Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 8, Number 2, April, 1937.

Printed in the Union of South Africa by the Government Printer, Pretoria

Studies in Mineral Metabolism XXXVII.

The Influence of Variations in the Dietary Phosphorus and in the Ca : P Ratio on the Production of Rickets in Cattle.

By A. THEILER †, P. J. DU TOIT, and A. I. MALAN, Section of Biochemistry, Onderstepoort.

INTRODUCTION.

EARLIER investigations by Theiler, Green and du Toit (1927) and du Toit, Malan and Groenewald (1932) on minimum mineral requirements in cattle included work on the phosphorus and calcium requirements of cattle and the effect of dietary Ca and P upon their health and production.

This work has been extended in the present investigation to include variations in both the intake of P and the ratio of Ca to P, while an extensive microscopical examination has been carried out of the bones of the animals with a view to establishing the nature of the conditions produced respectively by aphosphorosis and acalcicosis. This part of the work is being continued, especially with regard to calcium deficiency for the study of which new material obtained in later experiments will soon be available and will be reported on in due course.

While no attempt will be made to review the vast amount of literature on calcium and phosphorus metabolism a few salient points should be mentioned.

In a recent paper Shohl and Wolbach (1936) pointed out that in the absence of vitamin D rickets may be produced in rats receiving high calcium low phosphorus diets, or low calcium high phosphorus diets and also with low calcium low phosphorus diets, which implies that rickets may be produced with any ratio of Ca to P and that this factor has lost much of its significance.

It would seem that under conditions of calcium or phosphorus low diets or both the absolute intake of these elements, at all events in the absence of vitamin D, according to Shohl's work, determines the production of rickets and not the ratio in which they happen to be present in the diet. Our own view has been that rickets may be produced by limiting the absolute intake no matter what the ratio is while it may also be cured by increasing the intake in spite of retaining an undesirable or abnormal ratio. It is equally true, however, that a calcium phosphorus intake may become rachitogenic merely by altering the ratio but such cases are usually true only when the calcium or phosphorus intake or both approaches the minimum requirements of the animal in question. A correction of the ratio in such cases would naturally produce a cure after the onset of rickets. The utilization of calcium and phosphorus seems to be undoubtedly affected by the proportions in which these elements are present in the diet but the extent to which this relationship affects their mutual metabolism seems to us to have been exaggerated. Shohl's latest work strongly emphasizes the importance of the absolute amounts of calcium and phosphorus in the diet of rats in the production of rickets and in a previous communication Brown and Shohl (1932) demonstrated that the ratio of Ca to P was inadequate to define the rachitogenic properties of a diet but that the absolute amounts of these two elements present should also be given.

Hitherto work at this Institute has stressed the importance of the absolute intake of minerals in the production of osteodystrophic diseases in the larger domesticated animals. Theiler, Green and du Toit stated in 1927, subsequent to the experimental production of rickets in bovines on a daily diet containing 6·9 gm. CaO and 5·1 gm. P_2O_5 and therefore showing a " normal " ratio, that " while realising the obvious fact that a particular ratio may represent the " optimum " we have rather regarded the current emphasis upon mineral ' balance ' as exaggerated ". Rickets has subsequently been produced on diets containing " normal " ratios of Ca to P in cattle, sheep, goats and pigs. In all these cases the causal factor seems to have been phosphorus deficiency as will be evident from a further discussion of this work.

It should be mentioned that, as cases of natural rickets in bovines—clinically recognisable and known as " styfsiekte " or stiff-sickness—occur in this country under conditions of bright sunshine frequently ranging from 7-9½ hours daily, experimental work on rickets has been carried out until recently without any attempt to limit vitamin D. In this respect the food being grown under South African conditions provided a good source of vitamin D while, regular and automatic exposure of the experimental animals to sunlight in the course of the experiment justifies the statement without fear of contradiction that vitamin D was supplied in abundance and that it cannot therefore be regarded as a factor in the production of the bone lesions in the experiments referred to.

Several investigations have been carried out here on the rôles played by calcium and phosphorus under conditions of vitamin D abundance, in the production of rickets in bovines, sheep, goats

and pigs. This work has provided considerable data on the production of rickets by limiting the calcium or phosphorus intake or both without considering the ratio in which these elements happen to be present in the diet as reference to the following table will clearly indicate: —

Date.	Species.	Daily Intake (grm.)		$CaO : P_2O_5$.	Diagnosis.
		CaO.	P_2O_5.		
1926	Bovine.....	6·9	5·1	Normal	Rickets.
1930–1932	2 Bovines...	6·8–8·3	8·4–15·4	Normal	Severe rickets.
1930–1932	2 Bovines...	50	8·4–15·4	3–6 : 1	Severe rickets.
1933–1935	2 Bovines...	5·0	10	1 : 2	Severe rickets.
1933–1935	2 Bovines...	62	10	6·2 : 1	Severe rickets.
1934–1935	2 Bovines...	3·8	3·9	Normal	Severe rickets.
1930–1932	5 Sheep.....	7·0	1·0	7 : 1	Severe rickets.
1930–1932	5 Sheep.....	7·0	1·5	4·6 : 1	Rickets.
1934–1935	4 Sheep.....	·7	1·0	Normal	Marked rickets.
1934–1935	4 Sheep.....	1·4	1·0	Normal	Rickets.
1934–1935	4 Sheep.....	2·1	1·0	1 : 2	Marked rickets.
1935	2 Goats.....	9·0	2·0	4·5 : 1	Florid rickets.
1935	2 Goats.....	1·0	2·0	Normal	Florid rickets.
1934–1935	2 Pigs.....	8·4	3·3	3·6 : 1	Slight rickets.
1934–1935	2 Pigs.....	1·1	3·3	1 : 3	Very slight rickets.
1935	2 Pigs.....	2·8	·25	11 : 1	Florid rickets.
1935	2 Pigs.....	·16	·25	Normal	Slight rickets.

It is at once apparent from the figures given above that instances of rickets produced by diets low only in calcium and diets containing enough or excess phosphorus are conspicuously absent. Such rations have been included in the experiments as a glance at the following will prove, but in no case was a condition produced which is histologically identical with rickets:

Osteoporosis was produced in two heifers in 1926 receiving a ration which contained 8·2 gm. CaO and 24 gm. P_2O_5. In 1935 a two-year-old heifer remained normal after receiving a ration containing 6 gm. CaO and 25 gm. P_2O_5 daily for 18 months. The experimental mate of this heifer showed marked bone atrophy at the end of the experiment but no rickets. Two five-months-old and two eight-months-old calves showed osteoporosis but no rickets after receiving a ration containing 3 gm. CaO and 30 gm. P_2O_5 for 6 months.* A ration containing 0·7 CaO and 10 gm. P_2O_5 produced marked atrophy and osteoporosis in goat lambs. It is now well known that low calcium and relatively high phosphorus, i.e. a wider ratio than 1 part of the former to 3 of the latter, will produce osteofibrosis in horses [Theiler 1934), Niimi and Aoka (1927), Kintner and Holt (1932)] and is also known to occur in goats, pigs and dogs. Some of the experiments on low Ca diets mentioned above are being continued and the diagnoses have been made from time to time by removing a portion of a rib at the chondro-costal junction. As osteofibrosis may take a considerable time to develop in horses, depending on the width of the Ca:P ratio it is perhaps too early to draw any conclusions in regard to the ultimate nature of the bone lesions that will be brought about by low calcium in these experiments.

* Osteodystrophia fibrosa has developed since the above was written.

It is, however, very significant that in all the investigations on osteodystrophic diseases referred to above, rickets or osteomalacia was invariably brought about by diets low in phosphorus, the severity of the condition depending on the degree of the deficiency, the P requirement of the animal and the duration of the experiment, while parallel experiments on group mates of the animals used for the P deficiency work, have not in a single instance produced rickets or osteomalacia when the diet was low in Ca but contained sufficient P. Osteodystrophic disease undoubtedly existed in most of these cases and could be recognised clinically as stiff gait, poor condition, enlargement of the maxillae and even tetany but microscopic examination of the bones revealed at most osteoporotic conditions which could not be considered as genuinely rachitic on account of the absence of pathognomic osteoid formation and the presence of an insufficient amount of osteoid to justify a diagnosis of rickets. Besides osteoporosis the bones of some species as for instance the horse, showed alterations of the marrow, fibrous tissue and increased multinuclear osteoclasts resembling osteodystrophia fibrosa.

The basis of the differentiation of the osteodystrophic diseases produced in these experiments has been described and discussed by one of the authors (Theiler, 1934, 1932).

The significant difference between the results obtained by Shohl and his co-workers under conditions of vitamin D deficiency in rats and those described above with domesticated animals where abundant vitamin D was present is that whereas low calcium in the diet produced rickets in the rats in the former case the latter workers report after feeding calcium low diet osteoporosis or osteodystrophia fibrosa or both, which conditions should not be confused with true rickets in the immature animal or osteomalacia in the adult.

Huffman and his co-workers (1936, 1935) produced and described rickets in calves under conditions of low vitamin D intake. In their earlier work (1933) on the phosphorus requirement of dairy cattle, histological work was apparently not undertaken, although it can safely be assumed from their reports on the animals in the experiment that rickets was produced by the phosphorus deficient diets. Incidentally it may be mentioned that their findings that a growing bovine requires daily about 10 gm. P for maintenance and normal growth is in close agreement with the observations made at this Institute (1927, 1932).

Like Shohl and his school, Huffman and co-workers produced rickets in dairy calves on rations which, in addition to their slow phosphorus or calcium content, or both, did not suffice the vitamin D requirements of the calves and their results on calcium low diets may therefore not be directly applicable to those obtained here where vitamin D deficiency did not exist and may be excluded from detailed consideration.

While clinically recognizable osteodystrophic diseases are produced in domesticated animals during comparatively short periods of feeding low phosphorus diets in presence of abundant vitamin D, calcium low diets with a sufficiency of phosphorus leave the animals apparently undisturbed and clinically healthy for much longer periods. This conclusion is based upon the results of experiments

on the production of aphosphorosis and acalcicosis during the last dozen years at this Institute. From the above it would seem, therefore, that a factor like vitamin D, which is closely associated with Ca and P metabolism, might produce microscopic bone lesions associated with a phosphorus deficiency per se (viz. rickets) more easily or sooner than it would produce results directly due to acute acalcicosis. It is further possible that indications of a shortage of phosphorus whose function in the animal body is complex and very varied, as for instance in bone formation, carbohydrate metabolism, fat, lipoid and protein metabolism, etc., are more easily effected than those of a deficiency of the functionally less complex and varied calcium. Even in the presence of abundant phosphorus in a diet containing relatively low calcium the comparative absence of vitamin D might invariably produce the easily obtained bone lesions of low phosphorus or abnormal phosphorus metabolism which would account for the constant diagnosis of rickets by investigators of low calcium diets under conditions of vitamin D deficiency. Attempts have been made here to feed diets low in calcium to bovines and pigs kept in dark stables in order to create conditions of vitamin D deficiency but up to the present the exclusion of light has apparently been without effect when rib sections removed periodically under anaesthesia have been compared with those of animals kept on identical diets and intake but in presence of abundant sunshine. Apparently the hay and other feed grown under conditions of practically daily exposure to sunlight, contain sufficient vitamin D to satisfy the requirements of the animals. This observation is in agreement with that made by Huffman (1931) that hay cured in the sun contains sufficient vitamin D to protect calves from rickets fed a ration otherwise low in this vitamin.

Wallis, Palmer and Gullickson (1935) carried out balance trials on young growing calves receiving a basal ration with and without mineral and vitamin D supplements. Both calcium and phosphorus retentions were considerably reduced when vitamin D was absent from the dietary supplement. The latter remark also applies when phosphorus or calcium was supplemented and vitamin D withheld. Histological diagnoses were not made of the condition produced in these experiments but in the light of other work on phosphorus deficiency in absence of vitamin D it may safely be assumed that on a normal Ca : P ratio and a high calcium low phosphous diet rickets was produced.

Rupel, Bohstedt and Hart (1933) produced rachitic conditions in calves by limiting the vitamin D intake even when the calcium phosphorus ratio of the diet could be considered distinctly normal. The calves remained healthy and grew well when vitamin D was supplied. A calcium low P high diet was not included in their series of experiments.

