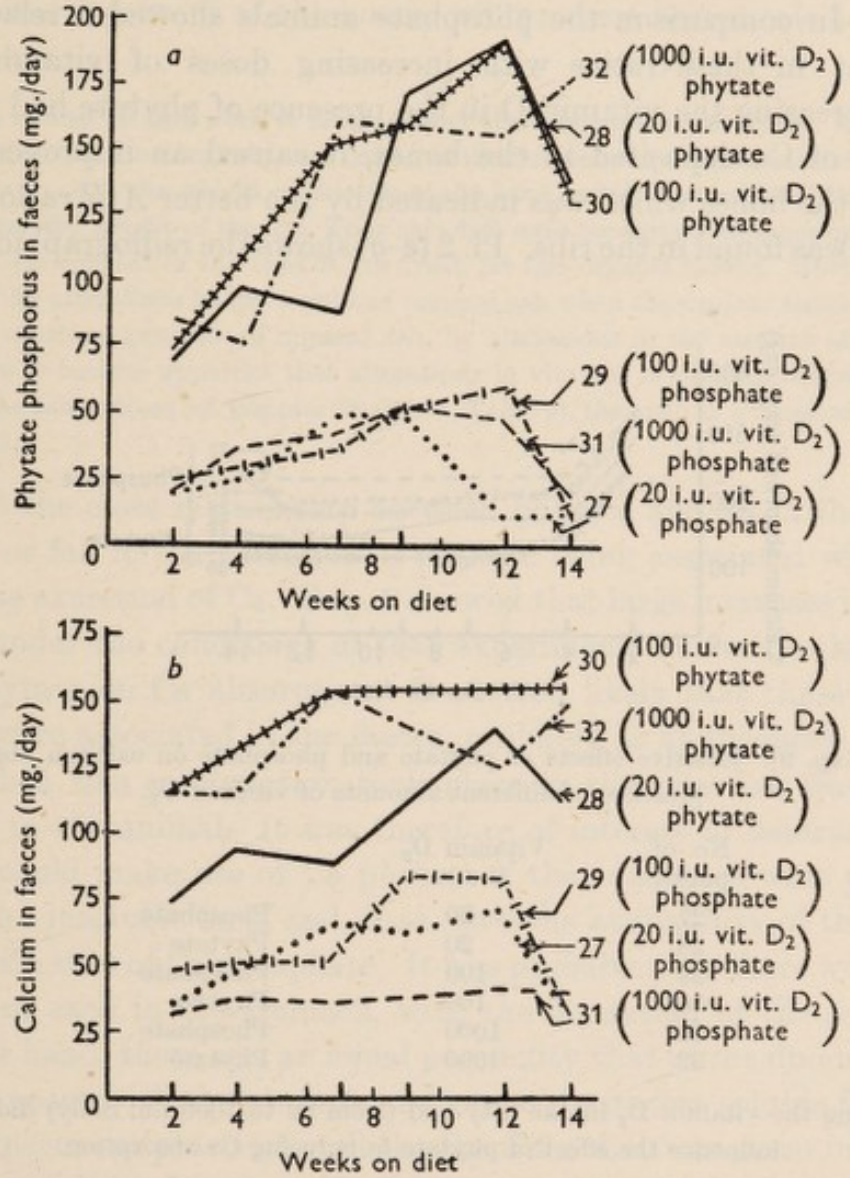
Dietary conditions						Bone results		
	Phosphorus added to basal diet (mg./day)							
	Na phytate		Na phosphate*					
No. of puppy	Min.	Max.	Min.	Max.	Vitamin D_2 (i.u.)	Combined weight of ash of humerus radius and ulna (g.)	Combined weight of ash of 5th, 6th 7th and 8th ribs (g.)	*A/R* ratio of femur shaft
27	0	0	159	509	20	6·555	0·987	1·13
28	150	480	9	29	20	5·762	0·678	0·87
29	0	0	159	509	100	6·449	0·958	1·25
30	150	480	9	29	100	5·053	0·724	1·07
31	0	0	159	509	1000	6·732	0·929	1·26
32	150	480	9	29	1000	5·385	0·695	1·19

* Na phytate hydrolysed by phytase of yeast.

the conditions of Exp. 9 the amount of phytate in the food again assumed a position of great importance and greatly affected the amount of Ca excreted. It will be seen later in Exp. 12, where oatmeal was the source of the dietary phytate, that increasing the vitamin D above 20 i.u. increased Ca absorption under some circumstances. Whether this was due to the fact that the phytate was a constituent of the oatmeal and not, as in Exp. 9, free Na phytate, or whether it was due to other variations between the two experiments is not known. Clearly 20 i.u. of vitamin D_2 daily are near the 'limiting' amount, i.e. the level of intake which produces optimum absorption of Ca from the gut.

Whilst little or no rickets could be produced under the conditions of Exp. 9 because of the presence of vitamin D, the effect of phytate would be to reduce the amount of Ca in the bones. This can be seen to have happened, since the average ash weight of the humerus, radius and ulna of the three phytate animals was 5·4 g. and that of the corresponding bones of the phosphate animals 6·579 g. Thus, the relative effects of phytate and phosphate on the absorption of Ca from the alimentary canal (as seen in Text-fig. 11) were reflected in the amount of calcium in the bones. It has also been seen that variations in the daily amount of vitamin D did not cause significant differences in the amount of Ca absorbed from the gut, either in the phytate or in the phosphate animals. In keeping with this result, it will also be noticed that the combined mineral ash of the humerus, radius and ulna was similar, namely 6·555, 6·449, and 6·732 g. in the phosphate animals and 5·762, 5·053 and 5·385 g. in the three animals taking phytate. Thus, the amount of Ca salts deposited in the bones, while greatly affected by phytate as compared with phosphate in the food, was not signifi-

cantly affected by increasing the amount of dietary vitamin D above a certain quantity.



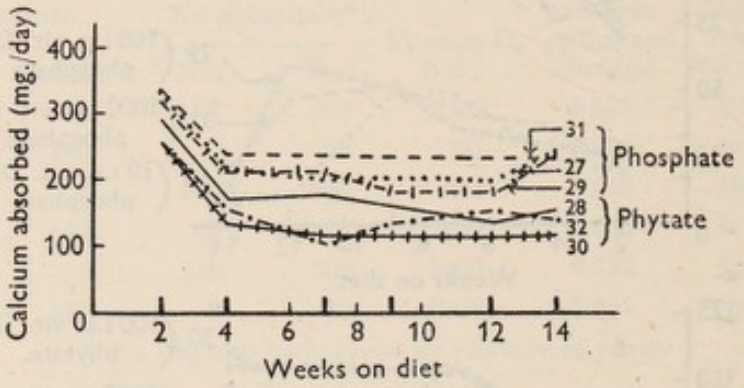
Text-fig. 10 (Exp. 9). Effects of increasing the dietary vitamin D_2 on the faecal excretion of phytate and Ca.

No. of puppy	Vitamin D_2 (i.u.)	
27	20	Phosphate
28	20	Phytate
29	100	Phosphate
30	100	Phytate
31	1000	Phosphate
32	1000	Phytate

Note. (1) Increasing the dietary phytate at the expense of phosphate increased both the phytate (*a*) and to some extent the Ca (*b*) in the faeces; (2) a large increase of vitamin D_2, i.e. from 20 to 1000 i.u. daily, did not significantly alter the effect of the dietary phytate on phytate (*a*) and Ca (*b*) excretion.

When, however, the A/R ratios of the femurs are considered (Table 9), it is evident that increasing the dietary vitamin D caused an improvement in the

quality of the bone formed. This was especially the case in the phytate animals, where the A/R ratios were 0·87 (20 i.u. vitamin D), 1·07 (100 i.u.) and 1·19 (1000 i.u.). In comparison the phosphate animals showed a relatively small improvement in these ratios with increasing doses of vitamin D. Thus although increasing the vitamin D in the presence of phytate had no effect on the amount of Ca deposited in the bones, it caused an improvement in the structure of the bones which was indicated by the better A/R ratios. The same relationship was found in the ribs. Pl. 2 (*e–g*) shows the radiographic appearance



Text-fig. 11 (Exp. 9). Relative effects of phytate and phosphate on calcium absorption in the presence of different amounts of vitamin D_2.

No. of puppy	Vitamin D_2 (i.u.)	
27	20	Phosphate
28	20	Phytate
29	100	Phosphate
30	100	Phytate
31	1000	Phosphate
32	1000	Phytate

Note. Increasing the vitamin D_2 intake fifty-fold (from 20 to 1000 i.u. daily) did not appear to influence the effect of phytate in reducing Ca absorption.

of the costochondral junctions of 27, 28 and 32, and it will be seen that, in the presence of 20 i.u. of vitamin D_2, a diet in which the P was mainly phosphate (*e*) produced well-formed bones, whereas when the phosphate was largely replaced by phytate of equal P content, the bones were thick and osteoporotic (*f*). Thus, in the presence of equal dietary vitamin D, replacing phosphate by phytate lowered Ca absorption (Text-fig. 11), reduced the mineral content of the ribs (Table 9) and, as would be expected, reduced the quality of the bones (Pl. 2*e,f*).

The effect of raising the vitamin D intake from 20 to 1000 i.u. daily (*g*) with equal dietary phytate was more interesting, for it greatly improved the structure of the bone (cf. *f* and *g*) without significantly altering the Ca absorption (Text-fig. 11) or the mineral ash of the bone (Table 9), and the thin compact bones of puppy 32 (*g*) were comparable in radiographic appearance to those of puppy 27 (*e*).

It is obvious, therefore, that, when phytate forms a substantial part of the diet, raising the vitamin D intake, although not ensuring a higher Ca absorption or higher mineral content of the bones, will produce better calcified and more compact bones.

