Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 9, Number 1, July, 1937.

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

Studies in Mineral Metabolism XXXVIII.

Calcium and Phosphorus in the Nutrition of Growing Pigs.

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Bethke and associates (1933) and Dunlop (1935) conclude that growing pigs require approximately 0·6 per cent. P in their rations and that maximum growth is obtained with a Calcium-phosphorus ratio between 1:1 and 2:1. Bethke et al. furthermore state that as the proportion of Ca to P exceeded 3:1 the pigs became more rachitic and their vitamin D requirements increased. Our own experience here has been that while the ratio of Ca to P is undoubtedly important, osteodystrophic diseases may be produced in cattle, and probably in other species, merely by limiting the Ca or P intake or both, even when the ratio of these two constituents to one another was normal, when judged by the usually accepted standards; these observations have been summarized and discussed in the light of the findings of other investigators by Theiler and others (1936). Shohl and Wolbach (1936) report similar results with rats.

It would appear therefore that the relation between the ratio of Ca to P of a diet and the absolute intake of these two constituents is by no means settled, nor is it certain whether relatively high Ca with respect to P supplied in deficient amounts produces the same bone disease as the reverse, viz. high P and low Ca in the ration. Pigs were selected as the experimental animals as most of the more recent experiments at this Institute have been carried out on cattle and sheep, the results of which need obviously not apply to pigs.

Marek and his school (1932) have carried out experiments on Ca:P metabolism in pigs for the last twenty years and more and have expressed their conclusions in regard to the production of osteodystrophic disease subsequent to what they regard as abnormal Ca:P metabolism in pigs as follows:—

If the difference per 100 gms. dry feed consumed between Ca + Mg and P expressed as oxides does not fall within the comparatively narrow range of 20 and 25 mgm.-equivalents the development of osteodystrophic diseases follows. Magnesium should not exceed a third of the calcium content of the feed. It is not clear from Marek's work whether the above is generally true or only when vitamin D is present in deficient quantities. The above conception is clearly a modification of the Calcium-phosphorus ratio and does not make allowance for the presence of insufficient amounts of Ca and P when the difference referred to above, i.e. Erdalkali-Alkalizität (E.A.) of Marek, can be made to lie within the limits laid down by him. It must be admitted, however, that although an E.A. of 20-25 is regarded as normal by Marek he reports several cases of osteodystrophic disease in pigs on rations whose Ca, P, Mg contents gave normal values and again several animals remained healthy when the E.A. was distinctly abnormal. For instance pig No. 6 receiving daily per 10 Kg. body-weight on an average 3·435 gms. CaO and 5·591 P_2O_5 showing a strongly negative Erdalkali-Alkalizität remained healthy after 115 days in the experiment. Pig No. 27 received daily 3·576 gms. CaO and 1·9 gms. P_2O_5 per 10 Kg. bodyweight with an E.A. of +21·08 and developed severe rickets after 58 days in the experiment. Pigs No. 32, 33, 35, 39, 40 and 41 all remained healthy and with the exception of the last two received less than 1 gm. CaO per 10 Kg. liveweight but were given a supply of vitamin D by irradiation of the feed or in the feed; the E.A. ranged from slightly to strongly negative. Apparently the limits for E.A. hold good only when vitamin D is present in deficient amounts. If so, then it is not clear why pigs No. 27 and 28 should have developed severe rickets on an intake per 10 Kg. liveweight of approximately 3·5 gms. CaO and 1·8 gms. P_2O_5 with an E.A. of about 21 mgm. equivalents. In short, it would seem that, like the Ca : P ratio, Marek's Erdalkali-Alkalizität provides an inadequate basis for a discussion of the occurrence of osteodystrophic disease in pigs. If the intake of Ca or P, entirely apart from the ratio in which they occur or from the E.A. of the ration, can be made to determine whether or not bone disease develops, even if the animal can be made more sensitive to borderline intakes of Ca and P by altering the ratio, then surely the occurrence of osteodystrophic disease should invariably be related to the intake of Ca and P in the first instance unless of course the investigations are carried out under conditions of vitamin D deficiency when, generally speaking, the animal remains healthy within comparatively narrow limits of Ca : P intake, calcium phosphorus ratio or Erdalkali-Alkalizität. Abundant proof is available for the latter statement as a consideration of the work of Marek on E.A. and those of many investigators of Ca : P ratio will indicate (Shohl and Wohlbach 1936; Bethke and associates 1932, 2933; Dunlop 1935, etc.). But the occurrence of disease under conditions of vitamin D deficiency is the result of at least two factors, viz. abnormal ratio or intake of Ca and P or E.A. on the one hand and deficient vitamin D on the other. If however the latter factor be supplied the relation between abnormal Ca P metabolism and disease is a direct one which justifies investigation especially as this problem of abnormal Ca P metabolism, frequently caused by P deficiency, is unassociated with vitamin D deficiency in most sub-tropical countries.

An attempt was made in the preliminary experiments to be reported in this publication to study the effect on pigs of straight P or calcium deficiencies or both, while related factors such as E.A. and ratios of Ca to P naturally had to be considered.

EXPERIMENTAL DETAIL.

Uniform Large White piglets bred for experimental purposes at this Institute were selected from the available stock and divided into pairs. Individual feeding was practised for which purpose it was found best to run the pairs together and place each of a pair in a separate pen for feeding purposes from 12 noon until the following morning when the pair mates were let out into the adjoining common pen which was unprotected from weather conditions and hence afforded exposure of the pigs to the sun. No bedding was provided except for a short period before artificial heating of the piggeries had been installed when begasse or coarse fibrous sugar cane left after the cane sugar had been extracted, was supplied. For the rest a wooden board was placed in each pen on the concrete floor and the piglets generally accepted these as their beds without difficulty.

Water was always available in the common pen of each pair of piglets and by moistening the mash and feeding at maximum intake, but reducing feed whenever any was left over, food consumption was easily controlled and kept at maximum intake for each pair. All the pens were scrubbed and washed with water under pressure from a hose daily. This routine procedure was practised throughout the course of the experiments and left the animal undisturbed throughout the day and night except at feeding and washing time.

The piglets were weighed weekly, inspected daily for clinical symptoms of disease and blood was drawn and analysed for Ca, P and phosphatase at irregular intervals when technical assistance for the analyses was available; food consumption was recorded daily. Portions of the chondro-costal junction of the ribs were removed under anaesthesia from some of the piglets periodically for microscopical examination while others were killed at certain stages in the experiments for bone study—microscopical, physical and chemical. In some cases X-ray photographs were taken of the front legs of the animals which were placed under anaesthesia for this purpose.

Experiment I.

Ration Deficient in P but Adequate in Other Respects.

Pigs Nos. 987 and 993 aged approximately 6 months were given the following basal ration daily: 900 gms. maize-samp-meat meal mixture containing 10 per cent. of washed meat meal; 100 gms. green feed and 100 ml. fresh milk.

The meat meal contained approximately 80 per cent. protein and was washed twice in a very dilute HCl solution to reduce its mineral content and then with water several times to remove the acid. The washed product contained 0·1 per cent. P and ·06 per cent Ca. The milk was given after diluting it with 300 ml. of water and was immediately consumed. The samp-meat meal mixture was fed dry, while the green feed was placed alongside the trough on the concrete floor. $CaCO_3$ was added to the milk and water and well stirred in order to regulate the Ca intake according to the requirements of the experiment. As the appetite of the animals improved with growth the quantity of the samp-meat meal mixture was increased, the rest of the ration remaining constant.

The average daily intake of P and Ca throughout the course of the experiment was ·8 gms. and 6·0 gms. respectively with a ratio of Ca : P of 7·5 : 1.

The control animals, Nos. 989 and 990, were kept on the same basal ration except that unwashed meat meal was used instead of the washed product. Ca and P were added as $CaCO_3$ and Na_2 HPO_4 to ensure an average daily intake of Ca and P of 6·0 gms. and 3·0 gms. respectively; Ca : P, 2·0 : 1.

The experiment began in January, 1934. In March Nos. 987 and 993 on the P-deficient ration began to show signs of poor appetite while the food consumption of the control pair continued to increase. In May five months after the beginning of the experiment, each pig of the control pair was consuming 1,800 gms. samp-meat meal mixture, while Nos. 987 and 993 ate daily only 600 gms. and at the conclusion of the experiment in October the daily feed consumption of the remaining control and remaining P-deficient animal was 2,400 and 400 gms. respectively.

Pigs Nos. 987 and 993 showed signs of stiffness from June onwards but apart from that and, naturally, poor growth and condition, both lasted well. No. 987 was killed for bone study on 1.9.1934 and No. 993 2½ months afterwards on 20.11.1934. A control animal was killed on each of the two dates for purposes of comparison.

The weekly body weights of the two pairs of pigs are given in Figure I.

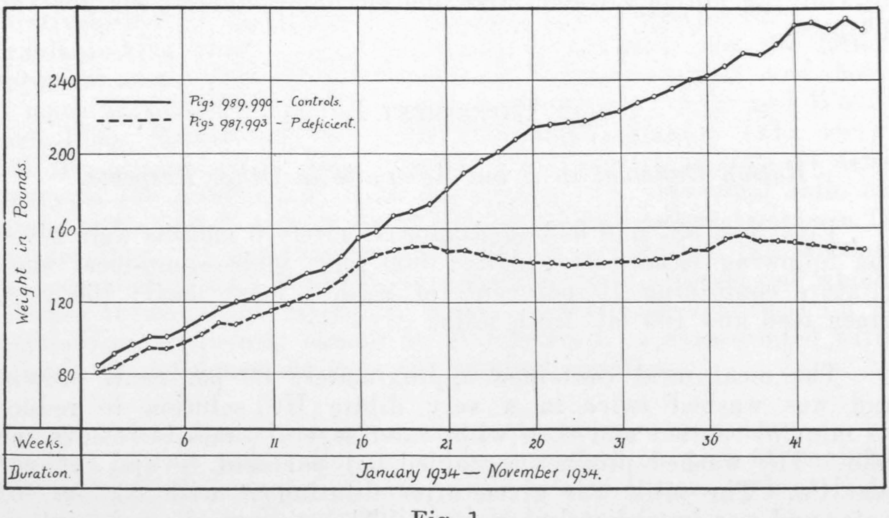

Fig. 1.

Weight increase appeared to be similar for the first five months of the experiment, i.e. until approximately 140 lb. was reached. From then onwards until the end of the experiment the pair on P-deficient diet showed no further increase, while the control pair continued to grow normally. The death of one pig in each group in September did not influence the increase in weight of the remaining pigs.

Inorganic phosphorus was determined in the blood in April, i.e. four months after the beginning of the experiment and the values obtained for the P-deficient group, viz. 3·6 and 3·7 mg P per 100 ml. blood suggest P deficiency while normal values of 8·8 and 9·0 mgm. were obtained for the two animals in the control group. The Ca content of the blood was determined only once during the course of the experiment, viz. in April when all the four values registered were approximately 10 mgm. Ca per 100 c.c. blood.

A femur of each pig was analysed at the end of the experiment and the results are shown in Table I.

TABLE I.—*On Green Weight.*

Nos.	Experimental details.	Green weight.	% fat and water.	% dry fat free bone.	Specific gravity.	% ash in dry fat free bone.
989	normal............	278·3	52·2	47·8	1·13	62·5
990	normal............	380·0	51·6	48·8	1·07	63·1
987	P. low.............	236·7	63·5	36·3	0·97	57·4
993	P. low.............	261·0	63·7	37·3	1·0	54·4

It will be noticed from the results given in the table that the quantity of bone material in the femurs of Nos. 987 and 993 is considerably less than that in the bones of the control group, viz. 36·8 per cent. as against 48·1 per cent. Furthermore the percentage ash, calculated on the fat free bone weight is only 55·7 in the femurs of the P-deficient group and 62·8 in the control group. There can be no doubt that the bones of the former group were abnormal.