Marek, Wellmann and Urbanyl (1935) describe rickets in calves which were given rations low in Ca and containing relatively large quantities of phosphorus. The vitamin D content of the feed was practically nil. The ratio of CaO to P_2O_5 in the diet was in the case of the one calf as 1 : 3·06 and in the other 1 : 3·90 while the Erdalkali-Alkalizität ($CaO + MgO - P_2O_5$ given in milligram equivalents per 100 gm. dry feed) was −10·77 and −16·25 respectively.

These authors are of the opinion that Erdalkali-Alkalizität is one of the factors which determine the development of rickets in herbivores receiving diets low in calcium or phosphorus, or both. This statement can be accepted readily because a change from what Marek and his co-workers regard as the " normal " Erdalkali-Alkalizität almost invariably involves a change in the calcium phosphorus ratio which admittedly plays an important rôle in the production of osteodystrophic disease in cattle receiving insufficient or even borderline quantities of either calcium or phosphorus, or both in their diets. Apparently Marek's conception provides a way of indicating greater sensitivity of the metabolic processes in the animal body to changes in the ratio of Ca to P than the ordinary expression of ratio would; for instance a change of E.A. from + 24 mgm. equivalent under such conditions to + 15 might be responsible for the onset of rickets according to Marek, whereas if this change is expressed in terms of an altered Ca : P ratio it would appear to be small and hardly likely to be responsible for the production of rickets.

Marek and his co-workers apparently kept the two calves under conditions of vitamin D deficiency for they state that the vitamin D content of the ration was practically nil and it can only be said therefore that the rickets produced was due to a complex of factors such as low calcium intake together with vitamin D deficiency and highly abnormal E.A. which, incidentally, was possible to obtain only by transgressing the accepted limits of " normal " calcium phosphorus ratios. This work does not reveal the nature of the bone lesions produced by a diet deficient only in calcium and containing abundant vitamin D, the absence of which might have been responsible for the development of rickets in Marek's experiment. It is also noteworthy that although Marek and Wellman (1932) frequently produced ostitis fibrosa in pigs receiving a diet relatively high in phosphorus this disease was never diagnosed when the diet contained low phosphorus and relatively high calcium. This result tends to strengthen the belief that rickets might be associated with phosphorus deficiency and ostitis fibrosa with a low calcium intake when abundant vitamin D is present in the diet of pigs. The applicability of this hypothesis to cattle will be discussed at a later stage in the present publication.

The present series of experiments reported in this paper was undertaken partly to gain further information on the nature of the osteodystrophic diseases produced respectively by phosphorus and calcium deficient rations in cattle.

EXPERIMENTAL DETAIL.

Twelve high-grade Friesland heifers, 15 months old, uniformly bred and whose full history since birth was known were selected for the investigation.

Details of the conditions under which the experimental animals were kept were essentially the same as those reported in the earlier investigation by du Toit *et al.* (1932).

All the animals in the experiment at some time or another showed a tendency to pick up and consume stones, sticks and any material that was to be found on the concrete floor of the exercising paddock. Such pica or depraved appetite is invariably noticed in our experimental animals when given rations very low in roughage and it is probably due to the artificial condition of the basal ration which is bound to contain a minimum of hay and other purified foods in order to keep its mineral content as low as possible. However, the fact that the control group, whose basal ration was supplemented with calcium and phosphorus showed normal growth is indicative of the adequacy of the basal ration for growth when its low Ca and P content was increased. The consumption of material such as stones, soil, manure due to pica was prevented by washing the concrete floor regularly and keeping the animals muzzled when they were allowed to exercise in the concrete paddock.

The animals were fed twice daily, hay in the early morning and concentrates in the afternoon. The little hay that was given was consumed almost immediately after which the animals were removed to the exercise paddock until feeding time in the afternoon. They were left in the feeding boxes overnight. Water was always available in the common paddock.

The daily basal ration at the beginning of the experiment consisted of—

3 Kg. maize-samp low in minerals.
·5 Kg. poor quality hay.
·5 Kg. greenfeed.
·25 Kg. meat meal.
25 gm. Mineral mixture.

This ration was supplemented with the necessary minerals in accordance with the aim of each trial. Feed was supplied according to the appetite of each pair of animals in a trial. Although rapid growth was aimed at the animals were not " forced " by ensuring maximum intake at all times. Occasionally a little feed was left over by some of the groups which was then weighed back but the complete consumption of the feed given was almost invariably the rule. The minerals if required were added to the concentrates just before feeding time and this method of administering minerals was found to be very satisfactory.

The composition of the basal ration is given in Table I.

The animals were inspected daily. Records of monthly weights, food consumption, oestrum and the health of the animals were kept. Blood was analysed for phosphorus, calcium and phosphatase at monthly intervals. X-ray photographs were taken occasionally as well as movies of the animals showing more pronounced clinical symptoms of the bone disease produced. Balance experiments on Ca and P were carried out periodically.

TABLE I.

Percentage composition of ration is given by the following:—

	Protein.	MgO.	CaO.	P_2O_5.	Na_2O.	K_2O.	Cl.	SO_3.
Hay teff.....	6·2	·3	·4	·24	·15	1·5	·2	·2
Samp.....	8·0	·008	·01	·12	·001	·06	·03	·14
Meat meal.....	85·0	·15	1·2	1·8	·27	·6	·16	·35
Green feed (fresh)....	3·0	·26	·09	·08	·07	·4	·3	·04
Mineral mixture.....	—	·5	—	—	1·7	0·8	0·9	0·7

Constituents of daily ration when fed in maximum amounts:—

	Protein.	MgO.	CaO.	P_2O_5.	Na_2O.	K_2O.	Cl.	SO_3.
Hay, 500 gm.....	31·0	1·5	2·0	1·2	·75	7·5	1·0	1·0
Samp, 4,000 gm.....	320·0	0·32	0·4	4·8	·04	2·4	1·2	5·6
Meat meal, 225 gm...	191·0	0·34	2·7	4·2	1·2	1·4	0·36	0·80
Green feed, 500 gm..	15	1·3	·45	·4	·35	2·0	1·5	·2
Mineral mixture, 25 gm.	—	1·9	—	—	6·9	3·2	3·6	2·7
TOTALS ± 5,000 gm. con.....	557·0	5·4	5·5	10·6	9·2	16·5	7·7	10·3

Most of the animals were killed at the conclusion of the experiment and chemical analyses and detailed histological studies of the following represenative bones were undertaken: Humerus, radius, femur, tibia, 6th and 9th ribs and the third lumbar vertebra.

Details of the individual experimental results are given in the following pages while reference should be made to the tables in the discussion for collective detailed information on all the trials to be reported in this publication.

RESULTS.

GROUP 1.

Controls.—Ration adequate in all respects. The basal ration was supplemented with Ca and P.

Two grade-Friesland heifers Nos. 5154 and 5163, approximately 15 months old, were used. Dehydrated Na_2HPO_4 and $CaCO_3$ were added to the basal ration; the average daily intake of Ca and P was increased to 24·0 gm. P_2O_5 and 25·5 gm. CaO. The experiment began on 8.12.33 and concluded on 12.8.35, when No. 5154 was killed for bone analysis and histolological study.

Both animals remained apparenty normal throughout the course of the experiment. Food consumption increased from 3 kg. samp during the pre-period to 4 kg. from the beginning of the experimental period at which figure it was kept constant throughout the trial and the feed was regularly consumed.

As seen on the individual weight curves in Fig. 1 both heifers increased uniformly in weight from an average of about 620 lb. to about 1,070 lb. at the end of the trial 20 months afterwards. Individual weights are given in Table 2.

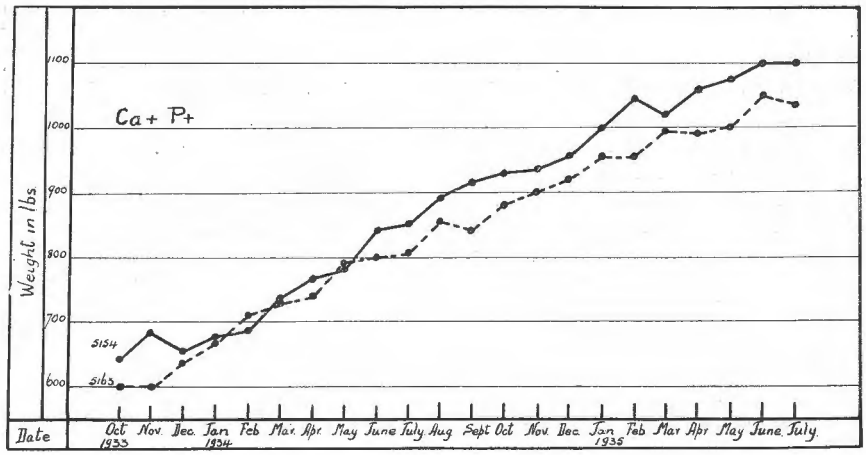

Fig. I.—Weights.

The calcium content of the blood remained normal throughout as reference to Table 3 will indicate. The inorganic phosphorus of the blood was rather low during the earlier part of the trial when the phosphorus intake was only about 15 gm. daily due to the addition of partly crystalline Na_2HPO_4 instead of the dehydrated form. All the figures indicate sufficiency, however, and are distinctly within normal limits. The inorganic phosphorus content of the blood of all the animals is given in Table 4 and of the pair under discussion in Figure 2.

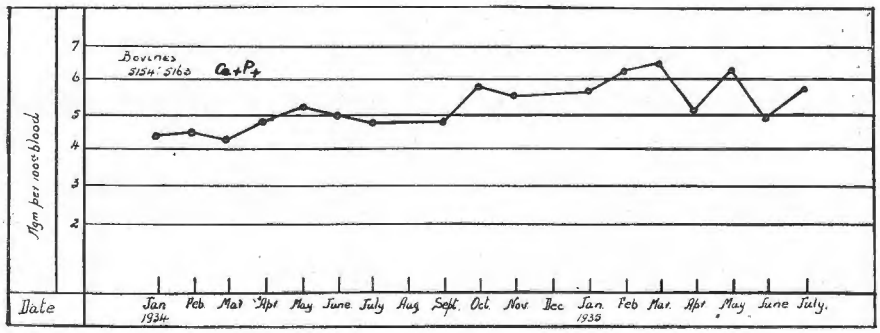

Fig. II.—Average Inorganic Phosphorus.

Blood phosphatase was determined towards the end of the trial and the results are given in Table 5.

The radiographs taken shortly before the end of the trial indicated normal bones which was confirmed microscopically in the case of No. 5154 after death in August, 1935. The bones showed no pathological changes. The calcification zone was complete and the presence of some osteoid seams was normal and indicated growth of a normal bone. The ash content of the femur calculated on green weight was 35·4 per cent., while the breaking strength of the metacarpus over a 6-inch span was 2,280 lb.—both values indicating normal bone. Details of the bone analyses are given in Table 6.

From a synopsis of 11 balance trials given in Table 7 and carried out on one animal of each pair during the course of the experiment it is evident that animal No. 5154 retained on an average 13·6 gm. CaO and 15 gm. P_2O_5 daily; these values account for 42·3 and 62·6 per cent. retentions of the two constituents respectively.

Group 2.

Ration low in P but adequate in other respects.

Low P (9·9 gm. P_2O_5), sufficient Ca (23·9 gm. CaO).

Two Grade Friesland heifers Nos. 5157 and 5155, approximately 15 months old, were used. Duration of experiment December, 1933, until August, 1935. $CaCO_3$ was added to the basal ration to increase the Ca to 31·6 gm. CaO.

The experimental procedure was essentially the same as that already reported.

Samp was given at the rate of 8 lb. per head per day from the start of the experiment in December, 1933. In March, 1934, No. 5155 showed signs of restlessness and poor food consumption. Both animals seemed to be lagging behind in growth. Occasionally feed was left over and the samp was reduced to 2 kg. for short periods on several occasions. For instance from 11.7.34 to 19.7.34 only 2 kg. of samp were given in the basal ration and then again increased to 4 kg. until February, 1935, when it was reduced to 3 kg. until the end of the trial. P_2O_5 intake consequently became reduced to 7·9 gm. instead of 9·9. Pica became very marked and in January, 1935, No. 5155 showed lameness in the near front leg. Both animals showed stiffness during the last six months of the experiment and were decidedly in poorer condition than the controls.

In July, 1935, these animals were examined rectally during oestrum and ovulation was found to have taken place normally. X-ray photographs were taken in July, 1935.

No. 5155 was killed on 16.8.35 for chemical and microscopical examination of the bones while No. 5155 was discharged.