Note In the course of this work it became clear that the A/R ratio (Chick, Korenchevsky & Roscoe, 1926; Chick & Roscoe, 1926) has only a limited usefulness as a measure of calcium retention. In this ratio, A is the weight of the ash of the bone and R the weight of the dried, fat-extracted bone less the weight of the ash. Thus the A/R ratio becomes a measure of the proportion of the mineral ash content of the bone to its dried, fat-free organic tissues. Obviously this ratio may be varied by alterations in the weight of mineral ash when the organic tissue is constant or, in the case of constant amounts of mineral ash, by alterations in the amount of organic tissue. When therefore it became apparent that alterations in vitamin D supplies might vary the A/R ratio and not the ash content (cf. puppies 28 and 32, Table 9), the ratio as a measure of calcification was abandoned.

In Exp. 8 the close relationship between phytate and Ca in the faeces was seen, a rise or fall in the excretion of phytate being associated with a similar change in the excretion of Ca. Exp. 9 showed that large increases in vitamin D could not, under the conditions of that experiment, overcome the depressing effect of phytate on Ca absorption. It seemed likely that these unabsorbed substances were associated in the faeces, probably as an insoluble Ca phytate, and that when this combination took place in the intestine the Ca became unavailable to the animal. It was therefore of interest to determine whether the animal could make use of Ca phytate if the substance were presented in the food in an insoluble form and, if so, how the availability of this substance compared with that of Ca phosphate. It was probable that there would be some breakdown of each in the stomach, with the formation of the soluble $CaCl_2$. On the other hand, there was an equal possibility that in the duodenum the Ca might again react with the phytate and return to its less soluble form. In the following experiment practically all the Ca of the diet was given in the form of either penta-calcium phytate or Ca phosphate; on the other hand, only about 70% of the total P intake came from the Ca phytate. The object was to see to what extent dogs could make use of the Ca and P of these compounds and whether a large increase of vitamin D in the diet facilitated their absorption.

Calcium phytate or calcium phosphate as source of dietary calcium

Exp. 10

The pre-experimental diet of the puppies contained no cod-liver oil for 3½ weeks prior to the beginning of the experimental period.

Basal experimental diet. White flour, 80–120 g.; lean meat, 15 g.; peanut oil, 10 ml.; NaCl, 1 g.; baker's yeast, 5% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1500 i.u. (Ca content: initial 22 mg.; final, 30 mg. P content: initial, 125 mg.; final, 175 mg.)

Note. No separated milk powder; hence Ca of diet low.

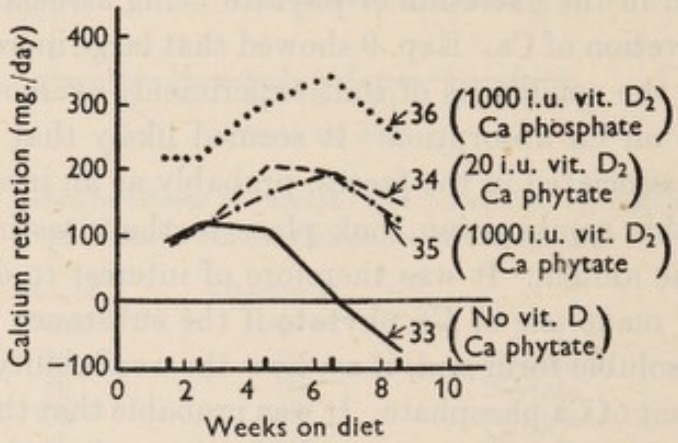
Daily additions to basal diet

No. of puppy	Penta-Ca phytate*	Ca phosphate	Vitamin D (i.u.)
33†	+	–	0
34	+	–	20
35	+	–	1000
36	–	+	1000

* Each batch was tested independently, the Ca:P ratios varying from 1·03 to 1·1.
† See note under Table 10.

Age at beginning of experiment: about 8 weeks; duration of experiment: 9 weeks.

It is seen from Text-fig. 12 that puppies 34 and 35, both of which had Ca phytate and vitamin D_2 in addition to the basal diet, absorbed sufficient Ca to maintain a positive balance of between 100 and 200 mg. daily throughout the experimental period, but that 35, receiving 1000 i.u. of the vitamin daily, absorbed no more than 34, which had only 20 i.u. Both, on the other hand,



Text-fig. 12 (Exp. 10). Relative effects of Ca phytate and Ca phosphate on Ca retention in the presence of different amounts of dietary vitamin D_2. About 85% of the Ca in the diet was given as Ca phytate in 33, 34, 35 and as Ca phosphate in 36. *Note.* In the absence of dietary vitamin D (33) the power to absorb Ca from Ca phytate was soon reduced. 20 i.u. of vitamin D_2 daily (34) promoted a higher retention of Ca, but 1000 i.u. (35) effected no further improvement. With equal amounts of vitamin D_2 (1000 i.u.), Ca phosphate (36) allowed a much higher Ca retention than did Ca phytate (35).

absorbed much less than puppy 36, which had the larger dosage of vitamin D_2 and the same amount of calcium as 34 and 35, but as phosphate instead of phytate. The calcium absorbed by the phytate animals during the experimental period could only have come from the Ca phytate in the food. The third of these, puppy 33, which had no vitamin D in its diet, lost its ability to absorb Ca after $6\frac{1}{2}$ weeks of the experiment, in spite of the fact that it did not eat as much food as the others, grew less, and at an early stage of the experiment had one dose of 200 i.u. of vitamin D_2 injected into the blood stream (see note, p. 513), which no doubt lengthened the period during which absorption of Ca took place. It is therefore probable that in the complete absence of vitamin D the Ca of Ca phytate was unavailable to this dog.

TABLE 10 (Exp. 10)

No. of puppy	Dietary conditions. Additions to basal diet: Calcium Total* Min.	Calcium Total* Max.	Calcium Phytate Min.	Calcium Phytate Max.	Phosphorus Total* Min.	Phosphorus Total* Max.	Phosphorus Phytate Min.	Phosphorus Phytate Max.	Vitamin D_2 (i.u.)	Bone results: Combined weight of ash of humerus radius and ulna	Bone results: *A/R* ratio of femur shaft	Bone results: Rickets† as judged by X-rays at P.M.
33‡	212	366	191	322	319	469	200	300	0	3·077	0·958	5
34	272	366	256	322	408	469	250	300	20	4·656	1·103	0
35	272	366	256	322	408	469	250	300	1000	4·586	1·106	0
36	308	366	0	0	432	469	44	55	1000	5·129	1·312	0

* These figures were obtained from estimations on the cooked food. The Ca as phytate figures were calculated.

† Rickets graded 1–10, the number increasing with the severity of the disease.

‡ As this animal did not eat well, 200 i.u. of vitamin D_2 were injected intravenously on the 7th day. The appetite improved but it was not possible to give it the same amount of cereal and Ca phytate as the rest of the family until towards the end of the experiment.

When bone calcification is considered it will be seen that the mineral ash weights (Table 10), as would be expected from the Ca absorption, were low in 33, high in 36 and equal at an intermediate level in 34 and 35. The *A/R* ratios show that 36 (phosphate) had better calcified bone than the three phytate animals, but the differences between 33 (D-deficient), 34 (20 i.u.) and 35 (1000 i.u.) were small. This may have been due partly to the short experimental period and relatively poor bone growth and partly to the injection of 200 i.u. of vitamin D_2 given to 33.

Thus the results of this experiment are in keeping with the findings of Exps. 8 and 9, namely that phytate in the presence of vitamin D can reduce both the Ca absorption and the amount of Ca deposited in the bones to a greater extent than phosphate of equal P content, and further that a large increase in dietary vitamin D does not offset this effect. It also shows that an animal supplied with vitamin D cannot only make use of Ca given as an insoluble penta-calcium phytate but can in some way deal with the phytate and reduce the amount excreted. Thus when the diet contained vitamin D (20 or 1000 i.u. daily) only about 40–60% of the P fed as phytate was recovered from the faeces.

The Interaction of Additional Calcium with Phytate in the Presence of Different Amounts of Dietary Vitamin D

It was shown in 1925 (Mellanby) that the addition of calcium to a low-calcium, vitamin D-deficient diet consisting largely of cereals resulted in improved calcification with a delay in the onset of rickets. In the following experiments an examination was made of the interaction of different amounts of dietary Ca with phytate as indicated by the absorption of Ca and phytate P and by calcification of bones.

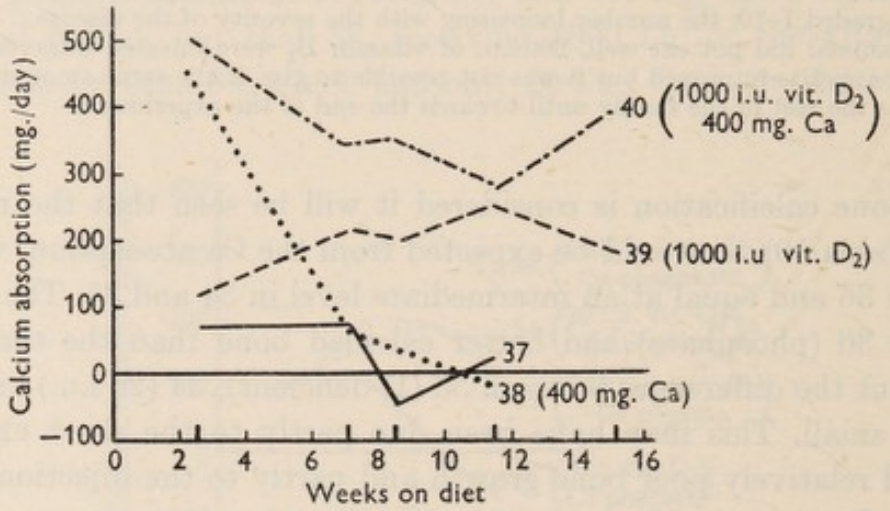
Exp. 11

Pre-experimental diet. For about 2 weeks before the experiment was begun each of the puppies had 2·5 ml. of cod-liver oil, daily, so that all would have moderate bodily reserves of vitamin D.