Sections of the ribs were examined microscopically and both animals were found to show rachitic lesions. Pig No. 993 which remained almost three months longer in the experiment than No. 987 showed lesions of marked rickets while slight rickets was diagnosed in the case of the group mate No. 987.

From a consideration of the data presented there can be no doubt that the ·8 gm. P supplied in the rations of pigs Nos. 987 and 993 was insufficient for normal growth. Increase in weight took place for the first 5 months of the experiment while the skeletal reserves of the pigs lasted and thereafter the bodyweight remained practically constant. Not only was growth absent but rachitic lesions developed due to the phosphorus deficiency. It is surprising that the animals lasted the full time of the experiment, which was probably due to the fact that they were 6 months old at the beginning and had built up a considerable mineral reserve in their skeleton. Hence it was decided to determine the effect of P deficiency on younger pigs.

Four 8-weeks-old piglets were accordingly selected. A difference in the method of feeding was introduced into this trial; the minerals were added to the samp of the group and mixed in bulk at the beginning of the experiment and were not fed in the milk. It was also thought advisable to introduce the vitamin D factor into this experiment. Hence in every group one pair of piglets was kept in semi-darkness and the other pair on the same ration but given free access to light.

The four control piglets Nos. 1133, 1143, 1138 and 1140 of which the latter two were kept in semi-darkness were given the following ration:—

100 ml. milk.
100 gms. green feed.
Mash according to appetite.

The mash consisted of—

90 parts maize samp.
10 parts high protein meat meal,
2·5 parts $Na_2\ HPO_4$ (19 per cent. P).
2·2 parts $CaCO_3$ (40 per cent. CaO).
1 part NaCl.

The Ca and P content of the mash was 1·0 and ·55 per cent. respectively.

Piglets Nos. 1135, 1144, 1131 and 1137 of which the latter two were kept in semi-darkness were given essentially the same rations as the controls, but the P intake was reduced to a minimum by omitting the $Na_2\ HPO_4$ from the ration and the effect of the P deficiency so created was made more severe by increasing the $CaCO_3$. The ration consisted of the following:—

100 ml. milk.

100 gms. green feed.

The mash which was fed according to appetite consisted of—

90 parts maize samp.

10 parts high protein meat meal.

4·9 gms. $CaCO_3$.

1 part NaCl.

The P and Ca content of the mash was ·709 and 2·0 per cent. respectively and the milk and green feed which were given contained ·21 gms. Ca and ·152 gms. P.

The darkness in the pens of the pigs kept in the dark was so intense that the troughs, etc., could be seen indistinctly and only after accustoming one's eyes to the darkness.

The experiment began in November, 1935; one animal in each group was killed in February, 1936, for bone studies.

A portion of the costo-chondral junction of a rib of the remaining group mates of the P-deficient group was removed in March and again in April, after which P was supplied and rib resections repeated on all the group mates in June when the experiment was discontinued.

Data in regard to the body weights are supplied in the following figures.

Figure 2 represents the comparison of the body weights of the pair of control pigs kept in darkness throughout the experiments and the pair given free access to light.

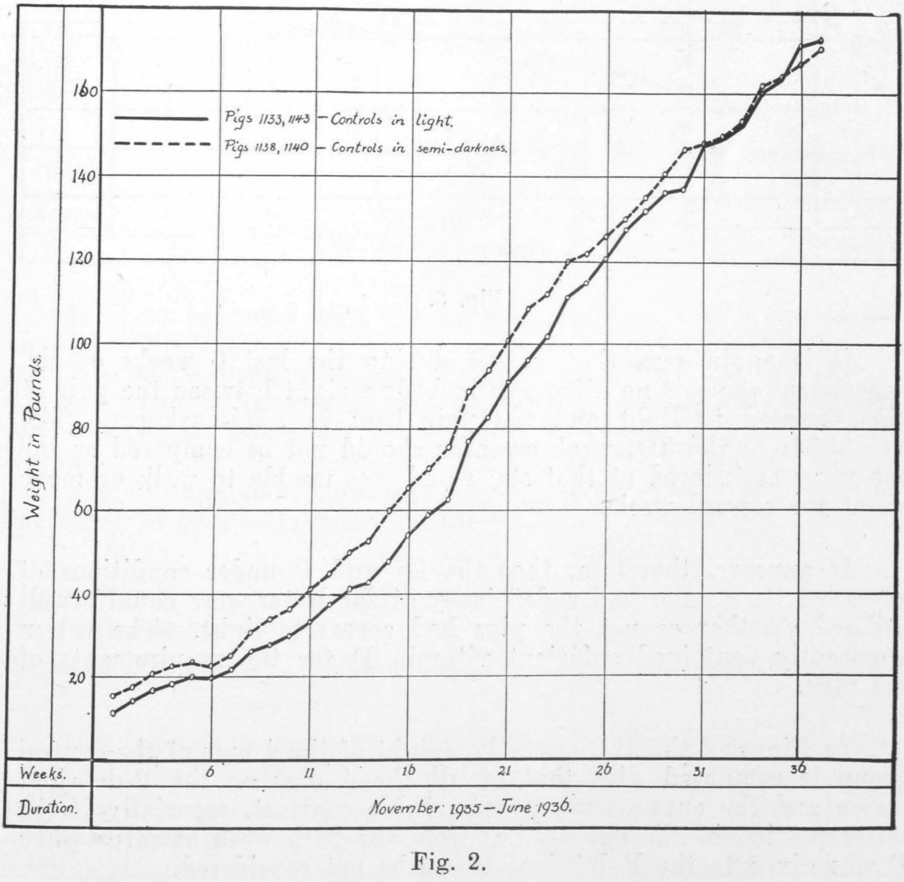

Fig. 2.

It is remarkable that after two months no significant difference existed in the body weight of the two pairs of pigs and that even after eight months the remaining pigs of each pair still showed practically the same increase in body weight. The increase in body weight undoubtedly suggests that the vitamin D content of the feeds used is sufficient for the normal growth of pigs for at least eight months when given adequate rations. This finding is in agreement with that of Huffman et al. (1935) with dairy calves given an adequate supply of hay. It would be interesting to know whether pigs may be reared to maturity practically in the absence of light and further work has been undertaken along these lines.

The body weights of the two pairs of pigs on the P-deficient diet are compared in Figure 3.

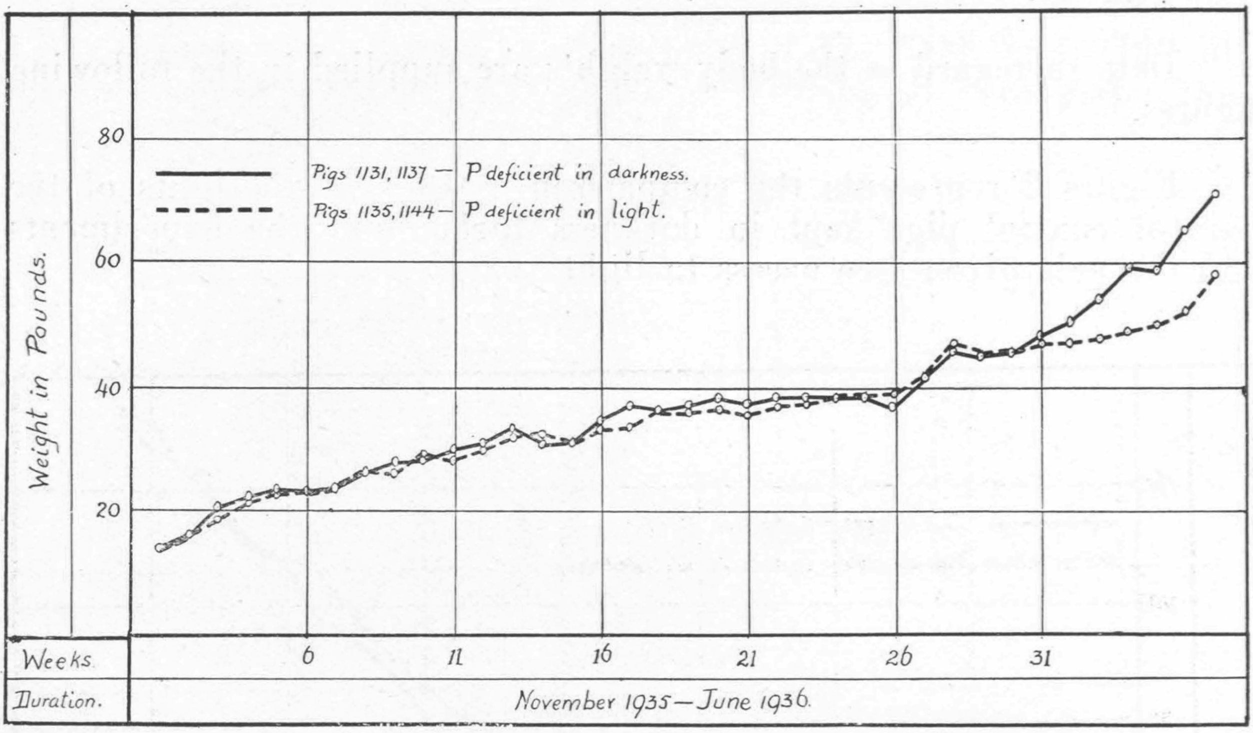

Fig. 3.

It is again seen that except during the last 6 weeks of the experiment there is no difference in body weight between the pair of pigs exposed to light and the pair kept in semi-darkness. The weights from the 31st week onwards should not be compared as the one pig was injured at that stage and was unable to walk or move about for several weeks.

It appears, therefore, that the Ca and P under conditions of excess of the former and a deficiency of the latter were equally well utilized whether or not the pigs had access to light. The ration apparently contained sufficient vitamin D for the requirements of the pigs.

In Figure 4 the average body weight of the 4 pigs of the control group is compared with that of all the 4 pigs on the P-deficient ration and the curves show a remarkable contrast, especially if the latter portion of the curves, i.e. from the 26th week onwards when P was given to the P-deficient group be not considered.