The weight curves of these animals are given in Fig. 3.

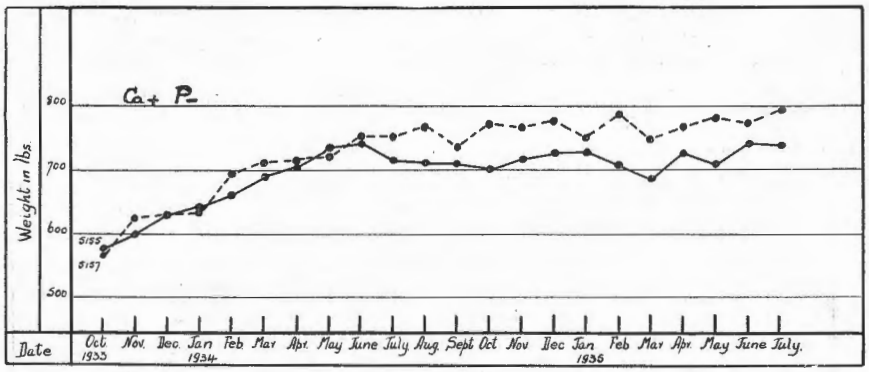

Fig. III.—Weights.

After an approximately normal increase for the first 3 months of the experiment both animals remained practically constant in weight until the end of the experiment at which stage they appeared less thrifty and thinner than the controls but of approximately the same height.

The curve for the inorganic phosphorus content of the blood given in Fig. 4 indicates aphosphorosis from the time the analyses were begun, one month after the starting date of the experiment. It would certainly have been interesting to have followed the present technique of removing portions of ribs for histological examination for a study of the development of the effects of aphosphorosis on bone structure.

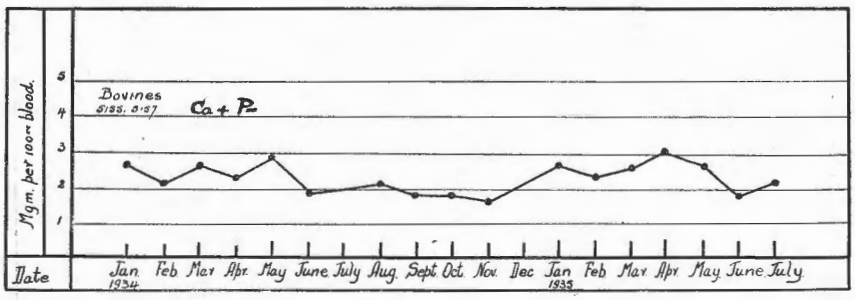

Fig. IV.—Average Inorganic Phosphorus.

The calcium content of the blood was similar to that of the controls (see Table 3).

The radiographs of the front legs of both animals suggest atrophy when compared with those of the control group as reference to Fig. 5 will signify.

The microscopic examination of the selected bones of heifer No. 5157 revealed some defects on the calcification zones but these had not been penetrated by the medullary tissue. No canals entered into the hypertrophic zone and they were all at about the same level.

It would appear that calcification was beginning to lack. The long secondary medullary canals indicated increased resorption. The presence of osteoid left no doubt about the diagnosis of rickets while excessive osteoclasia and the presence of very thin trabeculae indicated atrophy.

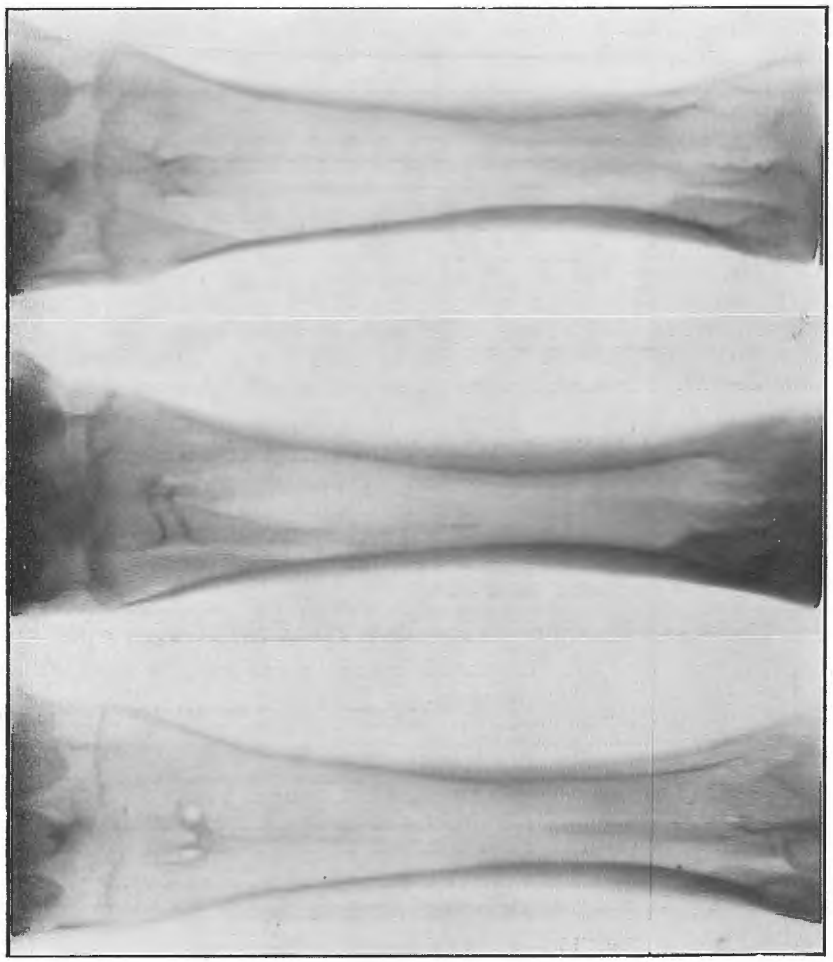

Fig. V.

Abnormally low values were obtained for the ash content of the green bones as reference to Table 5 will indicate. The breaking strength of the metacarpus over a 6-inch span was 1,800 lb. as compared wih 2,280 for that of the control animal (see Table 6).

Heifer No. 5157 retained on an average daily 6·3 gm. CaO and 0·78 gm. P_2O_5 or 19·4 and 10 per cent. respectively of the average consumption of these two constituents.

GROUP 3.

Ration low in P but containing excess calcium and adequate in other respects.

Grade Friesland heifers Nos. 5158 and 5159.

The average daily phosphorus intake was 6·9 gm. P_2O_5, while the Ca was increased to 60·6 gm. CaO by the addition of $CaCO_3$ to the basal ration.

The basal ration was given at the beginning of the trial (December, 1933), but the samp had to be reduced to 2 kg. six months afterwards to ensure complete food consumption. Poor food consumption was first noticed in March, 1934, and the group seemed to be lagging behind the controls. In June, 1934, the heifers showed rough coats and appeared to be emaciated. Samp was reduced to 2 kg. at this stage and even of this quantity traces were usually left in the mangers. In October the animals showed symptoms of styfsiekte and were in poor condition. In November, 1934, No. 5159 contracted and died from lamsiekte—*para-botulinus bovis* infection.

The remaining animal No. 5158 although poor in condition and suffering from aphosphorosis (styfsiekte) lasted until the end of the experiment when it was destroyed for bone studies. Its reproductive system was normal and showed normal ovulation.

This heifer was given 2 gm. P_2O_5 as Na_2HPO_4 daily from 20.3.35 to 7.6.35 to keep her in the experiment until its conclusion.

The weight curves of the heifers in this group are given in Fig. 6, which indicate poor weight increase during the course of the experiment when compared with the controls. As a matter of fact No. 5158 was only 60 lb. heavier at the end of the trial than at the beginning compared with an increase of about 450 lb. for the controls during the same period.

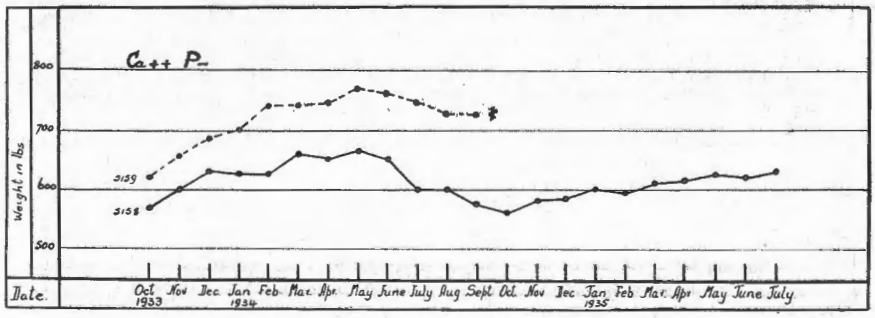

Fig. VI.—Weights.

The inorganic phosphorus in the blood given in Fig. 7 indicate aphosphorosis throughout the experiment except during a short period when phosphate was supplied.

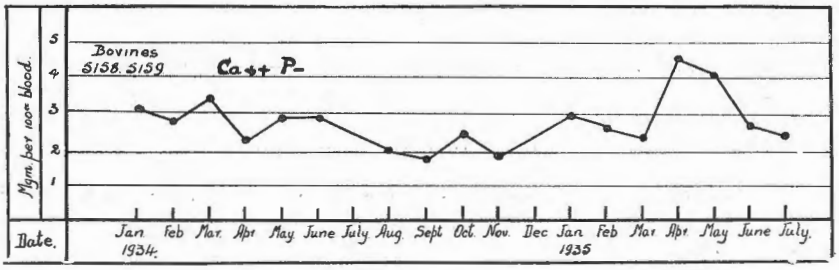

Fig. VII.—Average Inorganic Phosphorus.

On an average only 1·5 gm. P_2O_5 were retained daily by No. 5158 (Table 7) as against 15 gm. by the control, which supplies confirmatory evidence for the existence of phosphorus deficiency.

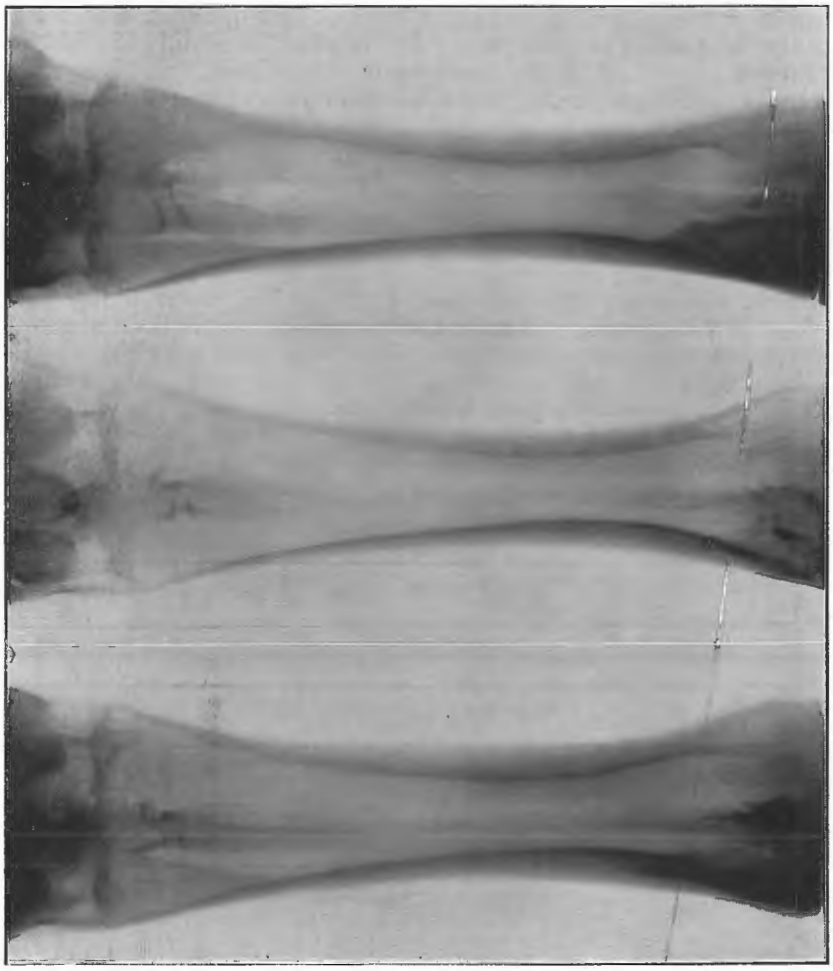

Fig. VIII.