Basal experimental diet. Bread, 64–150 g.; separated milk powder, 20 g.; lean meat, 7·5–17·5 g.; peanut oil, 5·6–13 ml.; NaCl, 0·75–1·75 g.; baker's yeast, 2·25–5·25 g.; ascorbic acid, 5 mg.; cabbage, 10·6–24·5 g.; Na phytate, 200 mg. (Ca content: initial, 290 mg.; final, 398 mg. P content: initial, 523 mg.; final 700 mg., phytate P content: initial, 216 mg.; final, 230 mg.)

Age at beginning of experiment: 6½ weeks; duration of experiment: 15–16 weeks.

Text-fig. 13 shows that, in the presence of abundant dietary vitamin D_2, increasing the calcium intake led to a greatly increased calcium absorption, especially in the earlier days of the feeding period (see 40 as compared with 39).



Text-fig. 13 (Exp. 11). Effect of different dietary calcium intakes on Ca absorption in the presence and absence of vitamin D_2. *Note.* Ca absorption was highest throughout in 40 and 39 (1000 i.u. vitamin D_2) but was specially high in 40 (extra 400 mg. Ca). After 7 weeks of the D-deficient diet (37 and 38) the power to absorb Ca was much reduced even in the presence of an extra 400 mg. of Ca (38). 200 mg. P as sodium phytate were included in the daily diets of all.

When there was no vitamin D in the diet, but the reserves were moderately good, a high calcium intake, as in the case of puppy 38, also caused a large calcium absorption. As the reserves were used up, however, most of the additional dietary Ca was excreted and the amount absorbed diminished, so that by the 7th week of the experiment only about 50 mg. daily were absorbed as compared with 450 mg. in the early days of the feeding period. After this interval there was but little or no difference in the amount of calcium absorbed by puppies 37 and 38, receiving the lower and higher calcium diets respectively.

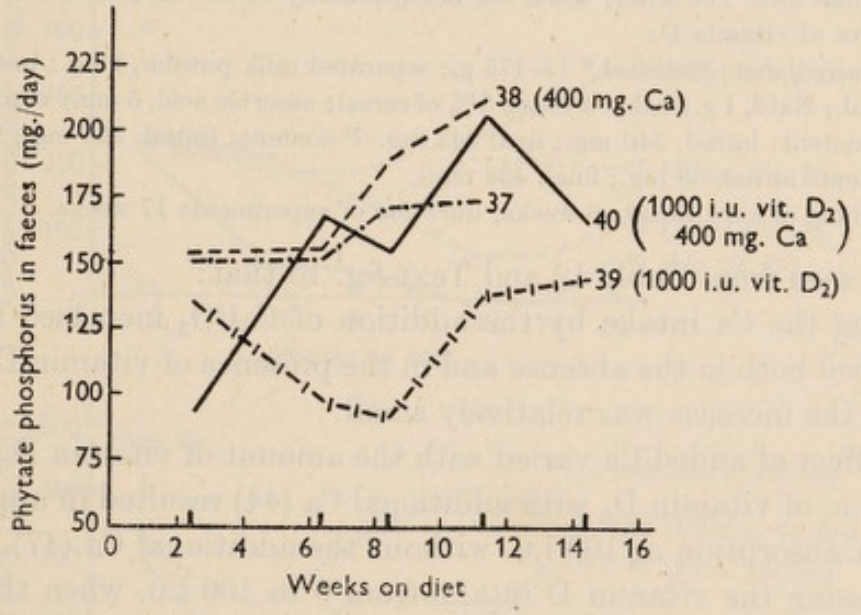
The effect of the large absorption of calcium by puppy 38 during the early weeks on the high Ca diet was greatly to delay the onset of rickets. After 11½ weeks of the experiment the radiographic appearance of this animal was nearly normal, whereas puppy 37, with no extra Ca, had developed very severe rickets and even at the end of the feeding period there was much less rickets in 38 than 37.

In view of the close relationship between phytate P and Ca excretion demonstrated in Exp. 8–10, it would be expected that the greatly increased excretion of Ca by puppy 38 following the loss of its vitamin D reserves would result in a corresponding excretion of phytate. Text-fig. 14 shows that this is not so, for, in the absence of vitamin D from the diet the additional Ca had but little effect

TABLE 11 (Exp. 11)

No. of puppy	Dietary conditions. Daily additions to basal diet: Ca as $CaCO_3$ (mg.)	Dietary conditions. Daily additions to basal diet: Vitamin D_2 (i.u.)	Bone results: *A/R* ratio of femur shaft	Bone results: Rickets as judged by X-rays at P.M.*
37	0	0	0·43	10
38	400	0	1·19	2
39	0	1000	1·24	0
40	400	1000	1·53	0

* Rickets graded 1–10, the number increasing with the severity of the disease.
In this experiment 200 mg. of phytate P were included in the basal diet of all puppies.



Text-fig. 14 (Exp. 11). Effect of additional dietary Ca on phytate excretion in the presence and absence of dietary vitamin D_2. In the presence of vitamin D_2 (1000 i.u.), the addition of 400 mg. of Ca daily (40) increased the faecal phytate (cf. 39). In the absence of the vitamin, the additional 400 mg. Ca (38) did not materially increase the phytate excretion, since much of the phytate ingested was being excreted without the additional Ca (37).

on the phytate excretion. Puppy 38 (high Ca) even after $11\frac{1}{2}$ weeks on diet excreted only about 38 mg. of phytate more than puppy 37 (low Ca). In the presence of dietary vitamin D_2 the addition of dietary Ca caused an increase in phytate excretion, the greatest variation between the puppy having additional Ca (40) and that not receiving it (39) being 77 mg. after $11\frac{1}{2}$ weeks on diet. It will be seen that when no additional Ca was given, 40–60% of the dietary phytate disappeared from the gut of the animal receiving vitamin D (39), whereas only 25–35% disappeared in the case of the D-deficient animal (37). There

was less margin, therefore, for the additional Ca to increase phytate excretion when the diet was deficient in vitamin D.

It is evident from this experiment that, even when there is no vitamin D in the diet, the body reserves of this substance can be very effective in promoting the absorption of any additional calcium there is in the food, but may have less effect on phytate excretion. The presence of dietary vitamin D promotes the absorption of some of the additional Ca, but that not absorbed appears to increase the excretion of phytate.

In the next experiment the effects of adding calcium to diets of animals containing smaller amounts of vitamin D_2 than were given in Exp. 10, namely 0, 5, 20 and 100 i.u. respectively, were tested as it was thought likely that the effect of raising the Ca intake might vary with the magnitude of the vitamin dosage.

Exp. 12

Pre-experimental diet. The litter, when fed independently of the mother, did not receive any additional source of vitamin D.

Basal experimental diet. Oatmeal,* 15–175 g.; separated milk powder, 25 g.; lean meat, 15 g.; peanut oil, 10 ml.; NaCl, 1 g.; baker's yeast, 5% of cereal; ascorbic acid, 5 mg.; vitamin A acetate, 1500 i.u. (Ca content: initial, 340 mg.; final 443 mg. P content: initial, 326 mg.; final, 945 mg., phytate P content: initial, 38 mg.; final, 438 mg.)

Age at beginning of experiment: 6 weeks; duration of experiment: 17 weeks.

It will be seen from Table 12 and Text-fig. 15 that:

(1) Raising the Ca intake by the addition of $CaCO_3$ increased the amount of Ca absorbed both in the absence and in the presence of vitamin D, but in the former case the increase was relatively small.

(2) The effect of added Ca varied with the amount of vitamin D given. Thus a dose of 5 i.u. of vitamin D_2 with additional Ca (44) resulted in approximately the same Ca absorption as 100 i.u. without the additional Ca (47).

(3) Increasing the vitamin D intake from 0 to 100 i.u. when the Ca of the food was low, increased the A/R ratios of the bones (0·85, 1·04, 1·11 and 1·27) but when the Ca content of the diet was higher the only increase in vitamin D intake which produced any significant improvement in calcification was that from 0 to 5 i.u. daily.

This experiment indicates, therefore, that under these conditions the addition of $CaCO_3$ to the diet promotes the absorption of Ca, and that the extra Ca content is of greater significance to the animal's economy when the dietary vitamin D is small.

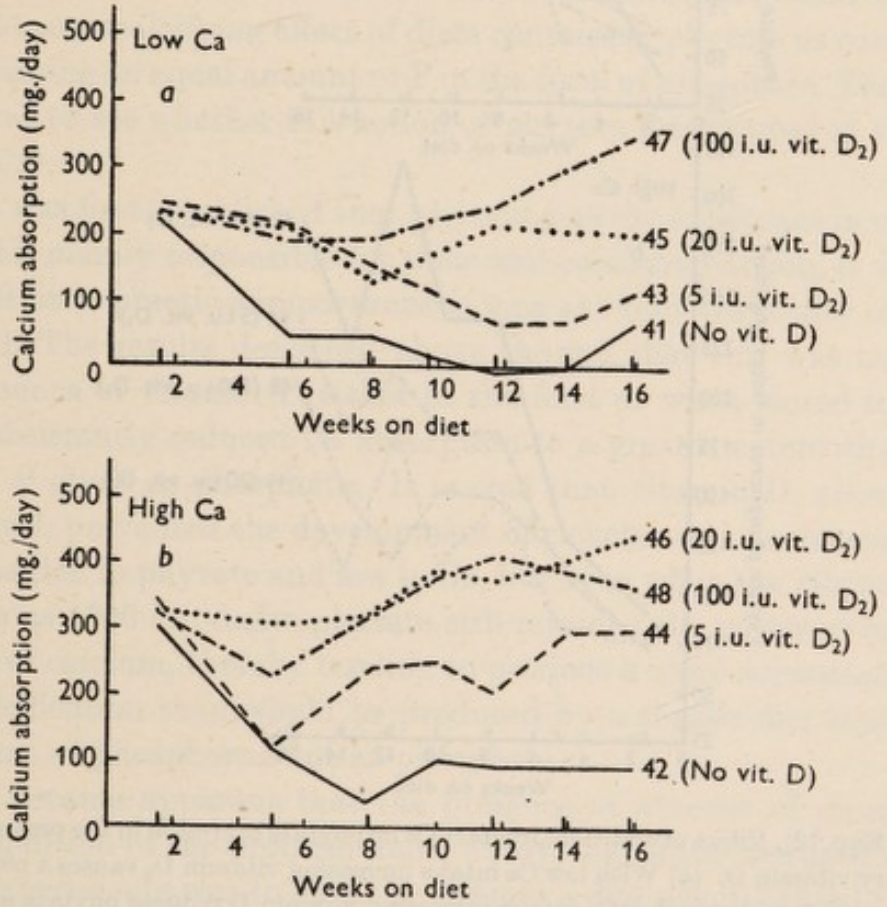
As regards bone calcification, animals 41 and 42 show that an increased Ca absorption in the absence of dietary vitamin D does not necessarily indicate improved bone quality. Thus puppy 42 (Text-fig. 15*b*) consistently absorbed more

* For a family of this size it was considered better to use oatmeal as a source of phytate than to attempt the preparation of large quantities of Na phytate.