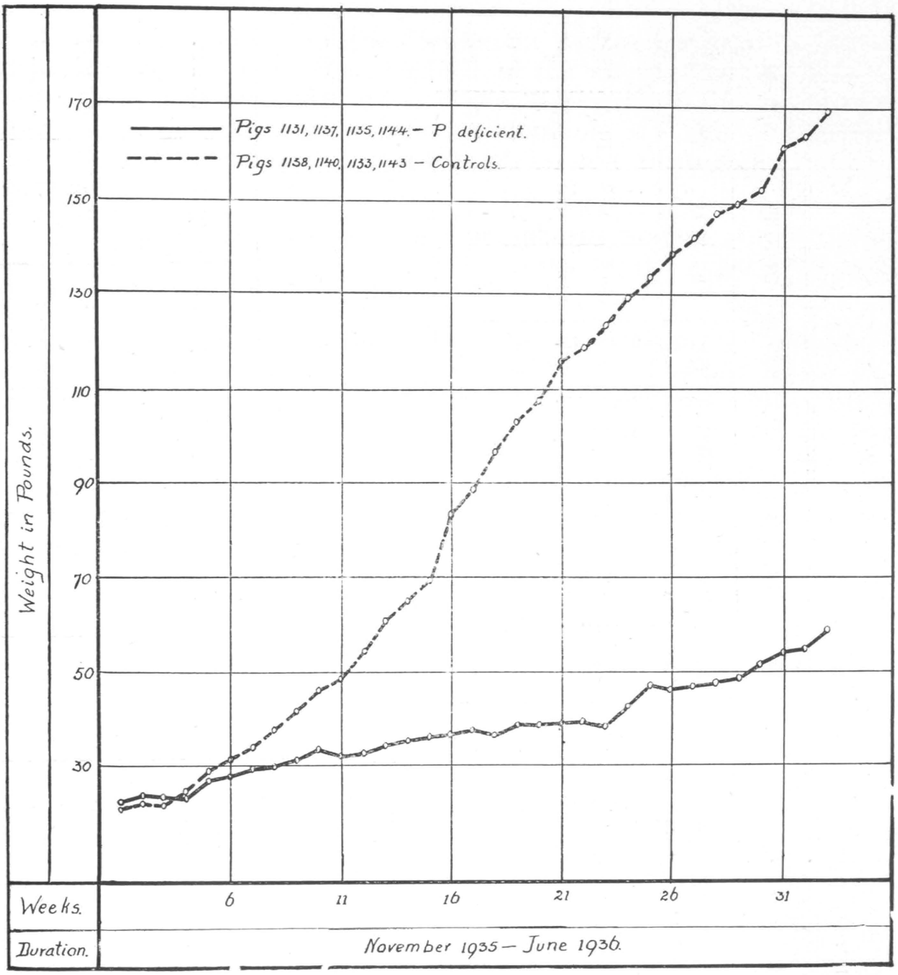

Fig. 4.

It is to be noted that the pigs receiving a P-deficient ration showed a total increase in body weight of only about 20 lb. during the 26 weeks experimental period as against 110 lb. for the control group. Growth in the P-deficient group was almost negligible which was also indicated by the poor food consumption and condition.

The average food consumption per pig per day calculated for the separate months is given in Table II.

TABLE II.

Weights given in gms.

No. of Pig.	Ration.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.
1138, 1140, 1133 and 1143	Controls in sun and darkness	370	750	1,100	1,300	1,500	1,600	1,700	1,750
1131, 1137, 1135 and 1144	P deficient in sun and darkness	400	535	720	460	350	720	740	1,000

TABLE III.

Phosphatase (Bodansky units) and Inorganic Phosphorus (mgms. per 100 ml. serum).

No. of Pig.	Experimental detail.	7/11/35.		4/12/35.		21/1/36.		11/2/36.		23/3/36.		5/5/36.	
		Ph.	IP.	Ph.	IP.	Ph.	IP.	Ph.	IP.	Ph.	IP.	Ph.	IP.
1138	Control pig in darkness	7·2	8·7	5·3	8·0	7·6	9·0	7·0	8·1	9·4	8·0	10·0	8·2
1133	Control pig in light	7·4	8·0	10·7	7·8	12·2	9·2	—	7·8	11·8	8·6	12·1	9·1
1131	P deficient pig in darkness	6·9	7·5	—	6·4	17·5	4·2	18·0	3·0	—	—	—	10·6
1135	P deficient pig in light	—	—	—	—	17·2	3·3	—	—	—	—	9·9	9·1

TABLE IV.

Analysis of femur.

No. of pig.	Experimental details.	Green weight. Gms.	On green weight.		On dry fat free bone.			On ash.		
			% fat and water.	% dry fat free bone.	% ash.	% Ca.	% P.	% Ca.	% P.	Ca : P.
1133	Ca+P+D+	100·1	57·6	42·4	62·3	23·7	11·6	38·1	18·6	2·05 : 1
1140	Ca+P+D−	106·2	58·2	41·8	61·4	22·9	11·3	37·3	18·4	2·03 : 1
1131	Ca+P−D+	63·4	60·1	39·9	48·9	17·7	9·0	36·2	18·4	1·97 : 1

Food consumption in the P-deficient group decreased continually until March when P was added to the ration and food intake more than doubled itself during the following month. During March, i.e. the month of poorest consumption, the control pigs ate more than four times the quantity of mash consumed by the P-deficient pigs.

Phosphatase and the inorganic phosphorus content of the serum were determined six times during the course of the experiment and the results are submitted in Table III.

The inorganic P content of the serum confirms P deficiency which was apparently most severe in February. The last values for Nos. 1131 and 1135 determined after P had been given for several months indicates a sufficiency of P in the ration. In the control group P sufficiency is suggested by the values of P throughout the experiment. It is noteworthy that the phosphatase values of the control pigs kept in the dark appear to have been consistently lower for several months of the experiment than those of the pigs receiving the same feed but allowed free access to sunlight. The normal values for phosphatase were less than 10 Bodansky units and the values of the pigs suffering from P deficiency increased to approximately 17 and were reduced to normal values after the addition of P in March, 1936.

Calcium was not determined except once in the blood of the pig killed in each group during February. The values all range from 9·4 to 10·2 mgm. per 100 c.c. blood and show no group differences. For the phosphatase determinations blood was drawn from the caudal vein and it proved to be difficult to obtain enough blood for both phosphatase and calcium.

A femur of each pig killed in February was taken for analyses and the results are tabulated in Table IV.

Unfortunately the femur of No. 1135 on a P-deficient diet and kept in the dark was not kept for analysis which reduces the value of the analyses given very considerably. It would seem, however, that a marked difference existed between the femur of the control pigs when compared with that of the P-deficient pig. The percentage bone material was less and so was the ash when P was present in insufficient amounts. In regard to the P and Ca content of the ash and the ratio in which they occur more will be said at a later stage in this article when more data can be discussed.

From a consideration of the data given one fact stands out clearly, viz. that P deficiency affected the pigs detrimentally although the extent to which that happened does not seem to have been influenced by the presence or absence of sunlight. It cannot be said that the control pigs were affected with regard to any of the observations made by the presence or absence of light. It would be interesting therefore to mention how the pigs reacted clinically to the conditions of the experiment.

The experiment, as stated, was begun in November, 1935, and except for the poor appetites displayed by the P-deficient groups no difference was noticed between the P-deficient groups and the controls until the end of December. Naturally the former groups appeared less satisfied and less lively than the latter. During the first week of January both the P-deficient groups appeared to be less inclined to move about and even suggested stiffness.

The hocks seemed to sag as if the weights of the bodies were too great to be held up properly by the legs. Towards the end of January No. 1137 on the P-deficient diet in the dark was slightly but decidedly stiff. On 21.1.36 this animal seemed to be in pain and so disinclined to walk or stand that it sat on its haunches most of the time. Two days afterwards all four pigs on the P-deficient ration were decidedly stiff, showed disinclination to walk and showed sagging of the hocks. No. 1135 died during the night of 24.1.36 after consuming most of its food. The cause of death did not appear to be associated with P deficiency. The remaining three pigs on the P-low diet (1131 and 1137 in dark and 1144 in light) gradually became worse. From 10.2.36 onwards rising was a supreme effort and was preceded by hard struggling. The hind legs continually slipped under their bodies when rising. On the 21.2.36 when No. 1131 was killed for bone studies all three were very weak in their hindquarters. The thickened joints which had gradually become more pronounced during the last month were very noticeable. The pigs moved from side to side when walking and were weak.

The remaining two pigs, Nos. 1137 and 1144, were given the P-deficient ration for another month, during which period they remained practically constant in weight, underfed and in great pain when forced to move. A portion of the costo-chondral junction of the 6th rib was removed from each pig on 27.3.36 after which they were given a daily supplement of Na_2HPO_4 when they showed an immediate improvement. Ribs were again removed for microscopical examination on the 3/4, 17/4 and 10/6. The animals were then discharged.

During the whole of the experimental period the control animals remained normal. No clinical symptoms were noticed at any stage and the pigs remained lively and healthy. No. 1140 (in dark) and 1133 (in light) were killed for bone study on 21.2.36 and ribs removed from the remaining controls on 10.6.36 at the end of the experiment.

From an examination of the histological sections of the ribs, bone formation was proceeding normally in Nos. 1140 and 1133 when they were killed in February, as was also the case with 1138 and 1143 in June when the resected ribs were examined. In contrast with these findings the rib sections of the pig No. 1131 on the P-deficient ration when killed at the end of February showed abundant microscopic lesions of marked rickets. The bones were soft and easily fractured by applying moderate pressure. The rib of No. 1135 which died in January also showed severe rickets. No. 1137 whose rib was removed in March suffered from florid rickets at that stage—the trabeculae consisting almost entirely of a mass of osteoid. After Na_2HPO_4 had been given to Nos. 1137 and 1144 for 7 days rib sections were again examined and still showed rickets but less severe lesions than those of the previous week. Even after a fortnight a diagnosis of rickets could still be made although the animals were moving about freely and showed very marked improvement. However, they did not seem to be recovering and in June, 1936, three months after the phosphate supplement had been given they appeared stunted and the legs still decidedly abnormal although microscopical bone sections showed normal bone formation and certainly no suggestion of rickets. They moved about freely and easily and their appetites were excellent.

Experiment II.

Ration Deficient in Ca but Adequate in Other Respects.

Pigs Nos. 983 and 978 aged approximately 6 months, like the first pair described in Experiment I, were used and given the same basal ration as in that case. The treatment was the same and both experiments were conducted concurrently.

The daily Ca intake increased during the course of the experiment from 0·45 gms. at the beginning in January, 1934, when food consumption was low to 1·2 in May when almost 2 Kg. of mash was consumed per head daily and never at any time exceeding this point until the conclusion of the experiment in November, 1934. The ratio of Ca : P was kept constant at 1 : 10.

Nos. 989 and 990 receiving on an average 6·0 gms. Ca and 3 gms. P daily as described in Experiment I, are regarded as the controls of the pigs in this experiment and the results will be presented accordingly.

During the second month of the experiment both pigs on the Ca-low ration (Nos. 983 and 978) appeared to be slightly stiff in the hindquarters. The quantity of feed consumed daily increased from 900 gms. at the beginning of the experiment in January to 1,700 gms. in April and then gradually decreased to 800 in August and showed a slight increase towards the end.

In August No. 978 showed digestive disturbances and refused its feed altogether after a week. It died on 27.8.34. The experimental mate lasted until the end of the experiment.

The weekly body weights are presented in Figure 5.

The average body weight of the group receiving low Ca did not differ significantly from that of the control group during the first 7 months of the experiment but after that period the difference in weight between the groups became more pronounced. Still, the Ca-low group continued to increase gradually in weight.

Blood analysis for Ca and P revealed no difference between the groups in March when the determinations were made.

The results of the analysis of the femur of pig No. 983 is given in Table V. The control pigs are included in the table for comparison; the femur of No. 978 was not analysed as this pig died during the course of the experiment and the results would therefore not be comparative.

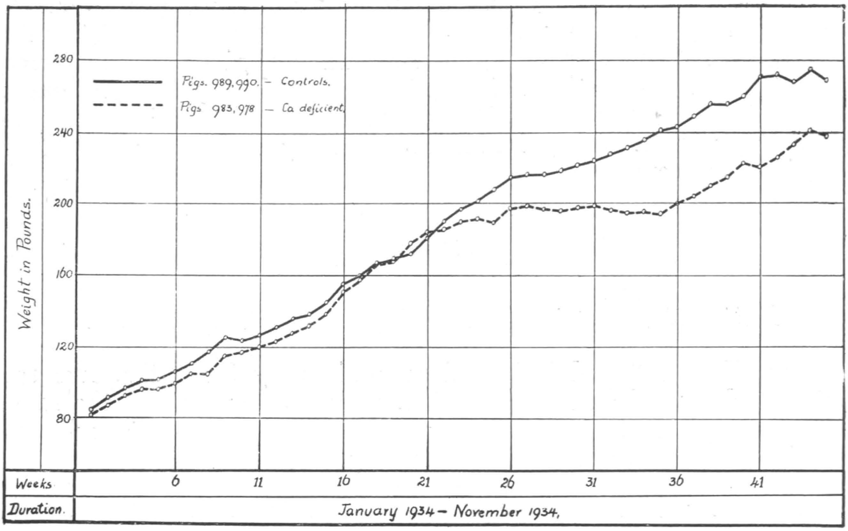

Fig. 5.