The radiographs reproduced in Fig. 8 show atrophy of the osseous tissue while the microscopic examination of the selected bone sections of No. 5158 indicate defects in the epiphyseal line and the presence of more than normal amounts of osteoid justifying a diagnosis of rickets.

The ash content of the femur was subnormal and the breaking strength of the metacarpus over a 6-inch span was 1,200 lb. or approximately half that of the bone of the control 5154 (see Table 6).

Group 4.

Ration low in both P and Ca but adequate in other respects.

Two Grade Friesland heifers No. 5160 and 5161 were placed in this group in December, 1933.

The average daily intake of Ca and P was 5·9 gm. CaO and 10·6 gm. P_2O_5.

The basal ration containing 4 kg. of samp was consumed daily throughout the experiment.

There was nothing unusual to report in regard to this pair of heifers until April, 1935, when No. 5161 slipped and broke her leg; her condition was not different from that of the controls.

No. 5160 began to show great reluctance to walk in May, 1935, although her condition did not suggest any deficiency. This disinclination to walk became more and more pronounced; in July No. 5160 showed signs of lameness and appeared to be suffering from sore feet while walking was obviously a very painful and slow process. Her condition was excellent and food consumption very satisfactory. Towards the end of July this heifer could not be induced to take more than a couple of steps and was obviously in great pain when the attempt was made. She was apparently quite comfortable when lying down and would eat normally. On 31.7.36 she was killed for bone studies.

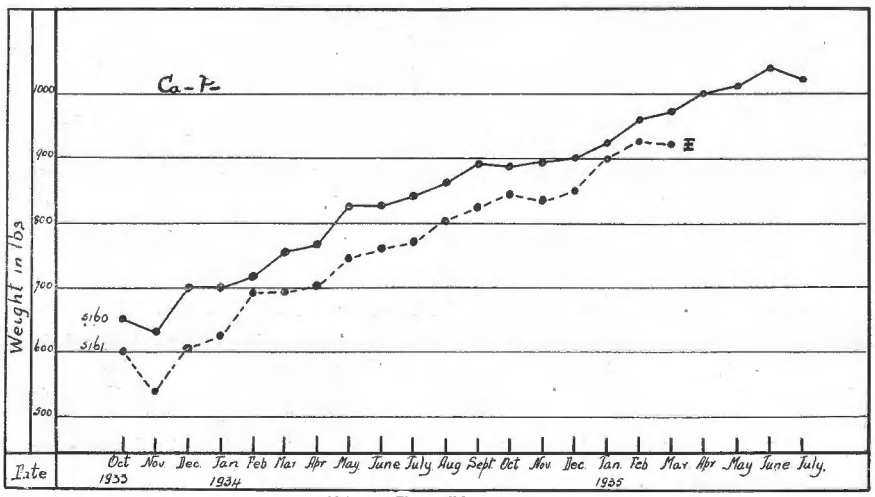

Fig. IX.—Weights.

Fig. 9 presents the monthly weights of the animals in this group. The weight increase does not appear to have been significantly affected by the deficiencies in the food.

Inorganic phosphorus in the blood suggested acute phosphorus deficiency in both animals for the greater part of the experiment as indicated in Fig. 10.

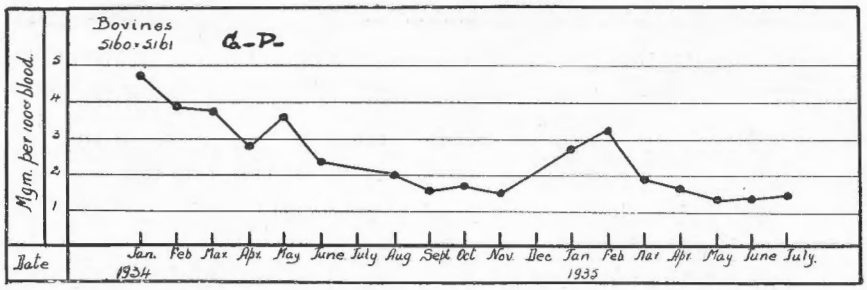

Fig. X.—Inorganic Phosphorus.

Blood calcium is given in Table 3.

The radiographs of 5160 indicate atrophy which is confirmed by the ash content of the femur of only 24 per cent. 1,375 lb. was registered as breaking strength of a six-inch span of the metacarpus compared with 2,280 for that of the control. (Table 6.)

Microscopical examination of the sections of selected bones of 5160 revealed all the lesions of rickets in an exaggerated measure.

The calcification zone was very defective, only portions of it being left; between these the medullary tissue penetrated into the hypertrophic zone. In the periphery of the cartilage calcification was completely lacking. The ossification zone was incomplete. The regular arrangement of the primary trabeculae was replaced by an irregular one. The spongioid zone was penetrated by cartilage tongues in places and the proximal spongiosa showed calcified trabeculae imbedded in osteoid. The periostal bone tissue consisted entirely of osteoid and the trabeculae of the osseous focus mainly of the same tissue.

Both calcium and phosphorus retentions were very poor. The average of the former was actually negative while that of the latter was only just positive. It appears that growth had taken place almost entirely by drawing upon the skeletal reserves of these two constituents with the result that only inferior bone could be produced as could be verified histologically and from the chemical data presented in Table 6.

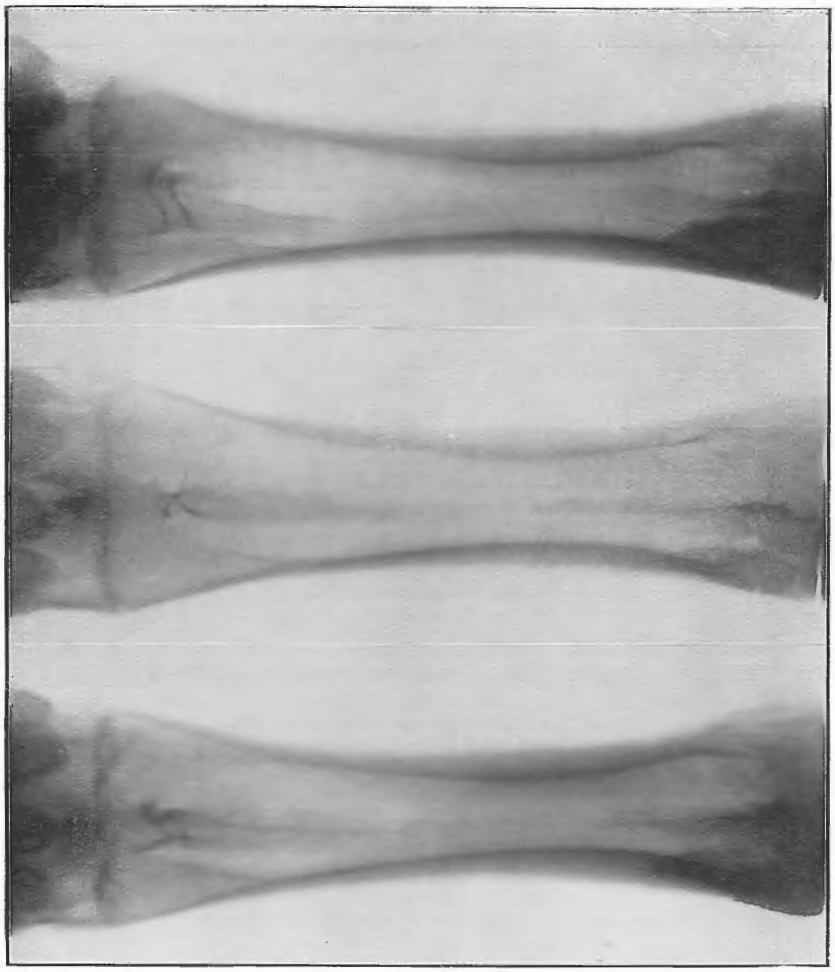

Fig. XI.

GROUP 5.

Ration containing excess Ca but adequate in all other respects.

Two grade Friesland heifers Nos. 5153 and 5167.

Average daily intake of Ca and P after supplementing basal ration with $CaCO_3$ and Na_2HPO_4 was 24·0 gm. P_2O_5 and 61·5 gm. CaO.

Both heifers were given the basal ration from the outset in December, 1933, and continued to consume it during the full course of the experiment. No abnormalities except pica were observed at any stage and heifer No. 5167 was killed in August, 1935, for bone studies.

The weight curves given in Fig. 12 show normal increase in weight when compared with the controls receiving adequate minerals.

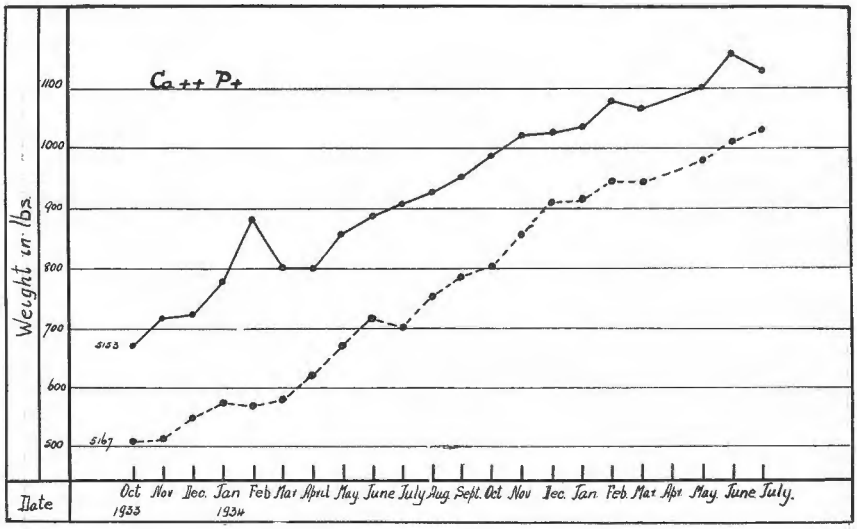

Fig. XII.—Weights.

The inorganic phosphorus content of the blood shows appreciable variation but suggest phosphorus sufficiency as is evident from Fig. 13.

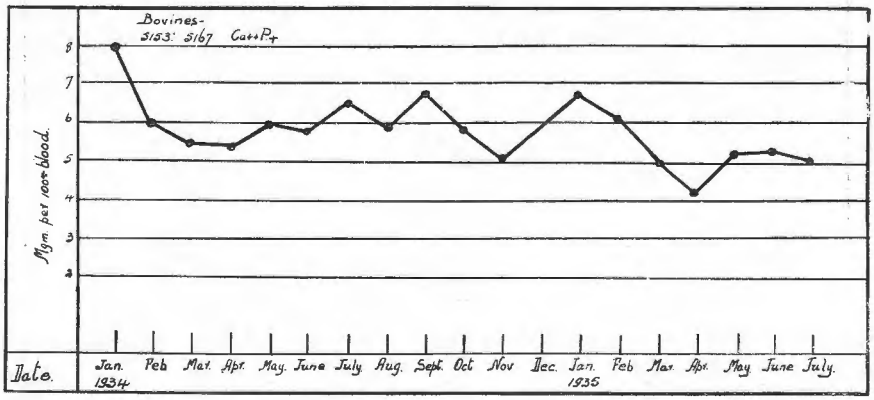

Fig. XIII.—Average Inorganic Phosphorus.

The calcium level of the blood is similar to that of the control animals (Table 3).

The bone ash and the breakng strength are not significantly different from the values obtained for the bones of the control animals, although the calcium retention was one and a half times, and P slightly less than that of the controls. (Tables 6 and 7.) Histological examination of the bone revealed normal bone structure which agreed with the X-ray photographs.

GROUP 6.

Ration low in Ca but adequate in other respects.

Grade Friesland heifers Nos. 5149 and 5147.

The average daily intake of Ca was 5·5 gm. expressed as CaO and 24·0 gm. P_2O_5.

Both animals received and consumed the basal ration during the full period of the trial. Pica was registered as in the other groups but otherwise no deficiency could be detected at any stage with the naked eye.

No. 5149 was killed in August, 1935, for bone studies.

A section of the nondecalcified rib showed the presence of the calcification zone which was thin in parts but complete. The medullary canals nowhere penetrated into the hypertrophic zone. An outstanding feature was the presence of fairly large medullary spaces which penetrated rather deeply but were always separated from the cartilage by the ossification zone. There were parts of the proximal spongiosa left and these showed well calcified trabeculae. The sections of other bones examined showed a marked ossification zone. An occasional red seam was present but certainly no lesions of rickets. The sections suggested normal bone structure.