TABLE 12 (Exp. 12)

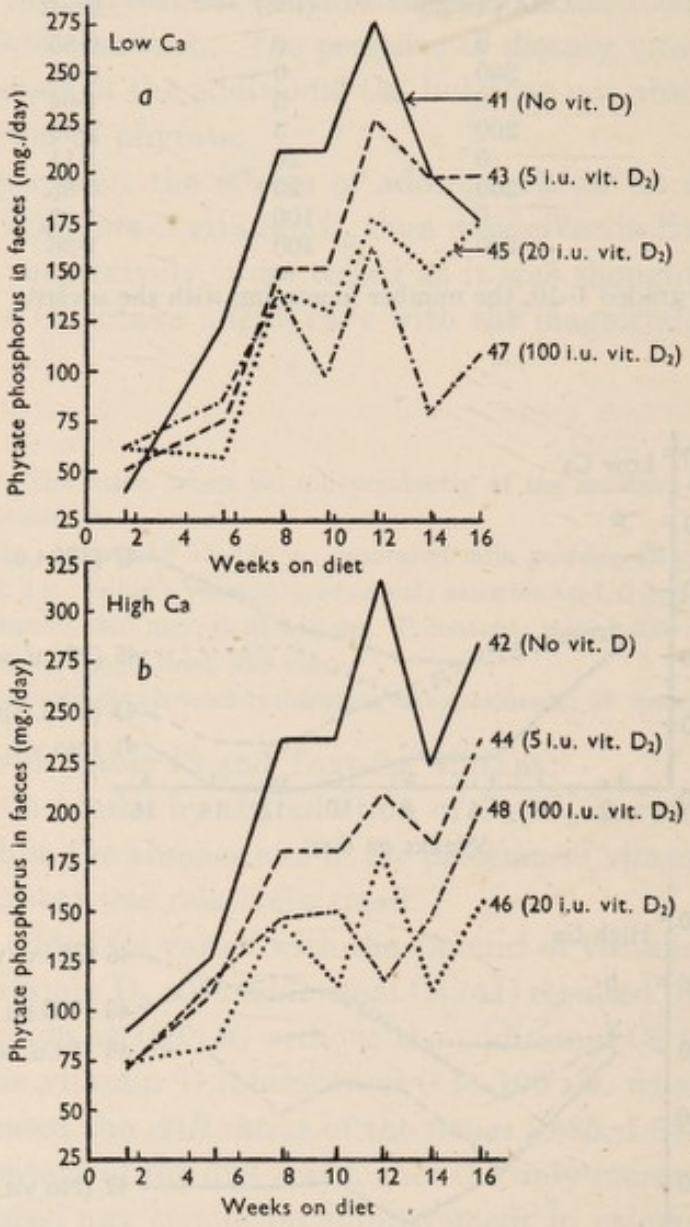
No. of puppy	Dietary conditions. Daily additions to basal diet: Calcium as $CaCO_3$ (mg.)	Dietary conditions. Daily additions to basal diet: Vitamin D_2 (i.u.)	Bone results: *A/R* ratio of femur shaft	Bone results: Rickets as judged by X-rays at P.M.*
41	0	0	0·85	9
42	200	0	0·88	8
43	0	5	1·04	3
44	200	5	1·30	1
45	0	20	1·11	1
46	200	20	1·35	0
47	0	100	1·27	0
48	200	100	1·36	0

* Rickets graded 1–10, the number increasing with the severity of the disease.



Text-fig. 15 (Exp. 12). Effect of additional dietary Ca on Ca absorption in the presence and absence of vitamin D_2. (*a*) With low Ca intake increasing vitamin D causes increased calcium absorption; (*b*) with a higher Ca intake increased vitamin D increases the absorption of Ca except that 46 (20 i.u.) and 48 (100 i.u.) are practically the same (cf. phytate excretion Text-fig. 16*b*). *Note.* Comparison of (*a*) and (*b*) shows (1) that at all vitamin levels extra dietary Ca has caused increased absorption; (2) that 20 i.u. of vitamin D_2 with an additional 200 mg. of Ca (46) had a better effect on Ca absorption than 100 i.u. without extra Ca (47). Similarly, 5 i.u. vitamin D_2 with 200 mg. extra Ca (44) brought about better Ca absorption than 20 i.u. vitamin D without extra Ca (45).

Ca than 41. The mineral ash content of the femur shaft was 23% greater than that of 41, but the A/R ratios (0·88 and 0·85) did not differ significantly and both animals had severe rickets. The converse of this, that equal ash content does not imply equal bone quality, was shown in Exp. 9.



Text-fig. 16 (Exp. 12). Effect of additional dietary Ca on phytate excretion in the presence and absence of dietary vitamin D. (*a*) With low Ca intake increasing vitamin D_2 causes a reduced excretion of phytate; (*b*) with a higher Ca intake increasing vitamin D reduces phytate excretion except that 46 (20 i.u.) and 48 (100 i.u.) are practically the same. *Note.* Comparison of (*a*) and (*b*) shows that (1) in the absence of vitamin D, additional Ca raised the faecal phytate; (2) when vitamin D was given in doses of 5, 20 and 100 i.u. respectively the effect of additional Ca on phytate excretion was small or absent.

Turning now to phytate excretion, Text-fig. 16*a* shows that with the lower Ca intake the phytate excretion was reduced as the dietary supply of vitamin D increased. In the presence of the additional Ca (Text-fig. 16*b*) diets containing 5 and 20 i.u. promoted progressively lower phytate excretions but increases

beyond 20 i.u. had apparently no further action. Comparing the effect of the additional Ca in animals with otherwise identical diets, it will be seen that in the absence of the vitamin the excretion of phytate was slightly increased, but as the dosage of the vitamin was raised from 5 to 100 i.u. daily the slight effect of the additional Ca disappeared.

The reduced effect of the additional Ca on phytate excretion in the presence of a supply of vitamin D may have been due to the fact that most of the additional Ca was absorbed, so that the amount available for precipitating phytate was similar in each pair of animals having the same amount of dietary vitamin D. Had larger quantities of Ca been added to the diet, a stage would undoubtedly have been reached when the faecal phytate would have been greatly increased.

DISCUSSION

Experiments have been described above which formed part of a further study of the anti-calcifying effect of diets containing phytate as compared with those containing an equal amount of P in the form of phosphate. The immediate problem was to see whether this action of phytate was exerted in the presence of vitamin D.

When it was first established that phytate was the substance in oatmeal and other cereals mainly responsible for their anti-calcifying action, it was thought to be of but little practical importance so long as there was plenty of vitamin D in the food. The results described above showed that this was not the case. In the presence of vitamin D either in the food or when stored in the body, phytate consistently reduced Ca absorption to a greater extent than an equal amount of P given as phosphate. It is true that vitamin D, above a certain minimal level, prevented the development of rickets and of osteomalacia when the diet was rich in phytate and low in Ca, but even when the vitamin D intake was as high as 1000 i.u. daily, phytate still retained the power of reducing the absorption of calcium, thereby tending to produce a more subnormal condition of bone calcification than would be produced by a similar diet containing the same amount of phosphorus but as phosphate.

When it became apparent that the presence or absence of vitamin D was a crucial condition of the problem under study, one of the methods used in the foregoing experiments was to give the puppies a diet which caused the gradual exhaustion of their body stores of the vitamin. A great reduction in the power to absorb Ca was regarded as coincident with the exhaustion of these reserves. The period necessary to produce this condition varied greatly according to the quantity of the vitamin consumed and stored before the experiment began. In some cases it took 2 or 3 weeks only, whereas in others it might take two or more months on a vitamin D-deficient diet before the growing puppy lost its power to retain Ca. In adult dogs where the demand was probably less and the stores greater it might take a year before the body was deprived of all traces

of the vitamin. In the early stages of the experimental feeding period before vitamin D depletion, phytate significantly lowered Ca absorption when compared with phosphate, but later this differentiation disappeared and the absorption was reduced in both cases, leading, if the experiment continued long enough, to negative balances of this element. It was not the effect of phytate in depressing Ca absorption which was fundamentally altered by the loss of vitamin so much as that inorganic phosphate changed from being relatively without effect on Ca absorption to having an inhibitory action of the same order as phytate.

In the foregoing experimental work two methods of assessing Ca metabolism have been used, (i) the determination of Ca and P absorbed and excreted by the gut under controlled dietary conditions, and (ii) estimation of the degree of calcification of the bones. Although it is generally true that more or less Ca absorbed from the intestine means better or worse calcified bones, the two measurements are not identical in their interpretation. Increasing the vitamin D supply of the body from nil upwards improves Ca absorption greatly once the stores of vitamin D are depleted, but soon reaches a point beyond which further increases of the vitamin have no such effect. It was found, for instance, that 20 i.u. daily of vitamin D_2, which will be referred to as the 'limiting amount', were often just sufficient with the experimental diets used to promote the maximum amount of Ca absorption, and that raising the dosage from 20 to 1000 i.u. in diets in which all other factors were constant did not materially increase the Ca absorption or retention.