Table V.

On Green Weight.

Nos.	Experimental details.	Green weight.	% fat and water.	% dry fat free bone.	Specific gravity.	% ash in dry fat free bone.
989	Normal............	278·3	52·2	47·8	1·13	62·5
990	Normal............	380·0	51·6	48·8	1·07	63·1
983	Low Ca............	267·5	63·1	36·9	1·00	59·9

It would seem from the values given that the femur of No. 983 receiving low Ca in its diet contained less bone material than the femur of the controls but the number of determinations is too limited to justify any serious consideration of the values.

Sections of the ribs of both animals were examined microscopically. The sections of No. 978 which had suffered from digestive disturbances prior to death showed extensive lesions of esteoporosis and atrophy and No. 983 osteoporosis and slight atrophy. The important fact of the diagnosis was that neither pig showed rickets microscopically and that the bones were not normal.

Summarizing the conclusion that the quantity of Ca present in the diet viz., approximately 1 gram per daily ration was insufficient for normal growth and bone formation, is justified. Apparently growth continued normally for the first 6 months of the experiment and only after that period could the skeletal reserves of lime no longer supply the body requirements of calcium, when growth began to suffer. The pigs were 6 months old at the beginning of the experiment which may account for the fact that they lasted well in spite of their low Ca intake. It was therefore decided to determine the effect of a low Ca intake upon younger pigs.

Four eight-weeks-old pigs were therefore selected and placed on the following daily ration:—

100 ml. milk.

100 gms. green feed.

Mash according to appetite.

The mash consisted of—

90 parts maize samp,

10 parts high protein meat meal.

5·8 parts Na_2HPO_4 (40 per cent. P_2O_5), and

1 part NaCl.

The P and Ca content of the mash was ·98 and ·11 per cent. respectively, while the daily milk and green feed supplied ·21 gms. Ca and ·152 gms. P.

This experiment was conducted concurrently with the second part of Experiment I and the same four animals, viz. No. 1138, 1140, 1133 and 1143 acted as controls to both experiments. As already stated all four animals were given the same normal ration and the only difference in management was that the latter two animals were allowed free access to direct sunlight when they were not feeding while the two former ones were always in semi-darkness.

The pigs receiving low Ca in their diets were also divided into two pairs. The one pair, viz. Nos. 1130 and 1145 was kept in semi-darkness whereas Nos. 1136 and 1142, receiving the same ration and treatment, were given free access to direct sunlight.

The experiment began in November, 1935, and continued for approximately eight months.

The average body weight of Nos. 1130 and 1145 on low Ca and in darkness is compared graphically in Figure 6 with that of Nos. 1136 and 1142 which had received the same ration but were allowed free access to light.

The body weights of the two pairs of pigs did not differ significantly during the first 22 weeks of the experiment, i.e. until March, 1935. Nos. 1136 and 1145 were killed for bone studies at the end of February and the remaining pig No. 1130 which was allowed free access to light developed severe diarrhoea shortly afterwards from which it suffered acutely for approximately two months and then died. Hence the body weights are not comparable after the 22nd week of the experiment. Prior to that period it seems that the body weights of the pigs kept on a Ca-deficient diet were not affected by the presence or absence of light.

The average body weight of pigs Nos. 1130 and 1145 on low Ca but kept in semi-darkness is compared with that of the corresponding control pair in Figure 7.

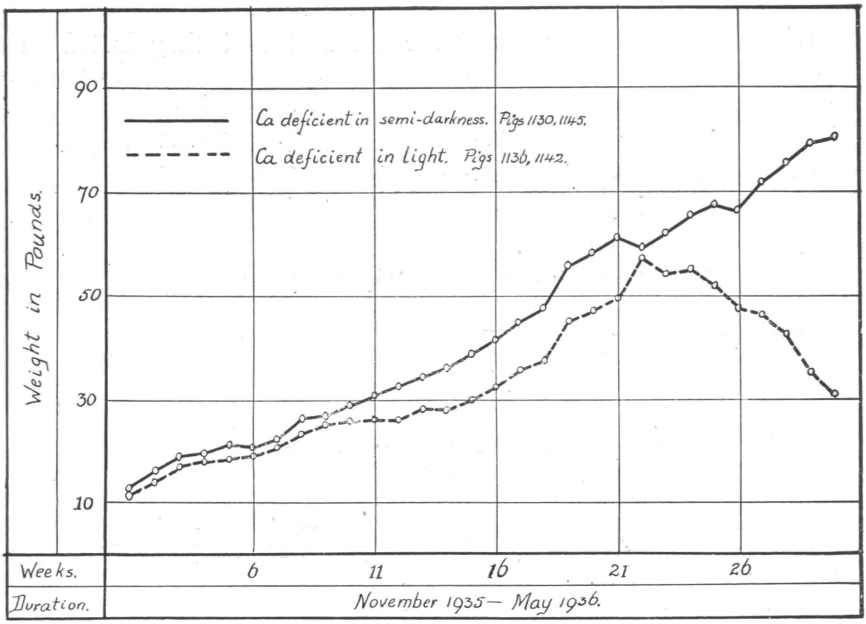
Ca deficient in semi-darkness. Pigs 1130, 1145.
Ca deficient in light. Pigs 1136, 1142.
Weight in Pounds.
90
70
50
30
10
Weeks.
6
11
16
21
26
Duration.
November 1935— May 1936.

Fig. 6.

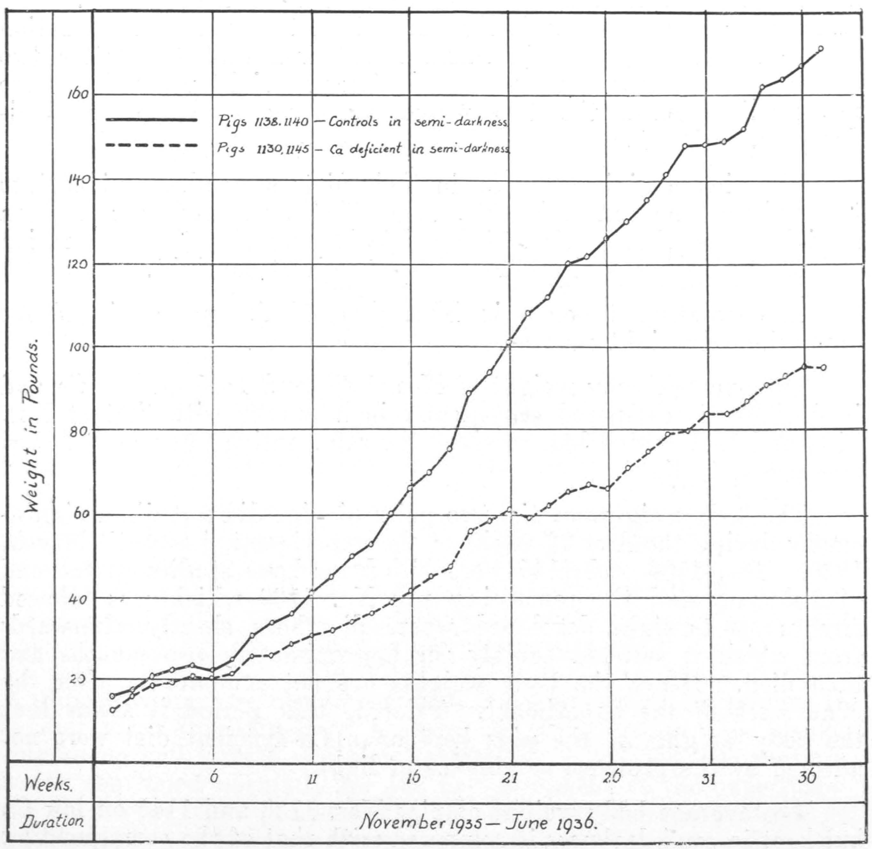
Pigs 1138. 1140 — Controls in semi-darkness
Pigs 1130, 1145 — Ca deficient in semi-darkness
Weight in Pounds.
160
140
120
100
80
60
40
20
Weeks.
6
11
16
21
26
31
36
Duration.
November 1935— June 1936.

Fig. 7.

Low Ca in the diet affected the body weight almost from the beginning of the experiment and was responsible for the difference of about 70 lb. at its conclusion eight months afterwards. In spite of a low Ca intake, however, a gradual increase in weight continued throughout the course of the experiment; there was no cessation of growth as in the case of the P-deficient pigs.

The average food consumption, which is given in Table VI, of the pigs on a Ca-low diet compared with that of the controls confirms the observations made in regard to the increase in body weight.

TABLE VI.

Daily Intake of Mash per Pig: Weights given in gms.

No. of Pig.	Ration.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.
1138, 1140, 1133 and 1143	Controls in sun and semi-darkness	370	750	1,100	1,300	1,500	1,600	1,700	1,750
1130, 1145, 1136 and 1142	Ca deficient diet in sun and semi-darkness	370	470	680	1,000	750	900	900	1,046

Food consumption was affected detrimentally by low Ca in the diet and more so as the experiment continued.

The values of phosphatase and the inorganic phosphorus content of the serum are given in Table 7.

It seems that neither phosphatase nor the inorganic phosphorus content of the serum was affected by the Ca content of the ration but the number of determinations is too limited to justify any conclusions being drawn in regard to phosphatase.

Calcium determined in the blood of the pigs of each pair that was killed in February revealed no change and was still apparently normal.

The femurs of the two pigs killed in February were analysed and the results presented in Table VIII together with the values of the control pigs.

The percentage bone material was considerably reduced in the green bone of the animals receiving low Ca in their rations. The ash content of the dry fat free bone was also significantly lower than that of the normal bones, although the percentage Ca and P of the ash remained practically constant.

Rib sections of the two pigs on a low Ca diet Nos. 1145 and 1136 killed in February, four months after the beginning of the experiment, were examined microscopically and showed osteoporosis but no lesions of rickets. It will be remembered that No. 1145 was kept in semi-darkness during the experiment and that No. 1136

TABLE VII.

Phosphatase (Bodansky Units) and Inorganic Phosphorus (I.P.) in mgms. per 100 *c.c. Serum.*

No. of Pig.	Experimental detail.	7/11/35.		4/12/35.		21/1/36.		11/2/36.		23/3/36.		5/5/36.	
		Ph.	I.P.	Ph.	I.P.	Ph.	I.P.	Ph.	I.P.	Ph.	I.P.	Ph.	I.P.
1138	Control pig in darkness	7·2	8·7	5·3	8·0	7·6	9·0	7·0	8·1	9·4	8·0	10·0	8·2
1133	Control pig in light	7·4	8·0	10·7	7·8	12·2	9·2	—	7·8	11·8	8·6	12·1	9·1
1145	Ca def. pig in darkness	6·8	9·2	—	—	8·0	6·5	—	10·3	—	—	—	11·1
1142	Ca def. pig in light	7·7	7·7	9·1	8·2	8·2	6·3	4·9	10·6	—	—	—	7·6

TABLE VIII.