The experimental mate of No. 5149, viz., No. 5147, was kept on her experimental ration subsequent to the destruction of the former. In April, 1936, i.e. after a further eight months a portion of a rib was removed under anaesthesia for microscopic study. Marked bone atrophy was revealed at that stage. Unfortunately the animal died as a result of the operation.

The weight curves of Nos. 5147 and 5149 given in Fig. 14 indicate that the weight increase of these two animals was only slightly poorer than that of the controls.

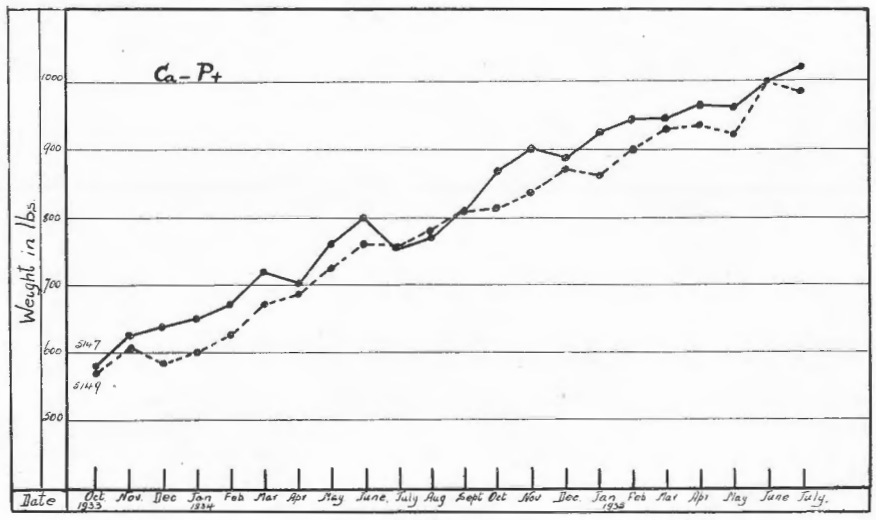

Fig. XIV.—Weights.

The inorganic phosphorus content of the blood suggests phosphorus sufficiency (Fig. 15), while the blood calcium does not show a significant decrease when compared with the values obtained for the other groups.

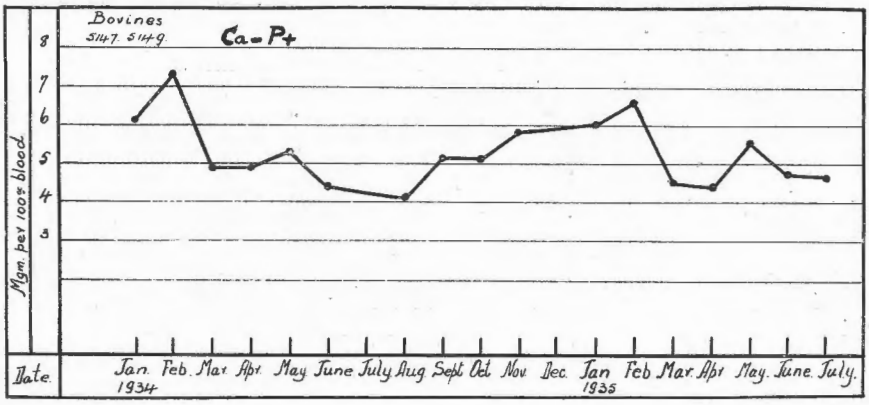

Fig. XV.—Average Inorganic Phosphorus.

The calcium retention of No. 5149 was poor although better than that of No. 5160 receiving the same quantity of Ca but less P, and phosphate about 70 per cent. of that of the control group (Table 7). In the light of the foregoing it is rather remarkable that the percentage ash is not more depressed when compared with that of the bones of the control animals (Table 6). The breaking strength of the metacarpus is similar to that of the metacarpus of the phosphorus deficient animal and is indicative of abnormal bone, but neither the radiograph nor the microscopic examination of bone sections confirms this finding.

DISCUSSION.

Many important points arise from a comparison of the results obtained in the different groups.

(1) Weight Increase and Food Consumption.

The weights of the animals are given in Table 2.

Poor increase in weight was shown without exception by the animals receiving a ration low in phosphorus but sufficient or excess calcium. Poor weight increase was invariably associated with poor appetite and food consumption. It would again appear that the lack of condition was associated with a decreased intake rather than with poorer utilization of food. This indication also consistently appeared in the earlier investigations (du Toit *et al*, 1931, etc.).

TABLE 2.

WEIGHTS IN LB.

Groups.

	I. Ca + P +		II. Ca + P−		III. Ca ++ P−		IV. Ca−P−		V. Ca ++ P +		VI. Ca−P+	
	5154	5163	5155	5157	5158	5159	5160	5161	5153	5167	5147	5149
October, 1934	640	600	580	570	570	620	650	600	670	510	580	570
November	685	600	600	625	600	655	630	540	715	515	625	605
December	655	630	630	630	630	685	700	605	720	550	640	585
January, 1935	675	670	640	635	625	700	700	625	775	575	650	600
February	685	710	660	695	625	740	715	690	780	570	670	625
March	735	730	690	710	660	740	755	696	800	580	720	670
April	765	740	705	710	650	745	765	700	800	620	700	685
May	780	790	735	720	665	770	825	745	855	670	760	725
June	840	800	740	750	650	760	825	760	885	715	800	760
July	850	805	715	750	600	745	840	770	905	700	755	755
August	890	855	710	765	600	725	865	800	925	750	770	780
September	915	840	710	735	575	725	890	825	950	785	805	805
October	930	880	770	770	560	Died	885	845	985	800	865	810
November	935	900	715	765	580	—	895	835	1020	835	900	835
December	955	920	725	775	585	—	900	850	1025	885	885	870
January, 1936	1000	955	725	750	600	—	925	900	1035	910	925	865
February	1045	955	705	785	595	—	965	925	1080	915	945	900
March	1020	995	685	745	610	—	970	920	1065	945	945	930
April	1060	990	725	765	615	—	1000	Died	1100	960	965	940
May	1075	1000	705	780	625	—	1010	—	1100	980	960	920
June	1100	1050	740	770	620	—	1040	—	1160	1010	1000	1000
July	1100	1035	735	790	630	—	1020	—	1155	1030	1020	985

Weight increase is inversely related to calcium intake in the phosphorus deficient groups. Poorest increase was registered in Group 3, where excess calcium was present and the best increase where both calcium and phosphorus were deficient. In other words the ratio of calcium to phosphorus when the latter was present in deficient amounts undoubtedly affected the animals, normal ratio beneficially and abnormal ratio adversely in regard to food consumption and the subsequent gain in weight. As the phosphorus intake of the three groups receiving low phosphorus diets was equal at the beginning of the trial and was reduced due to less food consumption of the groups receiving adequate and excess Ca respectively the decrease in food consumption can clearly not be associated with the decrease of the P present but rather with the abnormal ratio of Ca to P.

In Groups 5 and 6 where the phosphorus intake was adequate neither excess nor deficient calcium had any significant effect on weight increase or food consumption. In both these groups the ratio of calcium to P was abnormal, viz., 2·6 $CaO:1\ P_2O_5$ and 1 CaO: 4·3 P_2O_5 respectively but with adequate P intake the ratios seem to have been without effect on growth. Obviously, an answer to the question of the effect on weight increase and food consumption of increasing amounts of P when the Ca intake is low cannot be given from the data obtained in Group 6, but it could be mentioned here that subsequent work with bovines on a low Ca intake and increasing amounts of P does suggest rather strongly that appetite is not so sensitive to increasing amounts of P when calcium is low as to increasing amounts of Ca when P is low. So far (after 10 months) in the newer experiment on the same low Ca intake a ratio of 1 $CaO:5\ P_2O_5$ does not show a more pronounced effect on appetite than one of 1 $CaO:10\ P_2O_5$. Both in case of low P on the one hand and low Ca on the other the ratio of Ca to P is not without effect but apparently with a more pronounced and more easily obtained effect on appetite and gain in weight when P is deficient.

(2) Blood Analysis.

Blood calcium determinations were started 5 months after the beginning of the experiment and can be dismissed without further comment as there does not appear to be significant differences in this respect among the groups.

The inorganic phosphorus content of the blood is given in Table 4.

Phosphorus deficiency in Groups 2, 3 and 4 resulted as usual in low inorganic phosphorus of the blood. While these values were already low in Groups 2 and 3 receiving abnormal ratios of Ca and P one month after the beginning of the experiment, the blood inorganic phosphorus in Group 4 receiving a normal ratio decreased more gradually and did not reach the low figures registered in the other two groups until almost a year after the beginning of the experiment after which period the values showed a tendency of being even lower than those of the other two groups. It cannot be said, however, that the slight difference in the inorganic phosphorus content of the blood of the heifers in these three groups is primarily due to the

TABLE 3.

Blood Calcium in mgm. per 100 m.l. Blood.

Group No.	Bovine No.	1934.								1935.						
		9 May.	9 June.	10 July.	11 Aug.	12 Sept.	13 Oct.	14 Nov.	15 Dec.	16 Jan.	17 Feb.	18 Mar.	19 Apr.	20 May.	21 June.	22 July.
I	5154	10·6	9·8	11·0	10·3	10·2	9·9	10·3	—	10·0	10·1	8·5	11·2	9·5	8·7	8·7
	5163	11·3	9·8	10·0	11·1	10·8	10·5	10·2	—	10·5	11·0	10·0	8·2	9·2	8·6	9·8
II	5155	11·6	10·6	10·6	10·2	10·6	11·3	10·5	—	10·0	11·1	10·0	9·8	8·9	9·8	9·8
	5157	11·2	9·8	11·0	10·5	10·5	11·5	11·1	—	10·0	11·3	9·8	9·3	8·7	9·4	9·6
III	5158	11·8	10·0	10·6	11·1	10·0	11·0	10·3	—	11·0	11·1	9·6	8·9	8·4	9·4	10·0
	5159	10·7	9·6	9·6	11·2	10·8	11·4	10·1	—	—	—	—	—	—	—	—
IV	5160	10·7	10·0	10·0	10·6	11·0	11·5	110·6	—	10·3	11·2	9·8	8·8	10·8	9·8	9·5
	5161	10·2	11·2	11·0	11·4	10·5	11·3	11·0	—	10·1	10·7	—	—	—	—	—
V	5153	11·0	9·8	9·6	10·4	10·2	10·6	10·0	—	9·8	10·2	9·2	8·7	8·2	8·7	8·5
	5167	10·9	10·6	11·0	10·6	10·4	11·0	10·6	—	10·6	10·6	10·2	8·7	9·7	8·6	9·1
VI	5147	10·0	10·4	9·8	10·5	10·8	10·6	10·6	—	10·1	10·8	10·3	10·0	9·1	8·5	9·8
	5149	10·8	10·2	10·2	10·1	10·0	10·3	10·5	—	10·0	10·4	9·8	8·4	8·7	8·4	8·6

TABLE 4.

Inorganic Phosphorus in mgms. per 100 m.l. Blood.