Turning now to the question of the structure and calcification of bone, it would be expected that below the limiting amount of vitamin D, bone calcification would be poor and that rickets would be produced, since the amount of Ca absorbed was subnormal. Above this limiting intake, however, since the amount of Ca absorbed remained a maximum under those experimental conditions, it would be expected that the Ca content of comparable bones would also be a maximum, i.e. as much in amount in animals with low as in animals with higher vitamin D intake. This, indeed, proved to be the case but it did not mean that bones of animals receiving the larger amount of vitamin D were of the same structure, even though the Ca content was similar. In such cases the radiographic appearance and the A/R ratio of comparable bones sometimes showed that the amount of vitamin D which just promoted a maximum absorption of Ca was not sufficient to produce well calcified bones.

This fact can be seen in Pl. 2 (*e*, *f* and *g*) which shows radiographs of the costochondral junctions of three animals, two of which (*f* and *g*) had received a high phytate diet together with 20 and 1000 i.u. respectively of vitamin D. It will be seen that in puppy 28 (*f*) the ribs are thick and osteoporotic, whereas in puppy 32 (*g*), with the higher vitamin intake, they are relatively well calcified and well formed, yet the bones of these two animals contained approximately the same amount of mineral matter. In spite of this the A/R ratios of the femur

shafts were in line with the radiographic appearance of the ribs, being less in the animal with the lower vitamin D intake (Table 9).

Since the A/R ratio represents the proportion of the total mineral content of the bone to the dried fat-free organic matter, the bone with the lower A/R ratio, but with the same total amount of mineral matter, must have had a larger quantity of non-calcified or poorly calcified organic matter, a fact confirmed by radiological and histological examination. The bones of puppy 28 were more cancellous, i.e. they contained more marrow spaces and were also of a more elementary lamellar type of structure with fewer well-formed Haversian systems than those of puppy 32 (1000 i.u. vitamin D_2). Of course with a vitamin D intake below the limiting amount, under the experimental conditions chosen, the Ca absorption from the gut was depressed and frank rickets with a large amount of osteoid tissue was produced. In this case the A/R ratio diminished, both because the mineral content was low and because non-calcified tissue, including osteoid tissue, was relatively increased.

Here, then, is direct evidence that vitamin D not only has the function of controlling Ca absorption and of incorporating it into bone, but above the limiting amount this vitamin controls the quality and structure of bone laid down, i.e. ensures that not only the pattern of the bone is correct but also that what is formed is more fully calcified, by limiting the formation of some non-calcified or partially calcified organic matrix which may not be osteoid tissue. The amount of vitamin D necessary to produce perfect bones is therefore, under the experimental conditions studied, higher than the amount necessary to produce maximum Ca absorption from the gut and maximum Ca incorporation in the bone.

Effect of phytate

The effect of increasing dietary phytate in the presence of vitamin D is quite another matter. In numerous experiments described above it has been shown that substituting sodium phytate for phosphate, so as to retain the same total P in the diet, has two constant effects. In the first place it increases the phytate in the faeces and the more consumed the greater is the amount excreted in this way (Text-fig. 9*a*). It is true that a varying proportion of the phytate eaten disappears, i.e. does not appear in the faeces or urine, and that the amount which disappears is greater when the diet contains vitamin D than when the vitamin is absent from the body. But this action of vitamin D in bringing about the 'disappearance' of phytate from the gut is limited so that, even when the vitamin D intake is raised to a higher level, other conditions being constant, there is no further diminution in the amount of phytate excreted. In other words, the limitation, referred to above, of vitamin D in bringing about Ca absorption, is also accompanied by a similar limitation in causing the disappearance of phytate from the intestine.

The second effect of adding phytate to the diet is to increase the Ca excretion in the faeces. Thus an increase in faecal phytate is associated with an increase in faecal Ca (Text-fig. 9*b*), which means that less Ca is available for absorption. In growing animals this may result in the production of rachitic or osteoporotic bones according to the intensity of the effect and the state of the animal at the time. If the vitamin D intake is just sufficient with the basal diet used to prevent rickets, then the addition of phytate, even if it replaces phosphate, will result in frank rickets. With higher vitamin D supplies, instead of producing rickets, the addition of phytate will tend to produce a more osteoporotic condition of the bone. This action of phytate on bone structure can be seen in Pl. 2 (*e*, *f* and *g*), showing radiographs of the costochondral junctions of three puppies. The ribs of (*f*) are thick and osteoporotic while those of (*g*) are thinner and more normal. Both these animals received 20 i.u. vitamin D_2 daily and the osteoporotic appearance of (*f*) is due to the larger calcium-depriving effect of phytate as compared with that of phosphate (*e*).

Under some conditions phytate may even cause withdrawal of Ca from the body itself. This was shown experimentally in its grossest form by maintaining fully grown dogs on diets poor in Ca and vitamin D and rich in high phytate-containing foods such as oatmeal and maize (Mellanby, 1937). These animals developed severe osteomalacia and osteoporosis with great bone deformity. Additional vitamin D protected animals on these diets to some extent, while vitamin D and a sufficiency of Ca gave full protection. Animals on similar diets in which rice or white flour (poor in phytate) replaced the oatmeal and maize, did not develop these gross deformities although the bones were osteoporotic. Thus it will be seen that dietary phytate has the power not only of immobilizing Ca in food but may even be responsible for its withdrawal from the highly calcified tissues of the body such as bones and for causing its loss through the intestinal tract.

The importance of a high calcium intake with phytate

The practical issue, therefore, is how best to bring about perfect bone formation in growing animals when the diet is relatively rich in phytate, as, for instance, when it contains abundant cereals such as oatmeal or maize. The objective must be to increase the Ca absorption so that it can be incorporated to the best advantage in the bone and other tissues. Obviously a sufficiency of vitamin D is essential, but it is just as important at this stage to increase the Ca intake. There are two reasons for this. In the first place, under the experimental conditions chosen, calcium is often a limiting factor in the diet. Secondly, increasing the Ca makes the vitamin, especially if present in small quantities, much more effective. For instance, a puppy which received 5 i.u. of vitamin D_2 and an additional 200 mg. daily of Ca (Exp. 12) absorbed a quantity of Ca approximately equal to that absorbed by another puppy of the same litter

getting 100 i.u. of the vitamin without the extra Ca (puppy 47). This synergistic effect of Ca salts with the anti-rachitic vitamin has long been known and was described in 1925 when it was found that the calcium retention produced by butter was greatly increased if additional Ca carbonate or Ca phosphate was present in the food (Mellanby). It was this fact which formed the basis for emphasizing at that time the advantages of giving milk rather than an equivalent amount of butter for the calcification of bones in children.

It might be thought that increasing the calcium would only bring about the formation of more insoluble pentacalcium phytate in the intestine and thereby increase both the phytate and the Ca excretion, with no benefit to the animal. It is, of course, true that if the calcium added is excessive and the P intake low such an effect will be produced, but increasing the Ca intake within physiological limits, although causing a slight increase in phytate excretion, also increases the Ca absorption. The idea that the addition of calcium to the diet causes a great increase in the phytate excretion is probably dependent upon rat experiments, a point which will be referred to in more detail later. So far, however, as the dog experiments are concerned, it is undoubted that one way, and the most important practical way, of overcoming the Ca immobilizing effect of a diet rich in phytate, assuming that there is some vitamin D present in the body or diet, is by increasing the calcium intake. A diet which contains vitamin D and is rich both in calcium and phytate is compatible with perfect bone formation, but this is not the case when the diet is rich in both vitamin D and phytate and relatively (but not absolutely) low in calcium. Increasing the Ca intake when the body and the diet are devoid of vitamin D is of little use since much, or in some cases all, of the extra Ca is excreted, together with most of the phytate.

The main fact to be gathered from these experiments is that, once sufficient vitamin D is given, the level of Ca absorption is controlled by the amount of Ca and by the amount and proportion of dietary P given as phytate.

Inconsistency of dog and rat experiments as regards phytate effect

It may be asked why it has taken so many years for the anti-calcifying action of cereals to be generally accepted. Much of the criticism which has been directed against the work has depended on results showing that rickets in rats is controlled by the Ca:P ratio of the diet and the availability of P. Can the present results explain the apparent anomalies between rat and dog investigations? In order to produce rickets in rats with certainty it has been usual to deprive the mother of vitamin D during lactation, give a vitamin-D free diet to the young and use an experimental diet having a high Ca:P ratio. This unnatural dietetic technique and its results have largely dominated the subject of the human disease.

The effect of the complete deprival of vitamin D would certainly tend to increase the significance of the Ca:P ratio of the diet in the aetiology of rickets for, as was demonstrated in the above dog experiments, phosphate becomes under such conditions nearly as anti-calcifying, in the sense of preventing Ca absorption, as phytate. The second condition of the rat experiments, namely the high Ca-low P diet, must also have a special but abnormal significance. Clearly with diets containing four or more parts of Ca to one of P, the factor most powerfully determining the degree of calcification is the availability of the P, for however much Ca is absorbed it cannot be incorporated into growing bone unless there is a sufficiency of P with which to combine. The general effect of adding very large amounts of Ca to the diet is to immobilize by precipitation both the phytate and phosphate P of the diet and prevent its absorption. The significance of P as the limiting factor in experiments on rickets was particularly apparent when Bruce & Callow (1934) showed that with diets of high Ca content phytate in the presence of vitamin D was less effective than phosphate in promoting healing of rickets. The problem of the relative availability of phytate and phosphate in rats was further studied by Krieger & Steenbock (1940), both in the presence and absence of vitamin D, with diets the Ca:P ratio of which varied over a wide range. As a measure of availability of the P of phosphate and phytate they estimated the degree of calcification of the bones. They found that, in the absence of vitamin D and with an optimal intake of P and a Ca:P ratio of 1:1 the utilization of phytate P and phosphate P was not greatly dissimilar. With higher Ca:P ratios of 2:1, 4:1 and 6:1 the availability of the phytate P decreased rapidly, whereas the inorganic phosphate remained available to the optimum extent. When, on the other hand, there was vitamin D in the diet, the effect of altering the Ca:P ratios was not so apparent, for the vitamin improved the utilization of both phytate and phosphate P at all Ca:P ratios. Nevertheless, even in the presence of vitamin D, phytate P was never so readily available as the inorganic form.