No. of pig.	Experimental details.	Green weight. Gms.	On green weight.		On dry fat free bone.			On ash.		
			% fat and water.	% dry fat free bone.	% ash.	% Ca.	% P.	% Ca.	% P.	Ca : P.
1140	Control in darkness	106·2	58·2	41·8	61·4	22·9	11·3	37·3	18·4	2·03 : 1
1133	Control in light	100·1	57·6	42·4	62·3	23·7	11·6	38·1	18·6	2·05 : 1
1145	Ca low in darkness	76·8	68·6	31·4	55·8	20·8	10·3	37·3	18·5	2·02 : 1
1136	Ca low in light	57·0	69·4	30·7	55·3	19·9	10·4	36·0	18·8	1·96 : 1

had free access to light. Ribs were removed from the two remaining pigs on low Ca in March and again in June at the conclusion of the experiment. The sections of the ribs of both pigs showed marked bone atrophy or osteoporosis but no other lesions of osteodystrophic disease.

From a consideration of the data presented it is clear that ·11 per cent. of Ca in the mash of growing pigs was insufficient for normal growth and bone formation when 2·25 per cent. of P was present. It seems, however, that the deficiency was not acute enough to produce clinical symptoms of bone disease during the eight months that the experiment lasted. None of the four pigs on the Ca-low diet whether free access was given to light or not, showed signs of stiffness or any clinical symptoms which could be associated with calcium deficiency. The ration consisted of 100 ml. milk and 100 gms. green feed daily in addition to the mash.

A pair of pigs of the same age, viz, eight weeks, as those used in Experiment 5 was placed on a calcium-low ration when the latter experiment was conducted and the results obtained might briefly be reported here.

The daily ration given consisted of 100 ml. milk, 100 gms. green feed and mash consisting of 94 parts maize samp, 6 parts blood meal and 1 part of salt according to appetite. Na_2HPO_4 was added daily to the ration to ensure an intake of ·5 gms. CaO, 10·5 gms. P_2O_5 and a ratio of 1:21.

Both pigs (Nos. 1075 and 1089) showed poor appetite almost from the beginning of the experiment. No. 1089 developed severe diarrhoea and died of acute enteritis shortly after the beginning of the experiment. No. 1075 showed no digestive disturbances but did not relish its food. Approximately two months after the beginning of the experiment this pig showed unmistakable symptoms of stiffness and weakness. The hindquarters were apparently unable to support the body and especially on turning the pig would go down on its haunches and remain in that position for a few minutes. The pig appeared to improve slightly during the following week but on being driven to the scale for weighing it suddenly became lame and was quite unable to rise for several days. It ate its food in a lying position and was unable to change its position without assistance. During the remaining six weeks in the experiment this pig on several occasions developed lameness and subsequent inability to move around. It was always stiff when able to move about. The pig was killed on 13.8.35, i.e. after 105 days in the experiment for histological study of the bones. Numerous callusses were present in the ribs and the bones were obviously soft and brittle. Histological sections of the ribs showed marked atrophy and incipient osteodystrophia fibrosa but no rickets. The radiographs taken after the pig had been in the experiment for about two months also showed marked atrophy.

Only one pig was left on the Ca-low diet and the development of incipient osteofibrosis cannot therefore be ascribed definitely to low Ca but in the light of the results obtained with cattle, sheep and goats on Ca-deficient diets the possibility of a Ca-low diet leading to osteodystiophia fibrosa in pigs is tentatively suggested. Further work with pigs along these lines will be reported at a later stage.

EXPERIMENT III.

This Experiment was conducted concurrently with Experiments I and II and was an Attempt to Determine the Effects of a Combined Deficiency of Ca. and P.

It was realized that this experiment would throw further light on the effect of the ratio of Ca : P of the rations used in the first two experiments. Hence the P and Ca intakes were made to agree with those of Experiments I and II as the table given below indicates. A comprehensive table giving full details of all the experiments mentioned in this report is given on page 161.

Experiment.	Ca intake.	P intake.	Ratio Ca : P.
I. (6 months old pigs).............	6 gms.........	·6 gms........	10 : 1
I. (8 weeks old pigs)...............	2 per cent......	·11 per cent.....	10·9 : 1
II. (6 months old pigs).............	1 gm..........	10 gms........	1 : 10
II. (8 weeks old pigs)...............	·11 per cent.....	·98 per cent.....	1 : 8·9
III. (6 months old pigs).............	1 gm..........	·6 gm.........	1·7 : 1
III. (8 weeks old pigs)...............	·11 per cent.....	·11 per cent.....	1 : 1

It must be noted that, although the ratio of Ca : P in Experiment III was more favourable than that of Experiment I or II a double deficiency existed in Experiment III.

Two six-months-old pigs Nos. 988 and 994 were given the same basal ration as that used for the pigs of this age in Experiments I and II, viz. :—

100 gms. green feed.

100 ml, milk.

Maize samp plus meatmeal mixed in the proportions of 90 parts to 10 respectively.

The experiment began in January, 1934, and both animals lasted the full period of the experiment, i.e. until November, 1936. In February No. 988 appeared to be slightly stiff in the front legs for a short period and again showed slight stiffness in August in the hindquarters. Other clinical symptoms were not noticed at any stage in the course of the experiment.

The average body weight of pigs Nos. 988 and 994 is compared graphically with that of the control pair in Figure 8.

It is evident from the graphs that the body weight of the pair of pigs receiving low Ca and P in their diet was practically identical with that of the control pair for the first twenty weeks of the experiment. After that period a difference set in which reached about 50 lb. at the end of the experiment in favour of the control pair.

The inorganic phosphorus content of the blood was found to be 5·7 mg. per 100 ml. which is lower than the control value but appreciably higher than that obtained for the blood of the pigs on the P-deficient ration and excess Ca. Blood Ca was normal.

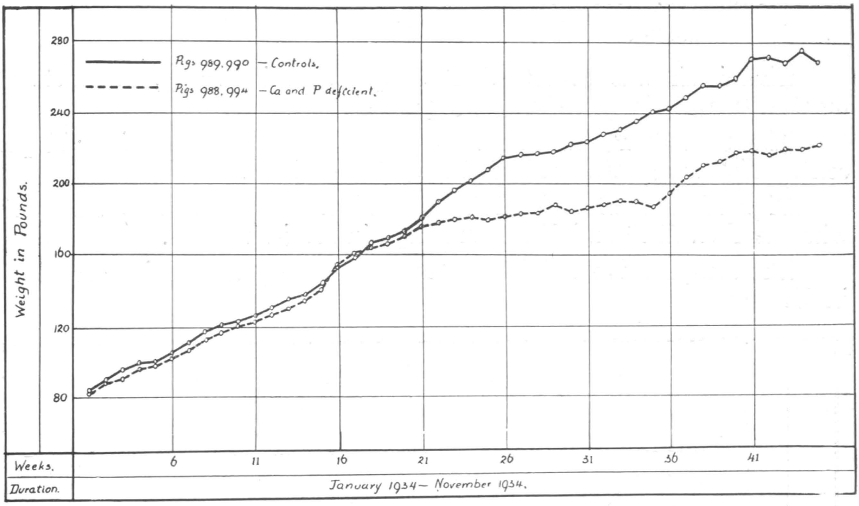

Fig. 8.

The results of the analyses of the femur are given in Table IX.

TABLE IX.

On Green Weight.

Nos.	Experimental details.	Green weight. Gms.	% fat and water.	% dry fat free bone.	Specific gravity.	% ash in dry fat free bone.
989	Normal............	278·3	52·2	47·8	1·13	62·5
990	Normal............	380·0	51·6	48·8	1·07	63·1
988	Low Ca and Low P.	248·8	58·1	41·9	1·00	57·8
994	Low Ca and Low P.	285·0	60·1	39·9	1·02	60·1

The percentage quantity of bone material on the femurs of Nos. 988 and 994 is less than that in the femurs of the controls which would indicate poorer bone formation; as a result the ash percentage of the former pair is also lower.

The rib sections of the Ca and P deficient pair of pigs showed microscopic lesions of extensive osteoporosis and indications of slight rickets; these facts corroborate the conclusions drawn from the bone analyses.

As already stated these pigs were approximately six months old when the experiment started and it was therefore decided to determine the effect of a ration low in both Ca and P on younger pigs.

Accordingly four eight-weeks-old pigs were selected and given the same basal ration as that of the pigs of the same age in Experiments I and II, viz.:—

100 ml. milk.

100 gms. green feed.

Mash according to appetite.

The mash was composed of 90 parts maize samp, 10 parts of meatmeal high in protein and 1 part of salt.

The Ca and P content of the mash was the same, viz. ·11 per cent. No extra mineral supplement was given.

Pigs Nos. 1132 and 1141 were given free access to sunlight, whereas Nos. 1139 and 1129 were kept in semi-darkness.

The comparative weight curves of the two pairs of pigs are given in Figure 9.

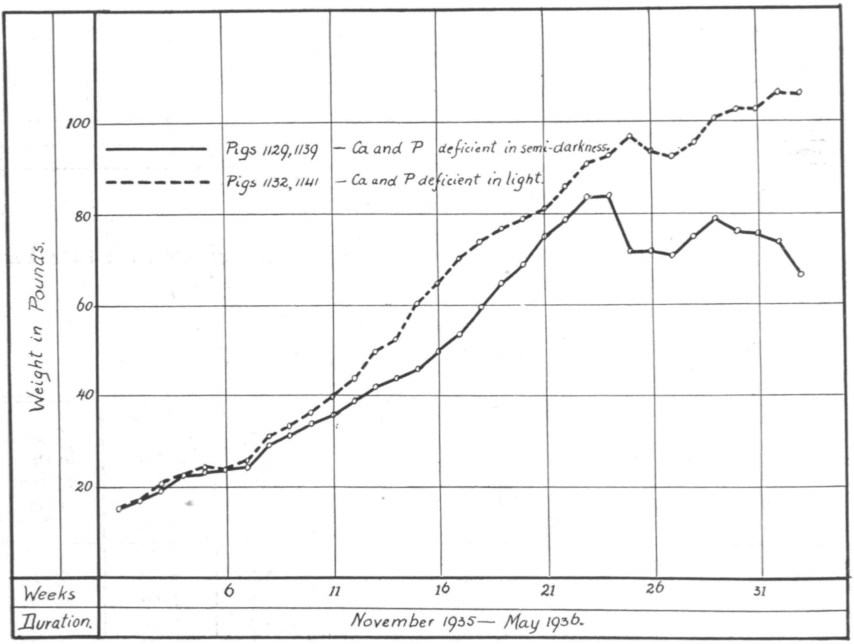

Fig. 9.

It is doubtful whether light had any effect on the body weights of the pigs before March, 1936, i.e. about five months after the beginning of the experiment. One pig of each pair was killed for bone studies towards the end of February and it was after that period that the body weights of the remaining pigs began to differ considerably. No. 1139 kept in the dark actually lost 20 lb. during the following nine weeks, while No. 1141, which was allowed free access to sunlight increased about 20 lb.

It would seem that the effect of the absence of light was being felt during this period. However, as only two pigs could be compared this conclusion must be drawn with reservation until further work has been done.

If the body weight of the pair of pigs Nos. 1132 and 1141 be compared with that of the control pair which was also allowed access to sunlight it is seen that low Ca and P began to affect the body weights of the pigs adversely about twenty weeks after the beginning of the experiment. Weight increase was poor after that period and a difference of 70 lb. was registered between the two remaining pigs at the conclusion of the experiment. (Fig. 10.)