Group.	D.O.B.	1934.												1935.						
		Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.
I.....	5151	4·2	4·5	3·4	4·6	5·4	5·0	5·2	5·0	4·6	5·9	6·5	—	6·1	5·9	7·7	5·8	7·5	5·2	6·7
	5163	4·6	4·5	5·0	5·0	5·1	4·9	4·4	4·6	5·1	5·5	4·5	—	5·2	6·7	5·1	4·5	5·3	4·7	4·8
II.....	5155	3·6	2·1	2·7	2·5	2·9	1·9	1·6	1·9	1·8	2·2	1·8	—	2·3	2·1	2·4	3·0	2·9	2·9	2·4
	5157	2·6	2·2	2·6	2·2	3·0	2·0	1·6	2·3	1·8	1·5	1·5	—	3·0	2·5	2·5	3·0	2·3	3·0	2·0
III.....	5158	3·4	3·1	3·5	2·7	3·4	2·3	2·0	1·8	1·7	3·4	2·4	—	3·0	2·6	2·4	4·6	4·1	4·3	2·4
	5159	2·8	3·5	3·3	2·0	2·4	1·6	1·9	2·3	2·0	1·6	1·4	—	—	—	—	—	—	—	—
IV.....	5160	5·1	3·5	4·0	2·6	3·6	2·2	2·1	2·2	1·7	1·5	1·7	—	2·4	3·0	1·6	1·6	1·3	1·3	1·4
	5161	4·6	4·3	3·6	3·1	3·7	2·7	2·0	1·8	1·6	1·9	1·3	—	3·0	3·4	—	—	—	—	—
V.....	5153	8·4	6·2	5·4	5·5	5·8	5·7	5·3	6·1	7·3	5·8	5·6	—	7·8	7·3	5·0	5·0	5·3	5·0	5·0
	5167	7·8	5·8	5·6	5·3	6·0	6·0	7·7	5·7	6·4	6·9	4·6	—	7·7	7·1	5·4	5·7	5·2	5·7	5·2
VI.....	5147	4·9	7·0	5·2	5·0	5·5	4·3	4·8	3·8	4·8	4·8	5·8	—	5·5	6·8	4·0	4·2	5·2	5·4	4·4
	5149	7·3	7·7	4·7	4·8	5·1	4·5	5·8	4·6	5·7	5·4	5·9	—	6·6	6·4	5·1	4·6	5·9	4·1	4·9

differences among the three ratios as the group receiving the best ratio certainly shows, if anything, lower values for the greater part of the experimental period. Phosphorus deficiency was probably greatest in Group 4 receiving the normal ratio of Ca to P, as growth was rapid and the animals showed an increase in weight of about 400 lb. compared with 60 and 170 lb. for the other two groups, respectively. Food intake should be equalized when the effect of the ratio of Ca to P upon blood analysis will undoubtedly show in animals receiving inadequate P in their ration. In Groups 1, 5, 6 receiving the same amount of P and consuming the same quantity of food daily the inorganic P content of the blood of group 1 is significantly less than that of Group 5 receiving excess Ca, and less than that of Group 6 on low lime. It is questionable whether the higher values in groups 5 and 6 can be interpreted to indicate poorer utilization of P by these two groups than by the control group whose figures are less but still indicate sufficiency of P in the diet. Food intake was the same in these three groups and growth rapid throughout.

Phosphatase was determined in the blood of the heifers in all the groups during the last five months of the experiment. These values are tabulated in Table 5.

TABLE 5.

Phosphate Content of Blood in Bodansky Units.

Group.	Bovine No.	1935.				
		March.	April.	May.	June.	July.
I.....	5154	3·5	3·3	4·6	4·4	3·9
	5163	5·2	1·6	5·0	5·1	5·3
II.....	5155	8·6	6·4	7·1	8·5	9·0
	5151	9·4	7·5	9·6	8·2	9·8
III.....	5158	9·4	3·8	5·5	7·7	8·9
IV.....	5160	13·9	9·9	16·2	11·7	12·1
	5161	8·5	—	—	—	—
V.....	5153	5·3	3·0	4·1	5·4	4·9
	5167	3·6	2·8	4·4	3·9	3·8
VI.....	5147	2·8	·5	5·2	4·0	5·2
	5149	4·9	2·5	4·2	4·1	2·6

A glance at Table 5 indicates that the phosphatase values of Groups 2, 3 and 4 are significantly higher than those of the groups receiving adequate phosphorus and suggest poorer bone formation in the three former groups.

(3) BONE ANALYSIS.

Reference should be made to Table 6 for a comparison of the results of the bone analyses.

TABLE 6.

Bone Analysis.

FEMUR.

Bovine.	Group.	Green Wgt.	Green Bone.				Breaking Strength per Sq. In. 6″ Span.	Dry Fat Free Bone.			Per cent. Ash of Green Wgt.
			Per cent. Water.	Per cent. Fat.	Per cent. Dry Fat Free.	Sp. Gr.		Per cent. Ash.	Per cent. Ca.	Per cent. P.	
5154....	I.....	2098	12·05	34·85	53·1	1·296	2850	66·5	38·7	17·7	35·4
5157....	II.....	1794	15·84	43·62	40·54	1·142	2650	61·8	39·4	17·1	25·1
5158....	III.....	1622	14·68	41·92	43·40	1·220	3020	63·1	39·4	17·3	27·4
5160....	IV.....	1700	14·10	45·50	40·40	1·157	1990	60·2	38·6	17·0	24·3
5153....	V.....	2288	12·68	37·52	49·80	1·271	3425	65·8	39·6	18·0	32·8
5149....	VI.....	1672	14·00	38·70	47·30	1·211	2990	64·7	38·4	17·4	30·6
							HUMERUS.				
5154....	I.....	1617	9·20	39·20	51·60	1·274	3080	66·6	38·5	17·9	34·4
5157....	II.....	1310	13·7	45·8	40·5	1·091	2500	61·0	40·7	17·9	24·7
5158....	III.....	1239	14·40	43·60	42·00	1·181	2800	63·3	39·4	17·7	26·7
5160....	IV.....	1343	15·10	45·50	39·4	1·148	2480	60·0	40·0	17·7	23·6
5153....	V.....	1732	11·50	40·60	47·90	1·211	2700	66·0	39·2	17·8	31·6
5149....	VI.....	1332	12·36	39·90	47·74	1·211	1950	65·0	39·2	17·7	31·1
							METACARPUS.				
5154....	I.....	449	11 57	21·80	66·63	1·522	2280	66·6	38·5	17·7	44·4
5157....	II.....	356	12·37	30·92	56·71	1·368	1800	65·8	38·4	17·7	37·6
5158....	III.....	345	13·80	23·30	62·90	1·395	1200	65·5	38·7	17·6	41·2
5160....	IV.....	352	13·50	32·2	54·3	1·375	1375	62·7	39·3	17·9	34·1
5153....	V.....	443	11·75	23·75	64·50	1·477	2650	67·1	38·9	17·8	43·3
5149....	VI.....	348	13·55	21·62	64·83	1·462	1725	66·6	38·6	17·6	43·1
							RIB.				
5154....	I.....	305	22·9	16·8	60·3	1·525	—	63·6	—	—	38·5
5157....	II.....	220	25·5	27·4	47·1	1·257	—	57·9	—	—	27·2
5158....	III.....	187	28·2	23·2	48·6	1·390	—	59·6	—	—	29·0
5160....	IV.....	262	31·2	22·5	46·3	1·312	—	54·6	—	—	25·3
5153....	V.....	358	23·5	19·7	56·8	1·432	—	62·2	—	—	35·2
5149....	VI.....	289	28·7	21·8	49·5	1·343	—	61·1	—	—	30·2

The difference among the weights of the same green bones of the animals in the various groups are quite appreciable, but as these values are partly dependent on size which in its turn is also determined by factors other than feeding, as for instance breeding, the green weights are obviously only of limited value for comparison and difficult to interpret correctly under the conditions of the experiment.

The percentages dry fat free bone calculated on the green weight, indicate, as would be expected, that Group 1 receiving Ca and P in sufficient amounts under optimum conditions show the highest values for all four bones analysed. Groups 5 and 6 receiving the same amount of P as Group 1 but respectively more and less Ca show the nearest approach in ash percentage to the normal group; these diminished values suggest disturbances in the process of normal bone formation in these two groups. The ash percentages of the bones of Groups 2, 3 and 4 which received P in inadequate amounts show similar values definitely lower than those of the normal group. The bone ash percentages of these three groups should not be compared with each other on account of the unequal intake of food and consequent growth and hence effect on bone formation. It would seem, however, that the difference in the percentage of bone ash of the animals receiving adequate P (Groups 1, 5 and 6) when compared with those of animals receiving insufficient P in their diet (Groups 2, 3 and 4) is closely associated with the P intake and that the ratio of Ca too had a minor influence or at all events did not alter the values appreciably.

The fat fraction of the bones is inversely related to the ash fractions and hence increases in the bones of the P deficient animals where ash is low when compared with the control.

In regard to the breaking strength the metacarpus is probably the most suitable bone for this determination and gives the most reliable values. The femurs are often crushed and do not register sudden breaks at all, while the shape of the humerus is such that it is difficult, if not impossible, to fit it into the breaking machine so that the effect of the application of pressure does not cause a slip towards the side. The metacarpus of No. 5149 on a calcium low diet shows a low breaking strength and it would appear that at most it can be said that Ca or P deficiency or both in the diet produced weaker bones than the animals in Groups 1 and 5 where no deficiency existed.

The bones of animal No. 5158 receiving excess Ca and inadequate P gave results which are rather puzzling. From a consideration of weight increase, food consumption and the development of clinical symptoms this heifer appeared to be worse than 5157 on a ration low in P but adequate in Ca. On theoretical grounds too, one would expect the latter animal, whose ration contained Ca and P in a more beneficial ratio (2·4 CaO : 1 P_2O_5) to have responded better than 5158 receiving Ca and P in the ratio of 9 CaO to 1 P_2O_5. It will be recollected from the description of the animals in the latter

group (Nos. 5158 and 5159) that 2 gm. P_2O_5 were given for about 3 months to No. 5158 after the death of No. 5159 to keep her in the experiment and the possibility exists that the bone analyses show the response to this extra intake of P as is also noticeable from the inorganic P content of the blood and the plasma phosphatase and from the balance experiments which indicate that appreciably more P was retained for that period.

(4) Ca and P Retentions.

Table 7 gives a summary of the determinations of Ca and P retentions on a representative heifer from each group in the course of the experiment.

The daily retention of P in Group 1 receiving adequate quantities of both P and Ca amounted to 15 gm. P_2O_5 and was the best of all the groups. This retention decreased considerably by respectively increasing or decreasing the Ca intake without alteration of the P supplied as was done in Groups 5 and 6. It would seem that the abnormal ratio so caused in these two groups affected the P retention adversely, although apparently not to a degree which was recognizable clinically or which affected food consumption and consequently weight increase significantly. Still, the bone analyses confirm the indication of a poorer utilization of minerals in Groups 5 and 6 than in Group 1 for which the abnormal ratio of Ca to P in the former two groups must be held responsible. It is uncertain of course whether this decreased utilization of P would have led to phosphorus deficiency ultimately, although such a result seems improbable in the light of the fact that no unfavourable effects were registered on food consumption, weight increase, blood analyses and bone structure during the rapidly growing period of the heifers from approximately 18 months to 3 years of age.

In regard to Groups 2, 3 and 4 receiving inadequate amounts of phosphorus it seems remarkable, especially in the last group that growth could have taken place at all. Very little P was retained in the body and the animals were apparently almost entirely dependent upon breaking down their skeleton and producing instead bone of inferior quality as revealed by the bone analyses. On this basis the production of markedly rachitic bones in Group 4 (Ca and P deficient) where growth was rapid and equal to that of the normal group, and of relatively only slightly rachitic bones in Group 3 where (excess Ca, deficient P) growth was almost negligible, is readily explicable. The outstanding feature of the P retention in these three groups (2, 3 and 4) is that the animals were able to remain alive on a P retention of little more than 1 gm. P_2O_5 per day for more than two years.

In regard to the retention of Ca the determining factor in all the groups undoubtedly was the levels of intake. It is remarkable that in Group 3 receiving excess Ca and deficient P about 10 times

TABLE 7.

Average Daily Retention of Calcium and Phosphorus.

Groups.	1 B. 5154.		2 B. 5157.		3 B. 5158.		4 B. 5160.		5 B. 5153.		6 B. 5149.	
	CaO.	P_2O_5.	CaO.	P_2O_5.	CaO.	P_2O_5.	CaO.	P_2O_5.	CaO.	P_2O_5.	CaO.	P_2O_5.
July	22·90	18·76	1602	3·45	19·76	3·27	1·26	3·25	29·44	18·76	1·67	15·38
August	9·01	16·32	6·82	·69	20·39	2·06	−0·55	1·05	24·64	13·02	2·49	7·89
September	11·89	13·27	9·94	− ·27	17·02	1·10	·39	3·14	37·10	17·19	2·04	12·29
October	11·13	11·95	2·10	−1·83	17·46	1·05	−1·16	1·40	23·85	14·71	1·60	14·90
November	11·16	14·55	2·28	·64	15·41	1·14	−0·42	·85	18·74	15·47	−1·04	9·24
January	18·25	13·07	3·26	− ·02	18·47	−1·51	−1·08	·59	11·47	5·72	2·54	9·15
February	8·49	13·84	5·25	−1·01	11·26	·80	−1·33	1·03	14·92	9·29	0·34	4·19
March	15·66	15·81	9·94	2·01	6·90	2·24	−0·29	2·65	13·85	8·70	2·45	4·60
April	13·29	18·63	6·33	3·02	11·88	3·27	0·24	2·61	23·74	11·24	2·03	12·56
May	12·62	16·84	7·35	1·40	14·66	3·18	0·34	1·92	13·11	13·07	2·06	12·65
June	13·80	12·88	3·11	·62	9·99	·50	0·45	·57	16·57	6·38	1·08	5·35
Mean	13·57	15·06	6·13	·80	14·82	1·56	−0·56	1·74	20·67	12·15	1·57	9·82
Mean intake	32·1	24·0	31·6	7·87	60·6	6·93	5·92	10·6	63·0	24·4	5·8	24·4
Per cent. Retention	42·3	62·6	19·4	10·2	24·4	22·4	—	16·4	32·8	49·8	27·0	40·2

as much of the former constituent was retained as of the latter. The retention of Ca in Group 6 on low Ca intake was very low and it is surprising that greater evidence of a calcium deficiency was not obtained when animal No. 5149 was killed. It is to be remembered, however, that the group mate No. 5147 showed marked bone atrophy almost a year after No. 5149 had been killed. In case No. 5160 actually no calcium was retained, the balance being on an average slightly negative for the entire period; the clinical symptoms in this case were probably partly due to the deficiency of Ca.