These experiments show that phytate and phosphate have different biological availabilities in rats irrespective of the presence or absence of vitamin D, and that these differences only become evident when the P supply to the growing animals is limited. They do not throw any light, however, on the main point at issue, namely whether phytate in the diet is more anti-calcifying than phosphate, i.e. more potent in preventing Ca absorption and utilization, especially under what might be regarded as normal dietetic conditions, when, for example, the Ca:P ratio is less than or approaching 1, and there is at least some reserve of vitamin D available. Green & Mellanby (1928) investigated the effect of such diets on rats and their results indicated that phytate (although not recognized at that time as the offending agent) had a more powerful anti-calcifying effect than phosphate. They showed among other things that oatmeal (high phytate) was more rachitic than white flour (low phytate). Unfortunately it

has proved impossible to confirm these earlier results on rats with any consistency and it must be assumed that some unrecognized condition was present in that work. Many experiments were carried out at that time and the results obtained were so consistent that it is impossible to put them aside as fortuitous. The later discovery of Patwardhan (1937) that the mucous membrane of the intestine of rats is rich in phytase suggests that these animals have a much greater power of hydrolysing phytate to inositol and phosphoric acid than other experimental animals tested and that it is this factor which explains the greater difficulty of depressing bone calcification by phytate in rats. The fact that in rats phytate P only becomes much more difficult to absorb than phosphate in the presence of a very high Ca intake supports this view. Possibly in the type of rat used in the work of Green & Mellanby or under the conditions of their experiments the phytase activity of the gut was abnormally small. However that may be, the evidence indicates that the anti-calcifying effect of phytate is much smaller in the rat than in the dog, and that this difference is probably due to the greater phytase content of the rat intestine.

It is clear, therefore, that if the interpretation of the aetiology of rickets as it occurs in human beings had to depend on past experimental work on rats, it would be a right assumption that the Ca:P ratio and the availability of P of the diet explained the action of cereals in this disease. There would indeed be little or no support for the view that cereals differed in their rickets-producing action and that this was dependent largely on their content of phytate. On the other hand, the present experiments have abundantly confirmed those of Harrison & Mellanby and shown that in dogs phytate as compared with phosphate has the specific property of reducing the absorption of Ca, especially in the presence of vitamin D. In the dog experiments the Ca:P ratio has proved to be but a poor indication of the relation of diet to rickets. The vitamin D content of the body is always dominant but the intensity of its action as regards calcification can be modified in one direction by adding Ca and in the other by adding phytate. It has been shown above that diets having the same Ca:P ratios and amounts may have different effects on Ca absorption and bone calcification according to the chemical nature of the P-containing substance; again, altering the ratio by increasing the phosphates of the diet often has but little effect on Ca absorption. Indeed, it can be said that the amounts of dietary Ca and P necessary to produce optimal bone calcification vary greatly according to the other constituents of the diet and cannot be regarded as having any constant significance. It will be seen that the basis of the ordinary technique used to produce rickets in rats, namely severe limitation of P and excess of Ca intake, has only a partial relation to the nutritional condition which produces the human or canine disease where the main determining factor is the absorption of Ca in a sufficient and a correct form and not a deficiency either in the supply or absorption of P. The rat, as an experimental animal in the

elucidation of the aetiology of rickets as it occurs in human beings, has in some respects proved a defective guide and has long prevented the acceptance of the fact that some cereals produce defective calcification and that this action is dependent on the phytate content and Ca limitation.

The toxamin or anti-vitamin action of phytate—phytate and phosphate as chemical analogues

It may be of interest to discuss briefly the anti-calcifying action of phytate in the light of other recent discoveries on the antagonistic action of structural analogues, which have not only greatly illuminated the subject of anti-vitamins in general but have opened up many other biochemical problems, including the important field of chemotherapy. It was stated in the introduction that when the anti-calcifying action of cereals, and especially of oatmeal, was first reported, the hypothetical substance responsible for this action was described as an anti-vitamin, but that later the word 'toxamin' was used as being more descriptive of its effects. As the chemical nature of the cereal anti-vitamin was not known at that time no suggestions as to the basis of the antagonism were possible. The word 'toxamin' was substituted for anti-vitamin in this instance in order to describe a substance present in some cereals which, by interfering with Ca metabolism, had a harmful effect on the body. It was thought at that time that vitamin D could effectively prevent this action. The present work shows that this is not entirely the case but that, although vitamin D can usually cloak the harmful action of phytate, especially as regards the production of florid rickets, it cannot by itself prevent some anti-calcifying effect. Kodicek (1948) has suggested that the term 'toxamin' should be applied to all substances which interfere with the action of vitamins and that the term 'anti-vitamin' should be reserved for substances which act by reason of chemical analogy. Phytate of cereals, under this definition, qualifies for the designation 'toxamin' because it interferes with the action of vitamin D, probably not by direct antagonism to the vitamin, but indirectly by limiting the amount of Ca available upon which the vitamin can work. It is recognized, however, that this explanation may not account for all the actions of phytate in relation to vitamin D.

There are obviously a number of ways in which a vitamin can be prevented from doing its work. It may be destroyed in the intestine, or if present in the food or formed in the gut by micro-organisms, it may not be absorbed for some reason, but these instances are not due to the presence of an anti-vitamin. Anti-vitamin action may depend on the presence of a substance in food which is a structural analogue of the vitamin and displaces it from the surface on which it catalyses certain essential chemical reactions. In such an instance, while the anti-vitamin has a structural resemblance to the vitamin, it also has a structural difference which prevents it from having the biological action of

the natural vitamin. Although this is a widely accepted hypothesis, advanced to explain certain cases of biochemical interference of this type, not only in the case of some vitamins but of other substances having a drug-like action, there are certainly other possible modes of interfering action of structural analogues. For instance, an anti-vitamin may not interfere directly with the vitamin but may act by removing the substrate from its sphere of influence. This latter type of action is more likely to be the main explanation of the present problem of interference of phytate in calcification. As regards Ca absorption from the gut, the antagonism between phytate and vitamin D does not seem to be direct, otherwise a more evident quantitative relationship would exist between the two substances. As has been shown in Exp. 8 above, however, increasing the vitamin D of the diet from 20 to 1000 i.u. did not further antagonize the power of phytate to reduce Ca absorption from the gut. On the other hand, as also shown above, there was good evidence of a direct antagonism between phytate and phosphate in regard to Ca.

In what form the calcium passes from the gut into the blood stream is not known, but it may be that its absorption involves a reaction of a complex containing Ca and phosphate in a form related to the compound which is ultimately deposited in bones. In the complete absence of vitamin D from the body, whereas little Ca is absorbed, the absorption of P may be relatively large. On the addition of the vitamin to the food the absorption of Ca is immediately resumed and with this the P absorbed is also increased. The assumption therefore is that, under the influence of vitamin D, Ca is absorbed in association with phosphate. The view that the absorption of Ca is the dominant factor in the action of vitamin D on the intestine is in agreement with that of Nicolaysen (1937). He demonstrated that, in rats on a low P diet, Ca absorption was greatly reduced in vitamin D deficiency, but that when dietary Ca was low and the P moderate the latter was absorbed to an equal extent both in the presence and absence of the vitamin. He suggested that the reduced absorption of P reported in vitamin D-deficient rats was due to the large amount of unabsorbed Ca in the bowel precipitating the P. It may not be out of place to remark here again that the unphysiological high Ca-low P diets used to produce rickets in rats may be misleading, for in dogs with diets of more natural Ca:P ratios it appears that the reduced P absorption in vitamin D deficiency is due to the fact that when the Ca is not absorbed less P is needed and not, as in rats, to the precipitation of the phytate P by the large amount of unabsorbed Ca in the gut. Assuming, therefore, that Ca is absorbed in some combination containing phosphate, it follows that phytate, by combining with Ca to form the insoluble, quickly precipitated penta-calcium phytate (Hoff-Jørgensen, 1944), effectively reduces the formation of any complex containing the more soluble and more slowly precipitated calcium phosphate and thereby the Ca available for absorption. Here, then, is an instance of structural analogues, phytate and

phosphate, competing for a third substance, Ca, the absorption of which is controlled by a vitamin.

Whereas this part of the antagonism between phytate and phosphate and vitamin D is reasonably clear, it is more difficult to understand how vitamin D prevents the phytate effect becoming complete master of the situation. It does this at least partly by establishing conditions favourable to the breakdown of a large part of the phytate in the diet. How powerful this action can be is seen in Exp. 10 above, where it was shown that, even when the sole source of calcium in the diet was in the form of Ca phytate, the body absorbed a fair amount of calcium when there was a source of vitamin D in the body or in the food. It is true that the amount absorbed was much less than when the dietetic Ca was in the form of calcium phosphate; for instance, in Exp. 10, 218–348 mg. of Ca were absorbed daily in the phosphate animals when practically the only source of Ca was calcium-monophosphate, whereas in the corresponding phytate animal only 85–199 mg. of Ca were absorbed. It is difficult to believe that so much of the Ca of the phytate compound would have been absorbed if some of the phytate had not been converted to phosphate, and there is evidence that this actually happens under these conditions. In Exp. 10 it was found that 40–60% of the phytate consumed disappeared from the gut. This disappearance of the phytate caused by vitamin D will be described and discussed in a later publication.