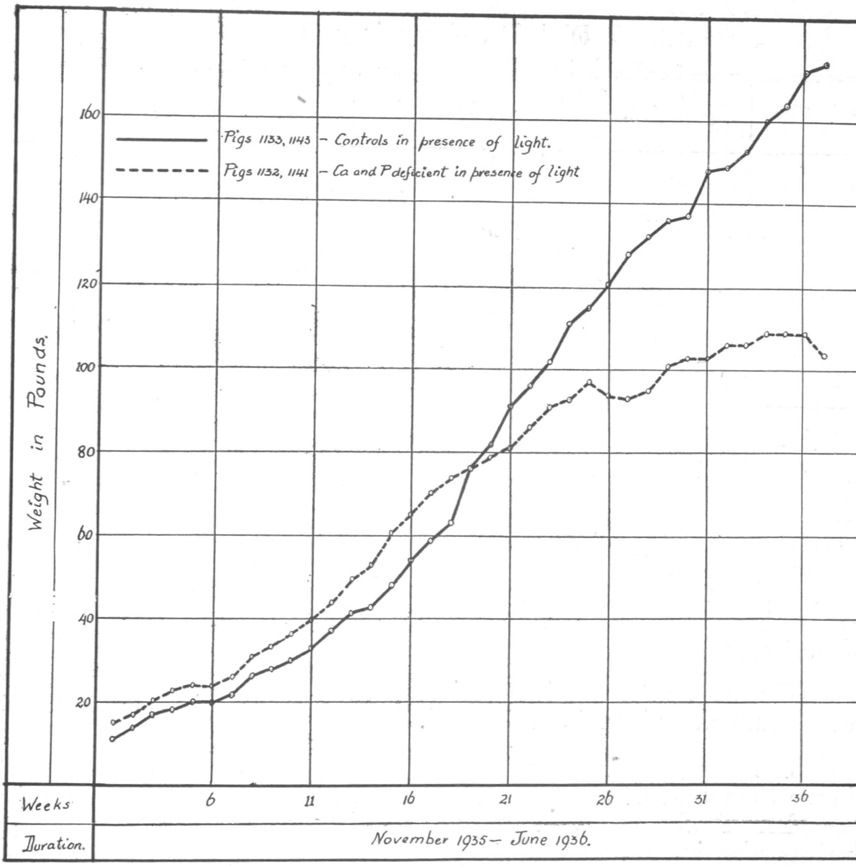

Fig. 10.

It is well to remember that the increase in weight of the pigs receiving a diet low in both Ca and P was by no means as poor as that registered in Experiment I, when P was deficient and Ca present in excess or as that given in Experiment II when Ca was deficient and P present in excess. The explanation of these apparently contradictory results, viz. that a deficiency of both Ca and P should produce better growth than a deficiency of either Ca or P, lies, of course, in the generally accepted view that the beneficial ratio in the first instance reduced the effects of the deficiencies while the abnormal ratio when either Ca or P was present in deficient amounts aggravated the effects of the deficiency to such an extent that it was actually more severely felt than when both elements were deficient.

Food consumption like the body weights shows reduction when compared with that of the controls but not to the same extent as when either Ca or P was present in insufficient amounts.

The average food consumption per pig per day during the separate months is given in Table X.

TABLE X.

Weights, Green in gms.

No. of Pig.	Ration.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.
1138, 1140, 1133 and 1143	Controls in darkness and sunlight	370	750	1,100	1,300	1,500	1,600	1,700	1,750
1132 and 1141	Ca and P deficient in light	370	713	1,080	1,200	1,000	1,026	1,239	993
1129 and 1139	Ca and P deficient in darkness	370	642	684	931	900	760	596	dead.

The food consumption of the pigs receiving a Ca and P low diet but allowed access to sunlight was not significantly different from that of the controls during the first two months of the experimental period but after that period the difference became increasingly greater. Nos. 1129 and 1139 showed poor consumption of food sooner and were significantly different from their group mates and also from the controls in this respect during the third month of the experiment.

The values for the inorganic P and the phosphatase content of the serum are given in Table XI.

The inorganic P content of the serum of Nos. 1132 and 1129 was lower than that of the controls but the reduction was not as marked as when P alone was deficient (Experiment I). The phosphatase values also indicated poorer calcification when P and Ca were deficient than in the control pigs. The blood Ca values gave an average of 9·5 in February, 1936, which was not significantly different from 9·7 obtained for the controls.

The results of the analysis of the femur are given in Table XII.

The percentage ash calculated on the dry fat free bone of the pigs receiving low P and Ca diets was reduced and considerably so in the case of No. 1129 which remained in semi-darkness during the experiment. The values suggest less bone material when Ca and P were present in insufficient amounts even if the ratio of Ca : P in the diet was a favourable one.

Summarizing the data presented it would appear that the presence of light was responsible for the differences in body weight and food consumption between the two pairs of pigs receiving rations low in both Ca and P, but it should be remembered that a

Table XI.

Phosphatase (Bodansky Units) and Inorganic Phosphorus (I.P.) (mgms. per 100 ml. Serum).

No. of Pig.	Experimental detail.	7/11/35.		4/12/36.		21/1/36.		11/2/36.		23/3/36.		5/5/36.	
		Ph.	I.P.	Ph.	I.P.	Ph.	I.P.	Ph.	I.P.	Ph.	I.P.	Ph.	I.P.
1138	Control pig in darkness	7·2	8·7	5·3	8·0	7·6	9·0	7·0	8·1	9·4	8·0	10·0	8·2
1133	Control pig in light	7·4	8·0	10·7	7·8	12·2	9·2	—	7·8	11·8	8·6	12·1	9·1
1132	Ca and P low (in light)	8·0	—	10·7	—	18·7	6·3	—	4·1	—	—	22·4	5·6
1129	Ca and P low (in darkness)	9·8	7·2	14·1	6·8	15·7	5·5	—	4·1	19·5	6·0	15·8	—

Table XII.

Analysis of femur.

No. of pig.	Experimental details.	Green weight. Gms.	On green weight.		On dry fat free bone.			On ash.		
			% fat and water.	% dry fat free bone.	% ash.	% Ca.	% P.	% Ca.	% P.	Ca : P.
1133	Control in light	100·1	57·6	42·4	62·3	23·7	11·6	38·1	18·6	2·06 : 1
1140	Control in darkness.	106·2	58·2	41·8	61·4	22·9	11·3	37·3	18·4	2·03 : 1
1132	Ca and P low in light	109·0	76·1	23·9	57·9	20·9	10·5	36·1	18·1	2·00 : 1
1129	Ca and P low in darkness	77·4	70·9	29·1	51·7	18·4	9·6	35·6	18·6	1·91 : 1
1139	Ca and P low in darkness	149·4	72·2	27·8	50·9	19·0	9·3	37·3	18·2	2·05 : 1

decided difference between the two groups, as far as body weight was concerned began to show only after one pig had been killed in each group during February, leaving only one pig for comparison. For this reason no conclusions can be drawn and further work has been undertaken to elucidate the point.

It should be pointed out that when either Ca or P was deficient no difference appeared to exist when light was excluded in spite of the abnormal ratio, but growth was considerably slower in these cases than in the pigs receiving a ration deficient in both Ca and P. More rapid growth in the latter case might have been responsible for the differences observed when light was excluded and for the difference in the clinical picture presented by the pigs, but surmise is dangerous at this stage.

No. 1132, allowed access to light and killed in February for bone studies, appeared clinically healthy throughout, with perhaps only a suggestion of being slightly stiff. Its group mate, No. 1141, which remained in the experiment until it was discharged in June, first showed signs of stiffness in March and remained so until the end.

In contrast with the above, the pair on the same ration but kept in semi-darkness fared less well. No. 1128 appeared weak on the legs at the end of January and lay down most of the time when not feeding. Especially the hind legs seemed to be unable to carry the weight of the body. During February it was very stiff and remained down when feeding. It could rise, however, when urged to do so. This pig was killed towards the end of February for bone studies. Its group mate, No. 1139 developed stiffness during February and became very stiff in March. Towards the end of March it had to struggle fiercely when rising. During April it was hardly able to walk after a struggling attempt to rise and stood shivering most of the time. Its condition became worse and the animal showed nervous symptoms in addition. It would scream continuously when approached and seemed helpless to move except with the greatest effort. Its legs were so stiff that it could not take longer steps than a couple of inches at a time. This pig died on 27.5.36 and the bones were collected for analysis. Histological sections of the 3rd rib of No. 1139 were examined microscopically both in March, when a portion of the costochondral junction was removed, and after death in May. In March marked bone atrophy was noticed but although some red osteoid seams were present the amount did not transgress physiological limits and a diagnosis of rickets was not justified. In May the bones still showed marked bone atrophy but no rickets and it would be difficult to compare the difference in degree of bone atrophy between the two pigs without a more detailed microscopical study of all the bones. It would seem, however, that the extra three months in the experiment provided insufficient time for the development of rickets or osteofibrosis under the conditions of Ca and P intake that existed in the experiment.

Nos. 1132 and 1141, kept under the same conditions of Ca and P deficiency as 1139 and 1192 but allowed free access to light, appeared to show better growth, as already stated. The rib sections of No. 1132, killed in February, showed marked bone atrophy and the presence of some red seams of osteoid but hardly rickets. Rib sections cut in June from the experimental mate of No. 1132, viz. 1141, also showed marked atrophy and more osteoid than normal or incipient rickets.

The clinical picture presented by the two pairs of pigs on Ca and P low diets favours the conclusion that the absence of light affected the one pair of pigs detrimentally. The same indication is found by examining the body weights, food consumption and some of the other data. The histological findings bear out the same point, viz. that the pair in the light developed indications of rickets which might follow better growth, while the pair in the dark showed no rickets, but as already stated the evidence is too limited to justify conclusions.

A fact which stands out clearly, however, and which must be emphasised is that Ca deficiency with excess P, and P deficiency with Ca excess, affected the pigs of both the ages used in these experiments more detrimentally as far as all the observations made were concerned than when the ration was deficient in both Ca and P. The abnormal ratio of Ca to P when only one of these constituents was present in insufficient amounts was undoubtedly responsible for the more drastic effects registered. It would appear, however, that rickets which develops rapidly when the P content of the ration is low is not produced as easily in pigs when both Ca and P are low. This observation does not apply to sheep, goats and bovines in which rickets is easily produced when both Ca and P are low in the diet. The possibility remains, however, that reduced quantities of P and Ca will produce rickets in pigs, as it should, in virtue of the low P, even when both are present in deficient amounts and in a favourable ratio.

Furthermore, as the ratio of Ca to P in the feed of pigs kept on rations deficient in one or both of the two constituents was mainly responsible for the differences registered between pigs receiving a normal and abnormal ratio respectively, it was decided to determine the effect of an abnormal ratio of Ca to P when these elements were present in sufficient amounts.

Experiment IV.

The Ration contained Excess of either Ca or P and a Sufficiency of the other Mineral; in Other Respects it was Adequate.

Two pairs of six-months-old pigs were selected and given the same basal ration as that reported in Experiment I for pigs of this age, viz.:—

100 ml. milk,

100 gms. green feed.

Mash according to appetite and consisting of 90 parts maize samp, 10 parts high protein meatmeal and 1 part of salt.

Calcium carbonate and disodium phosphate were added to the milk of the pigs but in such a manner that the one pair, Nos. 995 and 984, received sufficient Ca and excess P. viz. 6 gms. and 8·2 gms. respectively, while the other pair, Nos. 977 and 997, received sufficient P and excess Ca, viz. 3 gms. and 13·7 respectively. The ratios of Ca to P in the feed of the two pairs were 1:1·4 and 1:0·22.

The experiment began in January, 1934, and was concluded approximately nine months afterwards. All the pigs consumed their food at least as well as the controls did and at no stage did any show symptoms of abnormalities.

The curves representing the average body weights of the two pairs of pigs compared with the controls are given in Figure 11.