(5) Histological Findings.

The following is a summary of the diagnoses of the bonelesions produced in the different groups:—

No.	Group.	Diagnosis.
5154....	1. Ca and P adequate.....	Normal.
5157....	2. Ca adequate P low.....	Rickets.
5158....	3. Ca excess P low.....	Rickets.
5160....	4. Ca low P low.....	Marked rickets.
5161....	4. Ca low P low, died 4 months before 5160....	Very marked rickets.
5153....	5. Ca excess P adequate.....	Normal.
5149....	6. Ca low P adequate.....	Normal.
5147....	6. Ca low P adequate (after 32 months in expt.)	Marked bone atrophy.

In general the histological findings are in agreement with the results already discussed. The bones of No. 5160 showed marked rickets (osteomalacia). Clinically too, this animal showed the most severe symptoms of deficiency towards the close of the experiment. Food consumption remained good, growth rate was rapid, which was probably responsible for the development of more inferior bone than in the other two groups receiving insufficient P (Groups 2 and 3) where the animals showed better adaptation to the low P in the diet by consuming less food and increasing comparatively gradually in weight. The balance trials, bone analyses and even the inorganic P content of the blood all suggest that this animal would show the most severe bonelesions.

The outstanding feature of the microscopical examination of the bones is that osteomalacia or rickets was invariably associated with P deficiency, whereas Ca deficiency in Group 6 produced at most marked atrophy of the bones of No. 5147 but no rickets.

EXPERIMENT 2.

The results of another investigation into the effect of low P on cattle will be reported here very briefly as they corroborate in the main the results obtained in Experiment 1 but in addition provide extensions in some directions.

Detailed data of this work will be given by Otto (1937).

Four pairs of eighteen-months-old steers were placed on the same basal ration low in Ca and P. Ca and P were added to the basal ration in the form of $CaCO_3$ and Na_2HPO_4 to provide different intakes of Ca and P for each pair of steers; the relative proportions of these two constituents remained constant in three of the groups.

The daily basal ration consisted of—

3·5 kg. maize samp;

1·2 kg. teff hay;

0·24 kg. meat meal;

0·5 kg. greenfeed.

The basal ration contained approximately 13 gm. CaO and 10 gm. P_2O_5.

Details of the approximate mineral intake of the different pairs of steers are given below:—

Group.	No. of Animal.	CaO intake grams.	P_2O_5 intake grams.	CaO : P_2O_5.
1.....	4712	22	18	1·2 : 1
1.....	3478	22	18	1·2 : 1
2.....	3480	17	13	1·3 : 1
2.....	3465	17	13	1·3 : 1
3.....	3464	35	13	2·7 : 1
3.....	3467	35	13	2·7 : 1
4.....	3456	13	10	1·3 : 1
4.....	3464	13	10	1·3 : 1

A fifth pair of steers was subsequently added; in this case the Ca and P intakes were reduced to 3·8 and 3·9 gm. respectively when expressed as the oxides.

The experiment began in June, 1933, and was continued for two years. Weights and food consumption were recorded, blood was analysed for Ca, P and for phosphatase towards the end of the experiment, Ca and P balances were carried out periodically during the course of the experiment and bone studies—chemical and histological —undertaken at the conclusion of the trial.

The results of this experiment will be considered only in so far as they throw light on the inference drawn from the first investigation, viz., that osteomalacia or rickets was invariably associated with low dietary P apart from the Ca content of the ration.

The bones of animal No. 4712 receiving daily on an average 22 gm. CaO and 18 gm. P_2O_5 for 30 months showed no abnormal lesions. The calcification zone was well marked. It was nowhere interrupted and the primary trabeculae were in regular order and of fair length. The pillar trabeculae which entered the proximal spongiosa were all well calcified. The conditions were those found in a normal animal. The presence of an occasional red seam had to be expected in a fast growing animal and could not be looked upon as a pathological change. The bones were therefore considered to be of normal structure.

It should be remembered that the heifers showing normal bone structure in the first experiment were given daily a total of 24 gm. P_2O_5 and it would appear therefore that under the conditions of the second trial 18 gm. P_2O_5 were sufficient to provide for the requirements of a normally growing healthy steer.

In Group 2 whose diet contained 13 gm. P_2O_5 and 17 gm. CaO, blood analysis indicated that less P was present in the ration than in that of Group 1 and approached values at times that suggested P deficiency rather strongly. The other observations, viz., balance trials and bone analysis corroborated this view. The bones of animal No. 3480 showed a complete ossification zone with no interruptions and of fair thickness. Some of the primary trabeculae were very short. The pillar and other trabeculae belonging to the region showed thin conspicuous osteoid seams which were present in more than physiological amounts. There was a scarcity of pillar trabeculae. A diagnosis of rickets and atrophy was considered justified.

The development of rickets in Group 2 on low P but probably also inadequate Ca makes the results obtained in Group 3 significant and important. Here the P intake remained the same as in Group 2 but Ca was increased to 35 gm. producing a less beneficial ratio than that present in group 2. Judging from the inorganic phosphorus in the blood the animals in Group 3 suffered more from a P deficiency than those in Group 2 which was to be expected when considering the wide ratio. However, the animals in Group 3 responded similarly on the whole to those of Group 2 although this point is unimportant in this discussion as the ultimate object was to determine whether the bone lesions produced in Group 2 developed as a result of the P deficiency in the diet of the animals in that group or whether low Ca intake was also partly responsible for the rachitic lesions.

The 6th rib of No. 3467 revealed that the calcification zone was nowhere interrupted. The ossification zone had been reduced by the presence of large secondary medullary spaces. Osteoid was present in more than normal amounts. The trabeculae were very short and some of the secondary medullary spaces extended deeply into them. In other places some of the primary trabeculae were rather long. Marked resorption had taken place in the ossification zone. Diagnosis: slight rickets and atrophy.

It would appear that the presence of rickets is undoubtedly associated with the low P content of the ration in Group 3.

Both Ca and P were low in the ration of Group 4. Weight increase and food consumption were normal from the beginning of the experiment in July, 1933, until March, 1934, when one of the steers broke his leg and was destroyed. Blood analyses indicated aphosphorosis and poor calcification was confirmed from the bone analyses.

Microscopical examination of the bones of No. 3456 showed the presence of the provisional calcification zone. In parts it had a serrated surface due to the presence of chondroblasts in the calcified trabeculae. In some parts the calcified zone was very thin. The calcification zone was nowhere interrupted nor could medullary canals be seen entering the hypertrophic cartilage. The ossification zone was fairly wide in places but in others it had been resorbed and large medullary spaces occupied its place. The pillar trabeculae showed thin osteoid seams, while some of the primary trabeculae were lined with osteoid the amount of which certainly transgressed physiological limits. A diagnosis of incipient rickets and marked atrophy was made.

The rachitic lesions were more severe and extensive in Group 4 than in either of the two previous groups but it is to be remembered that the daily intake of P in the former group was only 10 gm. P_2O_5 compared with 13 gm. in the case of the two latter groups. Again it would seem therefore that the severity of the lesions was related to the quantitive intake of phosphorus.

The fifth pair of steers receiving daily in their ration approximately only 4 gm. each of CaO and P_2O_5 remained in good condition and showed considerable increase in weight, viz., 220 lb. for the first thirteen months of the experimental period. The inorganic phosphorus content of the blood indicated P deficiency and abnormal values for phosphatase that calcification was being interfered with but the appetites remained good and the animals did not present the classical picture of aphosphorosis. The progress of this pair of steers was very similar to that of the heifers Nos. 5160 and 5161 mentioned in the first investigation. It will be remembered that although severe rickets was diagnosed at the end of the trial these two heifers on low Ca and P increased in weight normally and were indistinguishable from the control group until shortly before the end of the experiment.

Steer No. 5430 was killed in September, 1935, nine months after the beginning of the experiment, for bone study. Bone sections were examined microscopically and found to justify a diagnosis of severe rickets and atrophy. The 10th rib for instance showed tongue-like projections of the cartilage into the subchondral zone; distally these projections were embedded in osteoid which was present in great amounts. The preparatory calcification of the proliferation zone was very irregular; occasionally the preparatory calcification zone was reduced to a very thin line and was even absent in some places. A conspicuous feature was the marked atrophy in the more distal portions.

A portion of a rib was removed under anaesthesia from the experimental mate of No. 5430, viz., No. 5431 three months after the former animal had been killed. This section also showed lesions of severe rickets and atrophy.

Summaries of the data collected appear in Tables 8-11 below:—

TABLE 8.

Weights of Animals in lb.

Date.	Group I.		Group II.		Group III.		Group IV.		Group V.	
	4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
June, 1933	821	800	812	767	798	818	756	836	—	—
July	841	819	831	790	796	831	779	857	—	—
August	880	860	863	814	834	854	814	855	—	—
September	890	865	873	827	851	860	820	860	—	—
October	861	861	863	816	832	845	797	836	—	—
November	894	887	883	839	839	845	833	839	—	—
December	900	886	819	844	820	844	855	838	—	—
January, 1934	906	914	824	850	815	871	863	848	—	—
February	934	934	850	865	805	865	883	855	—	—
March	949	983	872	874	816	880	Died	849	—	—
April	975	1000	885	888	830	895	—	850	—	—
May	995	1004	875	890	926	882	—	851	—	—
June	1020	1036	875	Died	853	890	—	875	—	—
July	1014	Died	910	—	892	896	—	869	450	400
August	1000	—	935	—	920	915	—	880	—	—
September	1040	—	925	—	915	945	—	845	470	440
October	1065	—	950	—	925	1000	—	845	490	460
November	1060	—	940	—	925	Died	—	820	510	500
December	—	—	—	—	—	—	—	—	500	508
January, 1935	1085	—	960	—	940	—	—	860	495	500
February	1100	—	1000	—	965	—	—	840	520	520
March	1125	—	970	—	1000	—	—	875	560	540
April	—	—	—	—	—	—	—	—	580	550
May	1170	—	1055	—	1050	—	—	895	615	600
June	1215	—	1100	—	1080	—	—	890	615	610
July	1265	—	1130	—	1105	—	—	885	625	620

TABLE 9.

Inorganic Blood Phosphorus in mgm. per 100 *m.l. Blood.*

Bovines.