In the complete absence of vitamin D, both from the diet and from the body, very little Ca is absorbed from the alimentary canal. As the vitamin D content of the diet increases, the amount of Ca absorbed increases, but now the antagonism between phytate and phosphate comes into play, the amount of Ca absorbed being lower in animals receiving dietary phytate. Vitamin D, probably by promoting the hydrolysis of phytate to phosphate, reduces this effect, but even a large addition of the vitamin does not permit destruction of all the phytate and the Ca absorption remains subnormal. Apart from its action in the gut, phytate appears to have little, if any effect, and vitamin D takes full control, improving the calcification of bones both by depositing a Ca-phosphate complex in osteoid tissue and by reducing the proportion of other organic as compared with inorganic material. Even with large supplies of vitamin D, however, unless additional Ca is given to satisfy the undestroyed phytate in the intestine, the calcification of the bones of animals receiving large amounts of dietary phytate will remain subnormal as compared with that of animals whose phosphorus is given as phosphate.

There is still one further aspect of phytate and vitamin D relationship which, while obviously a part of the general problem, evades explanation. This is a point which was indicated in Exp. 7, namely that dietary phytate seems to impose an increased demand on vitamin D. In that experiment it appeared that phytate hastened the disappearance of body reserves of this vitamin.

Inorganic phosphate may have a similar action, but, if so, it is of a lower intensity than that of phytate. The explanation of an antagonism of this kind, if confirmed by further work, would require more than the Ca-immobilizing effect of phytate, which seems to be the main explanation of the latter's action. The interplay of vitamin D, Ca, inorganic phosphate and phytate is still far from being understood.

Phytate and phosphate in human dietary

Finally it may be asked whether the factors described above apply to human beings. There is good evidence that they do. For instance, McCance & Widdowson (1942*a*,*b*) have shown: (1) that Ca was much less freely absorbed from diets consisting largely of brown bread than from those consisting largely of white; (2) the amount of Ca absorbed from brown bread diets could be raised by adding Ca to the diet; (3) the absorption of Ca from white bread could be prevented by adding sodium phytate to it; (4) the absorption of Ca from brown bread diets could be improved by dephytinizing the bread, i.e. by allowing the phytase of the flour to hydrolyse the phytate to inorganic phosphate.

Danish workers have also studied the problem and found that phytate interferes with the absorption of Ca in infants (Hoff-Jørgensen, Andersen, Begtrup & Nielsen, 1946) and in children (Hoff-Jørgensen, Andersen & Nielsen, 1946). So far as the general problem is concerned, namely the specific action of phytate in lowering the absorption of Ca from the gut, human beings undoubtedly react in the same way as dogs but probably unlike rats. The partial destruction of phytate also appears to take place in the human alimentary canal as in dogs and, according to Cruickshank, Duckworth, Kosterlitz & Warnock (1945) the amounts so destroyed may be large when the diets are sufficiently low in Ca to give a negative balance. The same workers showed that an addition of Ca to these diets diminished the phytate destruction if the Ca and the source of phytate were eaten at the same time. If there was an interval between the consumption of these two food factors, for instance, if the phytate as a component of oatmeal was given at breakfast and the addition of Ca in the form of milk at supper, there was no diminution in the amount of phytate which disappeared from the gut.

Some workers, including McCance & Widdowson (1942*a*) and Hoff-Jørgensen, Andersen, Begtrup & Nielsen (1946), when testing the action of vitamin D on Ca absorption in human beings, have obtained negative results. Such results can well be understood in the light of the foregoing dog experiments, for the amount of vitamin D necessary to promote a maximum Ca absorption is small and can be long supplied from the body reserves. Its administration might, therefore, result in little or no increase in Ca absorption, and might indeed have no effect which could be detected on the basis of our present knowledge, unless the body had previously been depleted of its stores. This long-continued effect

of body stores of vitamin D in man was evident in the work of Hannon, Liu, Chu, Wang, Chen & Chou (1934), who found that a small daily dose of vitamin D (amount unstated) given to adult osteomalacic patients over a period of 16 days, retained its influence on Ca absorption for at least 4 months after the administration had ceased. It is also well recognized that the assay of vitamin D can only be made on young animals after they have been depleted of their reserves of this substance. It now appears that in adults also the effect of vitamin D on Ca absorption only becomes evident when the body is depleted of or deficient in this vitamin.

Recently Walker, Fox & Irvine (1948), in confirming the reduction in available Ca that is produced in man by an increase in dietary phytate (as brown bread), have concluded that adults can accustom themselves to a higher phytate intake so that in time (about 6 weeks) a negative Ca balance is changed to a positive one. No good evidence of this adaptation has been seen in the present experiments on puppies, nor would it have been expected because the phytate content of the diet increased gradually during the experimental period.

While it is undoubtedly true that the body has some power of accommodating itself to changes in Ca intake, as indeed it has to many other environmental changes, it remains to be shown what the long-term effect of high phytate diets would be, and whether, assuming Ca equilibrium were maintained, the body reserves of Ca would be kept at a level consistent with optimal physical fitness. In this connexion it may be mentioned that Henderson & Kelly (1929) reported that a Ca balance could be maintained in growing boys at a daily intake of 430 mg. On giving additional Ca in a mineral mixture it was found that after the maintenance requirement had been satisfied, as much as 50% of any excess might be retained. The Ca of milk, on the other hand, was found to be utilized more efficiently, the corresponding retention figure being over 70%.

Meanwhile, it would be unfortunate if emphasis on such adaptations as may occur in adults were to obscure the major importance of a physiological sufficiency of available Ca, especially in children and growing animals. The practical objective, therefore, ought to be, not the Ca intake which just prevents a negative balance, but the Ca intake which promotes maximum absorption and retention, assimilated with sufficient vitamin D to ensure the optimum incorporation of the Ca in perfectly formed bones and teeth.

SUMMARY

1. A study has been made of the conditions affecting the action of cereal phytate in interfering with calcium metabolism and calcification processes in the growing animal (dog). The anti-calcifying effect of phytate is specific when the body contains vitamin D. In the complete absence of the vitamin from the diet and the body the specificity of action is largely lost and the anti-calcifying action of inorganic phosphate approaches that of phytate.

2. The anti-calcifying property of cereals was formerly ascribed to an anti-vitamin and later to a toxamin, a substance having a harmful effect which could be antagonized by the vitamin. Phytate does not seem to be an anti-vitamin in the sense that it directly antagonizes vitamin D, although this hypothesis may be necessary to explain some of its less understood interactions with the vitamin (see paragraph 9 below). Its main anti-calcifying effect depends on the fact that it competes in the intestine with its structural analogue, inorganic phosphate, for Ca. In this way phytate limits the amount of Ca available for absorption under the influence of vitamin D.

3. Although vitamin D in sufficient quantity prevents phytate from producing rickets in growing animals, it alone does not suppress the anti-calcifying effect of phytate. However high the vitamin D intake, phytate reduces the amount of Ca absorbed from the intestine and produces bones less well calcified and more osteoporotic than when it is absent from the diet or replaced by inorganic phosphate.

4. Increasing the phytate in the diet increases not only the phytate but also the Ca in the faeces, and in this way reduces the Ca available to the body.

5. A high phytate diet demands not only an adequate vitamin D supply, but, equally important, a high Ca intake. When the diet is rich in phytate, perfect bone formation can only be procured if sufficient Ca is added to a diet containing vitamin D.

6. Increasing the Ca of the diet may: (*a*) increase the excreted phytate if the absorption of Ca is not increased; or (*b*) reduce the phytate in the faeces if more Ca is absorbed.

7. Under the experimental conditions described, vitamin D does not remain passive to the antagonism of phytate but reacts by destroying some of it. This action is limited, and increasing the vitamin above a certain amount does not further reduce the phytate excreted, just as it does not increase the Ca absorbed.

8. Vitamin D not only promotes Ca absorption from the gut and its deposition in bone, but in growing animals adjusts the structure and bulk so as to produce better calcified bones in that they have a maximum of calcified tissue. More of this vitamin may be required to ensure perfect bone calcification than to procure maximum Ca absorption, and this is specially so when the diet is rich in phytate. Thus Ca absorption and retention may be good and yet the calcification of the bones be subnormal.

9. There is some evidence, not fully substantiated, that dietary phytate uses up the body reserves of vitamin D more rapidly than does inorganic phosphate.

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REFERENCES

Allen, R. J. L. (1940). *Biochem. J.* **34**, 858.

Briggs, A. P. (1922). *J. biol. Chem.* **53**, 13.

Bruce, H. M. & Callow, R. K. (1934). *Biochem. J.* **28**, 517.

Chick, H., Korenchevsky, V. & Roscoe, M. H. (1926). *Biochem. J.* **20**, 622.

Chick, H. & Roscoe, M. H. (1926). *Biochem. J.* **20**, 137.

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

Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.

Green, H. N. & Mellanby, E. (1928). *Biochem. J.* **22**, 102.

Hannon, R. R., Liu, S. H., Chu, H. I., Wang, S. H., Chen, K. C. & Chou, S. K. (1934). *Chin. med. J.* **48**, 623.

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

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

Hoff-Jørgensen, E. (1944). *Math.-fys. Medd.* **21**, no. 7.

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.

Imperial Bureau of Animal Nutrition (1937). *Methods for the Chemical Analysis of Biological Material in Nutrition Investigations.* (Technical Communications, no. 9.)

Kodicek, E. (1948). Communication to The Nutrition Society, London, 23 October 1948.

Krieger, C. H. & Steenbock, H. (1940). *J. Nutrit.* **20**, 125.

McCance, R. A. & Widdowson, E. M. (1935). *Biochem. J.* **29**, 2694.

McCance, R. A. & Widdowson, E. M. (1942*a*). *J. Physiol.* **101**, 44.

McCance, R. A. & Widdowson, E. M. (1942*b*). *J. Physiol.* **101**, 304.

Macy, I. G. (1942). *Nutrition and Chemical Growth in Childhood*, vol. **1**. Springfield, Ill.; Thomas.

Mellanby, E. (1919). *Lancet*, i, 407.

Mellanby, E. (1920). *Lancet*, i, 1290.

Mellanby, E. (1921). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 61.

Mellanby, E. (1922). *Brit. med. J.* ii, 849.

Mellanby, E. (1925). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 93.