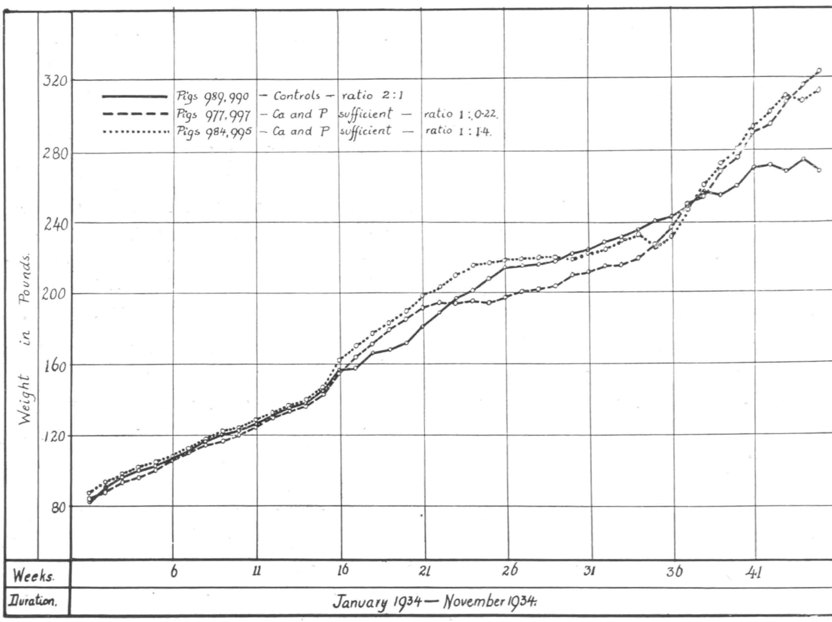

Fig. 11.

The excess of Ca in the feed of the one pair of pigs and the excess P in that of the other were apparently without detrimental effect on the weight increase of the pigs. It was only during the last eight weeks of the experiment, after one pig of each pair had been killed for bone studies, that the remaining experimental pigs appeared to increase more rapidly in weight than the control pig; this result cannot be regarded as significant therefore.

The inorganic P content of the blood determined four months after the beginning of the experiment show no difference between the control group and the one receiving enough P and excess lime. The values are 7·1 and 7·5 mg. P per 100 ml. blood for these two groups respectively. The inorganic P content of the blood of the pair receiving excess P and sufficient lime was 8·9 mgm. p.c. Blood calcium was normal in all three pairs, viz. 10·2 to 10·8 mgm. p.c.

The results of the analyses of the femurs are given in Table XIII.

TABLE XIII.

On Green Weight.

Nos.	Experimental details.	Green weight.	% fat and water.	% dry fat free bone.	Specific gravity.	% ash in dry fat free bone.
989	Control...........	278·3	52·2	47·8	1·13	62·5
990	Control...........	380·0	51·6	48·8	1·07	63·1
995	Suff. Ca excess P...	359·0	49·1	50·9	1·12	64·2
977	Suff. P excess Ca...	370·0	53·0	47·0	1·07	63·4
997	Suff. P excess Ca...	287·8	50·6	49·4	1·05	62·3

It is obvious from the figures given in Table XIII that not one of the values given was affected significantly by excess lime or P in the ration. The femurs of the pigs receiving excess Ca or P did not contain less bone material than those of the controls and as far as could be ascertained the bones were normal.

The histological sections of the ribs examined microscopically revealed no abnormalities.

It would appear therefore that if a sufficieney of P or Ca be given in the diet of six-months-old pigs an excess or Ca or P to the extent of that given in this experiment, viz. almost one and a half times as much P as Ca and in another case three and a half times as much Ca as P is without significant effect on body weight, food consumption, blood and bone analyses. In contrast to the above an abnormal ratio of Ca to P under conditions of Ca or P deficiency had disastrous effects on body weight, food consumption, blood and bone analysis of pigs as against less detrimental effects when the ratio was corrected but the deficiency retained as reported in Experiments I, II, III.

Although the experiment reported here by no means exhausts the study of the effect of abnormal ratios of Ca : P under conditions of a sufficiency of these two minerals it does suggest that an abnormal ratio under these conditions may not be as important as under conditions of a deficiency of P or Ca and work along these lines is being undertaken at present. Furthermore, arising out of the results of this work, it was decided to test the effect of an abnormal Erdalkali-Alkalizität in the ration of pigs under conditions of a sufficiency of Ca and P. Obviously changes in E.A. ($CaO + MgO - P_2O_5$ in mgm. equivalents per 100 gms. dry feed) involve changes in the Ca : P ratio.

EXPERIMENT V.

The rations contain a sufficiency of Ca and P but are abnormal with respect to the Erdalkali-Alkalizität in Marek's sense.

Briefly, Marek and his co-workers (1932 and 1935) claim that the best response to an adequate ration is obtained when the Erdalkali-Alkalizität lies between 20 and 25 mgm. equivalents. Furthermore, that if the E.A. is far removed from what they regard as the normal limits (20-25 mgm.) osteodystrophic disease is practically certain to develop. From a careful consideration of Marek's work it would appear that his results were obtained under conditions of vitamin D deficiency. If this is the case his results are not directly comparable with most of those reported in this experiment where vitamin D was present in abundance, nor is it then correct to claim that an abnormal E.A. is the sole cause of osteodystrophic disease is his experiments as, obviously, vitamin D deficiency was a complicating factor.

It should be pointed out that many rations which are considered normal are not such in Marek's sense and that unless special attention is paid to the E.A. of a ration the Erdalkali-Alkalizität most probably does not lie between the limits accepted by Marek. The control group in this experiment for instance showed normal growth and remained normal throughout the course of the experiment but did not receive a ration of which the E.A. lay between 20 and 25 mgm. equivalents. This point will be brought out more clearly, however, when considering the various rations fed.

Another factor which was considered important in this experiment was the production of rickets. Up to the present rickets has been produced here in pigs receiving adequate vitamin D only when the ration was deficient in phosphorus. This again is apparently at variance with Marek's work in which rickets was reported in pigs receiving sufficient P in their diet stated to be adequate in all respects except its E.A. Hence two of the rations given in our experiment corresponded as closely as practicable with the diets used by Marek and on which he produced severe rickets, our object was to produce rickets which could be proved to be due to a cause other than P deficiency.

The general treatment of the pigs was the same as that reported in Experiment I, and the experiment began in April, 1935, with 11-weeks-old pigs and lasted until the end of August.

The control pigs, Nos. 1076 and 1079, were given 100 ml. milk, 100 gms. green feed and mash according to appetite. The mash consisted of 94 parts maize samp, 6 parts bloodmeal, and 1·5 parts of salt. $CaCO_3$ and Na_2HPO_4 were added to the mash to ensure a daily intake of 7·2 gms. CaO and 6·3 gms. P_2O_5; the daily food supplied contained on an average ·44 gms. MgO. The Erdalkali-Alkalizität for the entire period was +6·5 mgm. equivalents.

These pigs weighed 13·3 Kg. on an average at the beginning of the experiment ond 68·4 Kgs. four months afterwards when they were discharged. Their appetite was good throughout and no indication of disease was noticed at any stage.

Radiographs were taken approximately two months after the beginning of the experiment and No. 1079 was killed an 30.8.35, for bone studies, i.e. after having been 120 days in the experiment.

The bones showed normal growth and development histologically and appeared to be perfectly normal from the radiographs. The percentage ash registered in the dry fat free bone was 54·8.

Several other pairs of pigs of the same age were fed concurrently with the pair mentioned above on rations showing a variation in their Erdalkali-Alkalizität as indicated below.

Nos. 1085 and 1090 were given a mash according to appetite and consisting of:—

Yellow maize meal	11 lb.
Barley meal	55 ,,
Wheaten bran	11 ,,
High protein meatmeal	11 ,,
Salt	1·5 ,,

$CaCO_3$ and Na_2HPO_4 were added to the daily ration to ensure a daily intake of CaO and P_2O_5 per 10 Kg. body weight of 10·8 and 4 gms. respectively. The ratio of CaO to P_2O_5 was 1:2·7 mgm. The Erdalkali-Alkalizität varied from 62 to 7·6 mgm. equivalents; the protein intake was 55 gms. per 10 Kg. live weight.

Another pair of pigs of the same age, No. 1084 and 1086, were given the same ration as that mentionod above, but these pigs were kept indoors permanently. It should be mentioned, however, that no attempt was made to exclude any but direct light and that it cannot be stated that this pair of pigs was kept under conditions of vitamin D deficiency. In addition the feed given, being grown under South African conditions and therefore exposed almost daily for 5 to 10 hours to direct sunlight during the growing and ripening period probably also supplied appreciable quantities of vitamin D. Still, direct sunlight was excluded to reduce the vitamin D present.

Another pair of pigs, viz. Nos. 1082 and 1087, was given the same mash as the two last mentioned pairs but 100 gms. green feed and 500 c.c. milk were given daily in addition to the quota of mash to improve the quality of the feed. $CaCO_3$ and Na_2HPO_4 were again added and the average E.A. for the entire period was found to be 65 mgm. equivalents.

Yet a fourth pair of pigs, viz. Nos. 1088 and 1074, was given the same mash which, incidentally, was responsible for the production of very severe rickets by Marek. Less $CaCO_3$ was added to the mash of this pair of pigs in order to create a more favourable Ca:P ratio and $Mg(OH)_2$ was added to the daily ration to keep its E.A. approximately the same as that of the other groups receiving this mash. The average E.A. for the period for this pair of pigs was 61 mgm. equivalents, and the $CaO:P_2O_5$ ratio 1·3 to 1 with an average intake of 11·5 gm. CaO and 9·0 gm. P_2O_5.

Results.

The weights of the individual pairs of pigs and the averages for each pair are given in Table XIV submitted below.

TABLE XIV.

Weights, given in Kgms.

1935.