Date.	4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
September, 1933	7·6	6·9	7·0	7·2	7·2	6·9	6·6	4·0	—	—
October	7·3	7·1	7·2	7·0	6·5	6·4	6·5	6·2	—	—
November	6·4	7·4	6·4	6·9	7·0	6·2	6·0	5·2	—	—
December	7·2	8·0	6·6	5·8	6·4	5·8	5·2	4·3	—	—
January, 1934	6·8	6·8	5·8	5·4	5·9	5·6	4·6	3·9	—	—
February	5·8	7·2	5·2	5·0	4·8	5·0	4·3	3·3	—	—
March	6·2	6·6	5·6	5·2	5·0	4·2	Died	3·6	—	—
April	5·8	7·2	4·6	4·9	3·6	3·5	—	3·2	—	—
May	5·4	7·6	5·8	Died	3·9	3·7	—	3·0	—	—
June	5·2	7·7	4·4	—	3·4	3·4	—	2·7	—	—
July	5·3	Died	3·0	—	2·8	2·6	—	2·5	—	—
August	6·1	—	3·5	—	3·2	2·9	—	2·9	7·0	4·4
September	6·2	—	3·7	—	3·3	3·4	—	2·6	6·4	5·0
October	6·2	—	3·9	—	3·4	3·1	—	2·7	5·5	4·0
November	6·2	—	4·7	—	3·5	2·7	—	2·8	4·4	3·2
December	6·1	—	4·6	—	4·0	Died	—	2·9	4·8	3·6
January, 1935	5·7	—	4·8	—	3·6	—	—	2·8	4·7	3·5
February	6·0	—	5·1	—	3·4	—	—	2·8	3·5	2·3
March	5·7	—	4·7	—	3·6	—	—	2·9	3·4	3·0
April	5·6	—	4·3	—	3·4	—	—	2·7	3·2	2·6
May	5·3	—	4·0	—	3·5	—	—	2·9	3·3	2·6
June	5·2	—	3·9	—	3·4	—	—	2·8	3·1	1·8
July	5·0	—	3·5	—	3·3	—	—	2·9	2·9	1·6

TABLE 10.

Blood Calcium in mgm. per 100 *m.l. Blood.*

Bovines.

Date.	4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
September, 1933	10·2	10·6	10·0	9·9	10·2	9·8	10·4	10·0	—	—
October	10·0	10·2	9·8	10·0	9·8	10·2	9·6	8·9	—	—
November	9·6	10·0	8·9	10·1	10·4	10·0	8·9	9·2	—	—
December	10·4	10·6	10·6	11·2	11·2	9·3	10·0	10·8	—	—
January, 1934	10·2	9·9	10·2	10·6	10·8	9·4	9·4	10·4	—	—
February	9·8	10·4	10·0	10·4	10·4	9·5	10·2	10·0	—	—
March	10·0	10·0	10·1	10·7	10·3	9·8	Died	9·6	—	—
April	9·4	10·6	9·8	10·5	10·7	9·7	—	9·2	—	—
May	10·6	9·8	10·0	Died	10·1	10·0	—	9·0	—	—
June	10·4	10·0	9·6	—	10·0	9·6	—	8·0	—	—
July	9·4	Died	9·5	—	10·6	9·5	—	10·4	—	—
August	9·8	—	10·0	—	10·7	10·3	—	11·2	10·8	10·5
September	8·9	—	10·2	—	10·4	11·2	—	10·6	10·4	10·7
October	10·6	—	10·1	—	8·9	10·8	—	10·4	10·6	10·2
November	10·2	—	10·4	—	9·6	11·0	—	8·4	10·4	10·5
December	10·0	—	10·6	—	10·4	Died	—	9·4	10·2	10·0
January, 1935	10·0	—	10·3	—	8·6	—	—	10·6	10·2	10·1
February	10·2	—	9·7	—	10·4	—	—	8·6	10·9	10·6
March	9·8	—	10·6	—	10·2	—	—	10·4	8·9	9·6
April	9·6	—	9·5	—	10·9	—	—	10·2	10·0	11·0
May	10·1	—	10·0	—	10·0	—	—	10·0	8·0	8·9
June	9·8	—	10·2	—	9·9	—	—	8·4	8·6	8·9
July	8·9	—	9·1	—	9·6	—	—	9·0	9·1	8·4

TABLE 11.

Summary of Ca and P Retentions (grms.)

	Group I. B. 4712.		Group II. B. 3480.		Group III. B. 3465.		Group IV. B. 3456.		Group V. B. 5431.	
	CaO.	P_2O_5.	CaO.	P_2O_5.	CaO.	P_2O_5.	CaO.	P_2O_5.	CaO.	P_2O_5.
October, 1933...	9·45	9·54	4·23	3·07	5·20	3·71	−1·95	−2·68	−2·32	1·17
November.....	8·10	6·61	4·93	3·94	8·61	4·65	−2·68	−1·40	−1·60	1·05
January, 1934..	12·30	10·39	—	—	8·94	3·23	2·42	1·51	−1·97	−1·69
February.....	9·36	10·18	—	—	6·09	3·94	4·24	1·46	−3·05	−1·76
March.....	9·71	9·50	—	—	6·78	4·97	4·02	3·73	−1·64	−1·37
April.....	9·13	11·72	—	—	9·92	3·48	2·84	2·15	−1·54	−0·11
May.....	9·08	10·82	6·29	6·48	6·97	6·94	2·72	5·90	0·46	0·14
July.....	8·95	11·26	3·21	4·92	5·22	6·20	3·46	5·70	0·22	0·66
August.....	8·66	12·43	5·50	5·81	3·08	6·70	3·71	5·03	0·59	0·55
September.....	9·36	8·35	3·40	5·95	3·84	5·35	3·68	4·62	0·25	0·16
October.....	11·06	7·51	5·08	4·42	4·31	2·91	0·34	0·85	1·02	1·05
November.....	7·87	9·73	6·12	4·81	6·55	4·03	0·20	1·28	0·81	1·35
January, 1935..	7·96	8·38	4·36	6·73	7·06	4·14	1·72	3·14	—	—
February.....	8·16	10·96	4·55	7·62	10·15	4·81	3·10	2·49	−0·49	0·11
March.....	5·60	9·86	6·81	5·68	11·54	3·68	4·41	2·01	—	—
May.....	9·44	11·74	6·62	5·58	7·90	5·90	5·55	3·55	—	—
June.....	7·14	8·31	5·43	3·94	8·91	5·42	4·52	2·26	—	—
	8·91	10·11	5·14	5·65	7·13	4·65	2·49	2·45	—	—

DISCUSSION.

Apart from the conspicuous association of rickets with aphosphorosis in all the groups on a low P intake mentioned in this experiment it was noteworthy, that the two steers Nos. 5430 and 5431 receiving a ration which contained approximately only 4 gm. each of P and Ca and in proportions accepted as normal, developed severe rickets, but that this condition was clinically hardly noticeable and never conspicuous to the naked eye at any stage of the experiment. Loss of appetite was not observed in this group nor was it reported in the case of Nos. 5160 and 5161 whose rations were also low in both Ca and P as mentioned in the first investigation. Although more Ca and P were contained in the ration of the latter group, these constituents were still supplied in inadequate amounts, viz., 5·5 and 10·6 gm. respectively daily. In spite of the development of severe rickets as diagnosed microscopically at the conclusion of the respective experiments, it will be remembered that growth remained normal, the appetites were good, while the groups receiving the same quantity of P as Nos. 5160 and 5161, viz., 10·6 gm. daily, but sufficient and excess calcium respectively suffered from partial loss of appetite and showed consequently poor gains in weight. It would seem that the loss of appetite which has been reported consistently in cattle on phosphorus low rations is not unassociated with the calcium content of the diet. Apparently a favourable ratio of Ca to P when the latter is present in insufficient amounts does not affect the appetite detrimentally to any appreciable extent and therefore incidentally

produces good growth, for food utilization is unaffected by Ca or P deficiency (Kleiber *et al* 1936; Otto 1932, Evans 1929). Furthermore such a ratio under conditions of P deficiency apparently indirectly retards the development of clinical symptoms of aphosphorosis in cattle. Still, the severity of the rachitic lesions does not appear to be invariably associated with that of the clinical symptoms and with condition, for whereas the bovines receiving diets low in both Ca and P showed bone lesions of florid rickets, while in good condition and clinically quite healthy, Nos. 5157 and 5158 receiving even less P than No. 5160 but more Ca showed microscopical bone lesions of rickets that were by no means as marked but in addition clinically recognizable symptoms of aphosphorosis, poor appetite and condition. The difference in the severity of the bone lesions is explicable on the basis that much more rapid growth took place in the case of No. 5160 and hence quicker bone formation than in the case of Nos. 5157 and 5158; the clinical symptoms of what has usually been regarded as those of a straight aphosphorosis shown by the latter animals should more correctly be regarded as the effects of a disturbance in Ca P metabolism rather than as those of a straightforward phosphorus deficiency. Excess Ca was probably as much responsible as the phosphorus deficiency. Even the values giving the daily retention of P (see Table 7) suggest that for their registered increase in weight Nos. 5160 and 5161 (low P and low Ca) retained less P than either Group 2 or 3 (i.e. Nos. 5157 and 5158) receiving low P but adequate and excess Ca respectively. If, therefore, the clinical symptoms were entirely the result of P deficiency these should have been most severe in Nos. 5160 and 5161. It seems more correct to regard the clinical symptoms as the sequel to mineral disturbance rather than to P deficiency. On this basis Ca deficiency might present at times a clinical picture indistinguishable from that of aphosphorosis and this has been observed.

Viewed in the light of the above the effects of a phosphorus deficiency under practical conditions are probably aggravated by relatively high calcium which is almost invariably present in phosphorus deficient pasture. Even if the main effect of the excess Ca is not increased elimination of P, for it can hardly be said that such was the case in animals Nos. 5157 and 5158 when compared with No. 5160 (Table 7) the increased retention of Ca where it is present in relatively large quantities (Nos. 5157 and 5158, Table 7) may be largely responsible for the development of clinical symptoms or perhaps the loss of appetite noted and subsequent poor growth and condition. In any case stiffness, poor condition, etc., which are common in cattle dependent upon P deficient grazing are possibly as closely associated with the relative Ca excess as with the low P intake.

In regard to the Erdalkali-alkalizität of the rations fed to the animals in the two experiments reported, the values ranged from approximately +64 to −20 mgm. equivalents and as in the case of the Ca:P ratios rickets was produced in animals when the Erdalkali-alkalizität was normal (Nos. 5157 and 5155) or abnormal (Nos. 5160 and 5161) while in other cases the animals remained healthy when the E.A. of the ration was distinctly abnormal (Nos. 5154 and

5163). E.A. like Ca:P ratio fails as an explanatory basis for the production of osteodystrophic diseases in bovines when the Ca and/or P intake is deficient under conditions of vitamin D sufficiency but is probably associated with the severity of the disease.

SUMMARY.

1. Young heifers and steers were fed basal rations supplemented with $CaCO_3$ and Na_2HPO_4 in such a manner that the intakes of Ca and P were different in the respective groups. Vitamin D was present in abundance.

2. The basis of the experiments was respectively deficiency and sufficiency of P with varying amounts of Ca in the rations.

3. The experiments continued for approximately 24 months during which period observations were recorded on weight increase, food consumption, blood analysis for P, Ca and phosphatase, clinical symptoms of disease and bone analyses—both chemical and histological.

4. The outstanding result of the experiments is that under the conditions mentioned P deficiency in bovines invariably leads to rickets or osteomalacia and that osteodystrophia fibrosa is not produced by P deficiency *per se*. With regard to the latter condition the suggestion is made that Ca deficiency may be the responsible factor.

5. Erdalkali-alkalizität, like Ca:P ratio of which it really is a modification, is not the factor which determines whether rickets will or will not develop under conditions of P deficiency but both are associated with the severity of the complex result produced; for instance if they are not always associated with the severity of the microscopical bone lesions then with the earlier effects upon food consumption, growth and the development of clinical symptoms.

6. From the data available it would appear that a ratio of $CaO:P_2O_5$ of 2·5:1, when P was present in adequate amounts did not affect the animals significantly in regard to the observations registered.

7. A daily intake of 19 gm. P_2O_5 of which 53 per cent. was retained by the steers and of 24·0 gm. P_2O_5 with a retention of approximately 63 per cent. by the heifers provided sufficient P for normal growth and development while 13 gm. and 10 gm. were insufficient for the steers and heifers respectively.

8. Decreased food consumption which has invariably been observed in cattle receiving insufficient amounts of P in their diets is not wholly due to the inadequacy of the P but is also associated with the calcium content of such a diet or apparently therefore an effect of a disturbance in the Ca:P metabolism of the animal.

9. With regard to blood analysis the phosphatase and the inorganic P content of the blood afford valuable assistance in following the development of rickets but Ca determinations have been found to be of little help in presence of vitamin D. The periodic

removal of portions of ribs causes the animal very little inconvenience and has advantages even over X-ray photography for studying the development of osteodystrophic diseases in the experimental animals.

ACKNOWLEDGMENTS.

The authors wish to acknowledge and record the technical assistance of Dr. Groenewald (animal husbandry), Dr. Thomas (pathology) and of Messrs. Otto and du Toit (chemistry), all of this Institute.

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