Mellanby, E. (1926). *J. Physiol.* **61**, xxiv *P.*

Mellanby, E. (1937). *Perspectives in Biochemistry*, p. 318. Cambridge University Press.

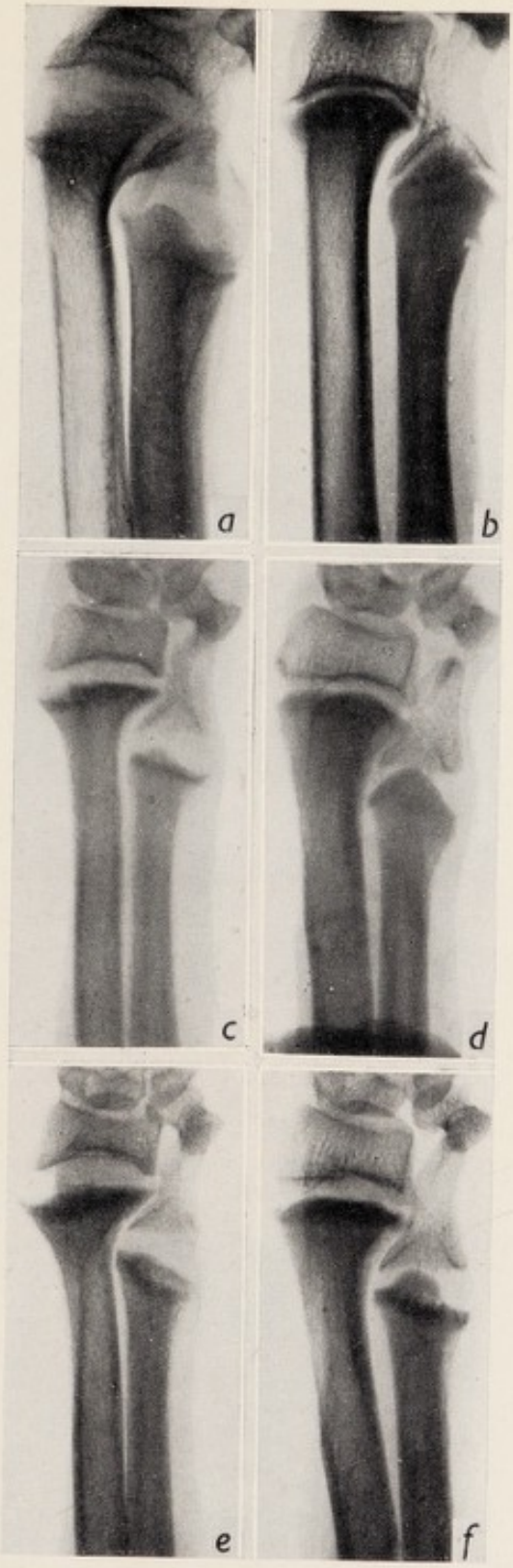
Mellanby, E. (1944). *Nature, Lond.*, **154**, 394.

Mellanby, M. (1929). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 140.

Nicolaysen, R. (1937). *Biochem. J.* **31**, 122.

Patwardhan, V. M. (1937). *Biochem. J.* **31**, 560.

Walker, A. R. P., Fox, F. W. & Irvine, J. T. (1948). *Biochem. J.* **42**, 452.



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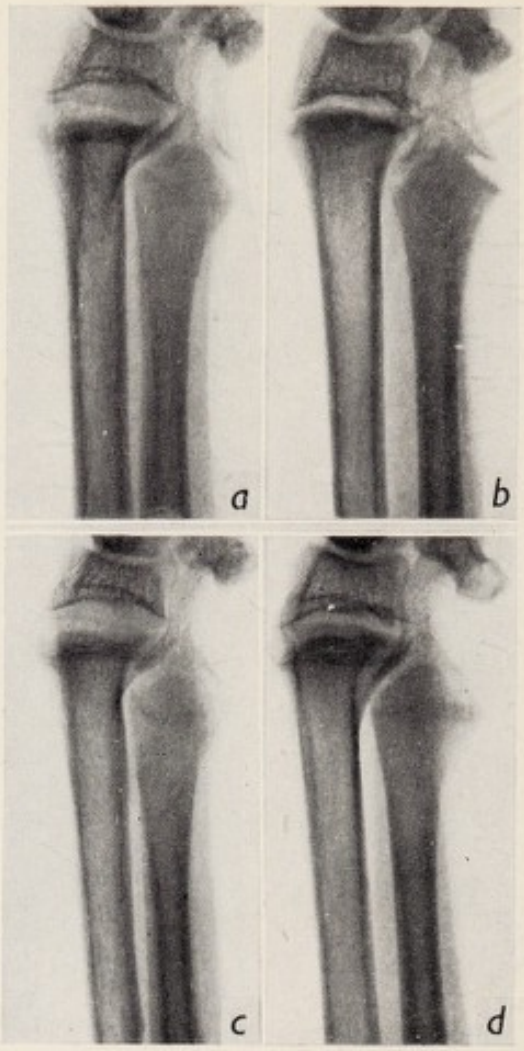
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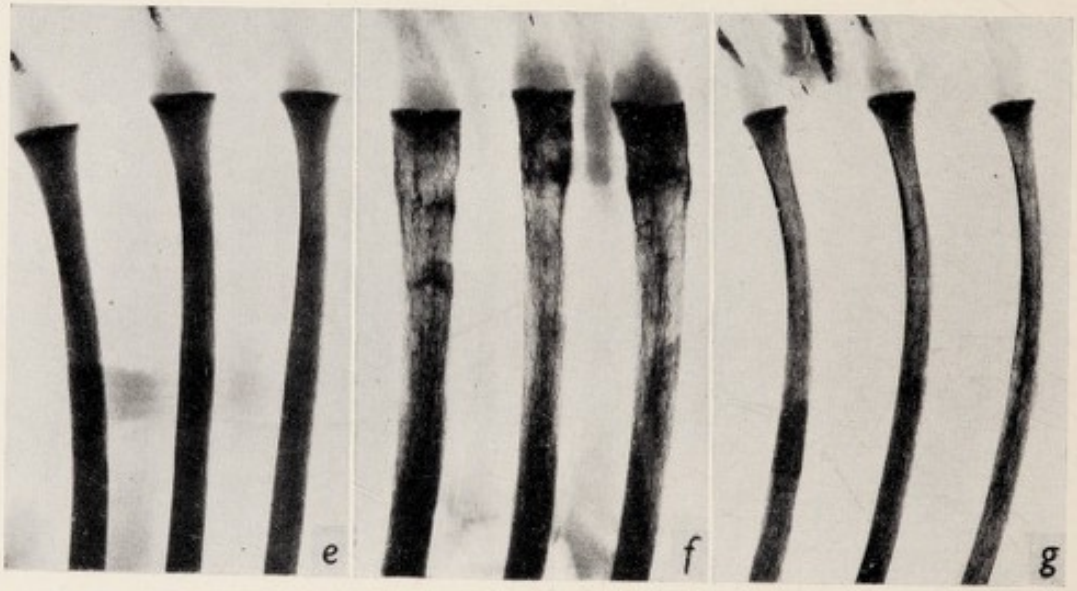
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EXPLANATION OF PLATES

Plate 1

Exp. 1. (*a*) and (*b*). Radiographs of forepaws at end of experiment, showing relative rickets-producing effect of phytate and phosphate in the presence of high vitamin D reserves. (*a*) Puppy 1. Untreated oatmeal (phytate intact). (*b*) Puppy 2. Oatmeal which had been boiled for 18 hr. with 1% HCl (most of phytate hydrolysed to phosphate). *Note.* Puppy 1 had severe rickets whereas puppy 2 was still normal after 11 weeks.

Exp. 2. (*c*)–(*f*). Radiographs of forepaws showing relative rickets-producing effect of phytate and phosphate in the presence of low vitamin D reserves. (*c*) and (*e*) Puppy 3. Untreated oatmeal (phytate intact). (*c*), 6 weeks on diet. (*e*), 8 weeks on diet. (*d*) and (*f*) Puppy 4. Oatmeal which had been boiled for 18 hr. with 1% HCl (most of phytate hydrolysed to phosphate). (*d*), 6 weeks on diet. (*f*), 8 weeks on diet. *Note.* After 6 weeks on the experimental diets (see (*c*) and (*d*)) puppy 4 (phosphate) had less severe rickets than puppy 3 (phytate), but 2 weeks later the condition of the two puppies was more similar (see (*e*) and (*f*)).

Plate 2

Exp. 3. (*a*)–(*d*). Radiographs showing the relative rickets-producing effect, in the presence of high and medium vitamin D reserves respectively, of oats before and after part of their phytate had been hydrolysed by germination and autolysis. (*a*) Puppy 5. Oats before treatment (phytate intact); high vitamin reserves. (*b*) Puppy 6. Oats after treatment (phytate hydrolysed to phosphate); high vitamin reserves. (*c*) Puppy 7. Oats before treatment (phytate intact); medium vitamin reserves. (*d*) Puppy 8. Oats after treatment (phytate hydrolysed to phosphate); medium vitamin reserves. *Note.* There is a greater difference in the degree of rickets between (*a*) and (*b*) (high vitamin reserves) than between (*c*) and (*d*) (medium vitamin reserves) after 17 weeks on diet.

Exp. 9. (*e*)–(*g*). Radiographs of costochondral junction. (*e*) Puppy 27. Received phosphate with 20 i.u. vitamin D_2. (*f*) Puppy 28. Received phytate with 20 i.u. vitamin D_2. (*g*) Puppy 32. Received phytate with 1000 i.u. vitamin D_2. *Note.* (1) Substituting phytate P (*f*) for equal amounts of inorganic P (*e*) resulted in thicker and more osteoporotic bones and reduced mineral ash content (see Table 9); (2) increasing vitamin D_2 from 20 (*f*) to 1000 i.u. (*g*) reduced the bulk and improved the quality of the bone although the mineral ash content was not increased (see p. 508 and discussion p. 520).