Nos.	E.A.	12/4	15/4	25/4	29/4	7/5	13/5	20/5	27/5	3/6	10/6	17/6	24/6	1/7	8/7	15/7	22/7	30/7	6/8	13/8	19/8	26/8
1076......	+6·5	14·9	14·0	15·6	16·6	17·7	19·5	20·6	23·1	24·9	29·2	32·6	37·1	42·5	46·9	46·4	55·6	64·1	68·1	71·9	75·6	79·2
1079......	+6·5	11·8	11·3	12·2	13·1	13·1	12·7	14·0	15·4	16·8	17·7	19·1	22·9	27·6	30·5	35·4	39·3	43·1	47·2	49·6	53·6	57·6
Average.....		13·35	12·6	13·9	14·8	15·4	16·1	17·3	19·25	20·8	23·45	25·8	30·0	35·05	38·7	40·9	47·45	53·6	57·6	60·7	64·6	68·4
1085......	+67	7·3	7·3	7·9	8·4	9·5	10·9	12·4	14·5	17·3	20·0	23·6	26·9	30·1	31·9	35·3	36·2	43·6	46·0	50·2	52·9	52·9
1090......	+67	6·6	6·4	7·3	7·7	8·8	9·5	11·1	13·1	15·6	17·7	20·4	23·3	26·3	27·3	30·1	32·3	37·7	38·9	41·3	42·9	46·3
Average.....		6·9	6·8	7·6	8·05	9·15	10·2	11·7	13·8	16·45	18·8	22·0	25·1	28·2	29·6	32·7	34·2	40·6	42·45	45·7	47·9	49·6
1086......	+67	7·0	7·3	7·7	8·2	9·5	10·0	11·8	13·3	15·9	19·1	22·2	24·0	27·2	30·8	32·6	34·6	38·9	40·4	43·6	44·7	47·4
1084......	+67	7·7	7·9	8·8	9·1	10·4	11·3	12·9	14·5	17·5	20·0	23·3	24·9	28·1	31·2	32·8	35·7	40·2	42·9	45·8	48·1	55·4
Average.....		7·35	7·6	8·25	8·6	9·9	10·6	12·35	13·9	16·7	19·5	22·7	24·45	27·6	31·0	32·7	35·1	39·5	41·6	44·7	46·4	51·4
1082......	+65	8·2	8·2	9·1	9·5	10·9	12·0	13·1	14·2	17·9	21·8	25·4	29·2	32·3	37·1	43·1	49·2	55·6	57·2	59·4	61·0	64·9
1087......	+65	7·5	7·7	8·6	9·1	10·4	11·3	13·1	14·2	17·0	19·1	23·1	26·3	29·4	33·0	36·8	39·8	45·6	46·7	49·6	52·2	56·9
Average.....		7·8	7·9	8·8	9·3	10·6	11·6	13·1	14·2	17·45	20·45	24·25	27·7	30·8	35·0	39·9	44·5	50·6	51·9	54·5	56·6	60·9
1088......	+61	8·2	7·9	8·8	9·3	10·9	12·0	14·2	15·1	18·2	22·2	25·6	29·0	33·7	36·2	43·5	46·5	53·3	55·6	60·5	62·8	65·6
1074......	+61	10·6	10·2	10·9	11·3	13·1	14·0	14·9	17·9	20·9	25·1	28·3	33·0	37·5	41·3	47·2	51·8	59·4	60·1	65·6	66·1	71·0
Average.....		9·4	9·0	9·8	10·3	12·0	13·0	15·0	16·5	19·5	23·6	26·9	31·0	35·6	38·7	45·3	49·1	56·8	57·8	63·0	64·45	68·3

A statistical analysis of the results shows that no significant differences existed between the group weights at the end of the experimental period.

None of the groups showed an adverse reaction to the conditions of the experiment. The food consumption records show no significant differences between the groups and all the pigs remained healthy throughout the course of the experiment.

X-ray photographs were taken one month before the conclusion of the experiment and revealed no differences in bone structure in the comparative groups.

One pig of each pair was killed at the conclusion of the experiment, i.e. after 122 days experimental period, and rib sections were examined histologically. These sections were found to be normal and revealed normal bone growth and development in every case.

DISCUSSION.

There is no doubt that under the conditions described, rickets was not produced in pigs which received for 122 days rations so compounded that their Erdalkali-Alkalizität was abnormal in Marek's sense. Furthermore, there was apparently no difference in the body weights, food consumption and microscopical bone structure between the pigs receiving a ration whose E.A. was +6·5 mgm. equivalents when compared with those on a ration of approximately 65 mgm. equivalents.

As vitamin D was present in abundance in the experiment reported here, whereas the same ration used by Marek contained negligible quantities of this vitamin the conclusion appears justified that the difference between our results and those of Marek is due to the difference in the Vitamin D content of the ration used by the two schools of workers. It is also obvious that the highly positive E.A. in Marek's experiment wos not responsible per se for the production of osteodystrophic disease. It would appear unwise, therefore, to emphasize the necessity of a correct Erdalkali-Alkalizität for normal growth and development in pigs unless it is stated that the E.A. of a ration is important when pigs are kept under conditions of vitamin D deficiency.

Concurrently with the above two pairs of pigs of the same age and litters as those used were given another of Marek's rations whose Erdalkali-Alkalizität was −25 mgm. equivalents and which produced severe rickets after 141 days.

The ration consisted of 500 ml. separated milk, crushed maize ad lib to which bonemeal and salt were added to ensure a daily intake per 10 Kg. body weight of approximately 35 gms. protein, 5·5 gms. CaO, 7 gms. P_2O_5 and an Erdalkali-Alkalizität of −25 mgm. equivalents.

This ration, as stated by Marek, is low in protein which was poor in quality. The pigs did not respond well and scouring was frequently present for short periods. Growth was poor on the whole and the pigs lagged behind those already mentioned and receiving the ration whose Erdalkali- Alkalizität was highly positive. The fortnightly body weights of these pigs compared with those of the controls are given below:—

TABLE XV.

1935.

Nos.	E.A.	12/4	24/5	7/5	20/5	3/6	17/6	1/7	15/7	30/7	13/8	26/8
1076.........	+6·5	14·9	15·6	17·7	20·6	24·9	32·6	42·5	46·4	61·1	71·9	79·2
1079.........	+6·5	11·8	12·2	13·1	14·0	16·8	19·1	27·6	35·4	43·1	49·6	57·6
Average....		13·35	13·9	15·4	17·3	20·8	25·8	35·0	40·9	53·6	60·7	68·4
1078.........	−25	11·1	12·2	14·2	16·4	18·2	23·1	26·7	30·8	34·6	36·8	43·8
1080.........	−25	9·3	10·2	12·9	13·6	16·1	18·2	21·3	23·6	26·7	29·4	30·8
1081.........	−25	9·7	11·3	13·3	15·1	17·0	19·5	23·6	27·6	29·6	33·7	36·3
1083.........	−25	8·6	10·2	12·0	12·4	12·9	15·4	17·9	20·5	22·9	24·9	27·6
Average....		9·9	10·9	13·1	14·3	16·05	19·05	22·4	25·6	28·4	31·2	34·4

The body weights of the pigs receiving the maize ration reveal, as would be anticipated, poor weight increase, viz. 24·5 lb. after four months, compared with 55 lb. for the controls. The pigs showed poor appetites from time to time and although obviously hungry did not relish their food. The radiographs taken after three months in the experiment show bone atrophy and the rib sections of pigs Nos. 1080 and 1083 killed after 122 days in the experiment confirm this finding. Osteoporosis was not extensive, but certainly present, while the amount of osteoid present did not transgress physiological limits; osteofibrosis and rachitic lesions were absent . It is not surprising that bone formation did not proceed normally if the inadequacy of the ration both in regard to the quality and the quantity of the protein is considered. The point that is most important, however, is that in spite of a strongly negative Erdalkali-Alkalizität neither rickets nor osteodystrophia fibrosa was produced as was found to be the case by Marek, working with the same diet but probably under conditions of vitamin D deficiency.

For the convenience of the reader the main features of the five experiments reported in this publication are shown below in tabular form. (Table XVI.)

A glance at the table given above reveals the fact that osteodystrophic diseases were produced only when the intake of P or Ca or both was low and that bone disease was absent whenever sufficient quantities of these minerals were supplied in spite of the Erdalkali-Alkalizität of the ration ranging from − 9 to + 65 milligram equivalents.

Here again, as in the case of a ration of strongly positive E.A., it would appear that the difference in the results obtained at this Institute when compared with those of Marek and his school lies in the difference between the vitamin D contents of the respective rations.

TABLE XVI.

No. of experiment.	Special feature of ration.	No. of Pigs.	Age at beginning of experiment.	Daily intake (Percentages.) Ca.	Daily intake (Percentages.) P.	Ratio. Ca : P.	Vitamin D. content.	Erdalkalialkalizität. (Mgm. equivalents.)	Diagnosis.
I	P low	2	6 mths.	0·67	0·09	7·5 : 1	Sufficient	+30	Rickets.
I	Normal	2	6 mths.	0·4	0·3	2 : 1	,,	+ 5	Normal.
I	P low	2	8 wks.	2·0	0·11	18·4 : 1	,,	+92	Rickets.
I	Normal	2	8 wks.	1·0	0·55	1·8 : 1	,,	+2·3	Normal.
I	P low	2	8 wks.	2·0	0·11	18·4 : 1	In semi-darkness	+92	Rickets.
I	Normal	2	8 wks.	1·0	0·55	1·8 : 1	,,	+2·3	Normal.
II	Ca low	2	6 mths.	0·06	0·6	1 : 10	Sufficient	−60	Osteoporosis, no rickets.
II	,,	2	8 wks.	0·11	0·98	1 : 9	,,	−86	,, ,,
II	,,	2	8 wks.	0·11	0·98	1 : 9	In semi-darkness	−86	,, ,,
II	,,	2	8 wks.	0·04	0·6	1 : 15	Sufficient	−50	(*a*) One died early in experiment. (*b*) Osteofibrosis in remaining pig.
III	Ca & P low	2	6 mths.	0·06	0·04	1·5 : 1	Sufficient	+ 2	Osteoporosis and slight rickets.
III	,,	2	8 wks.	0·11	0·11	1 : 1	,,	− 2	Osteoporosis and slight rickets.
III	,,	2	8 wks.	0·11	0·11	1 : 1	In semi-darkness	− 2	Osteoporosis but no rickets.
IV	Sufficient Ca & P in abnormal ratios	2	6 mths.	0·40	0·8	1 : 2	Sufficient	−19	Normal.
IV		2	6 mths.	1·1	0·2	5·5 : 1	,,	+23	Normal.
				CaO grms. (per 10 kg.	P_2O_5 body wt.)	Ratio			
V	Abnormal E.A.	2	11 wks.	7·2	6·3	1·1 : 1	Sufficient	+ 6·5	Normal.
V	,,	2	,,	10·8	4·0	2·7 : 1	,,	+62	Normal.
V	,,	2	,,	,,	,,	1·7 : 1	Probably sufficient	+62	Normal.
V	,,	2	,,	,,	,,	2·7 : 1	Sufficient	+62	Normal.
V	,,	2	,,	11·5	9·0	1·3 : 1	,,	+61	Normal.
V	,,	4	,,	5·5	7·0	1 : 1·3	,,	−25	Slight osteoporosis.

The control pigs to those mentioned in Experiments II, III and IV are those of the relative ages given as "Normal" in Experiment I.

Rations of strongly positive or negative Erdalkali-Alkalizität apparently do not produce rickets or osteodystrophia fibrosa in young, growing pigs if sufficient P and Ca are present, even after several months' feeding, unless the vitamin D content of the ration is below the requirements of the pigs. Marek's results therefore cannot be described as being directly due to the Erdalkali-Alkalizität of the ration but were apparently due to a complex of at least two factors, viz. E.A. and vitamin D shortage.

SUMMARY AND CONCLUSIONS.

1. Data are presented on the effect upon growing pigs of rations deficient in P or Ca or both. Observations were made upon the body weight, food consumption, blood analysis and bone analysis—physical, chemical and histological.

2. The effects of the Ca : P ratio and of the Erdalkali-Alkalizität of some of the rations were also considered and discussed.

Low P, Abnormal Ratio.

3. ·8gm. of P and ·1 per cent. P were found to be not only insufficient for the normal growth and development of young growing pigs but caused severe rickets as determined microscopically.

4. The absence or presence of light was apparently without influence on the effects of the phosphorus deficiency and this suggests that the ration, probably already contained a sufficiency of vitamin D.

5. The low P content of the ration was reflected as low inorganic phosphorus in the serum, while serum phosphatase (Bodansky units) gave significantly higher than normal values, suggesting poor calcification.

6. A considerably smaller percentage of ash was present in the bones of the pigs suffering from P deficiency than in those of the control group.

7. Clinical symptoms of P deficiency appeared shortly after the beginning of the experiment and gradually became more pronounced until severe rickets could be diagnosed with the naked eye, when the pigs were in a pitiable state and the experiment discontinued. The addition of phosphate to the ration at this stage cured the rickets but the pigs remained smaller than the controls and had a stunted appearance, nor did the legs become normal.

Low Ca, Abnormal Ratio.

8. ·1 per cent, Ca in one experiment and 1 gm. daily in another were found to be insufficient for the normal growth of young pigs.

9. As in the case of P deficiency, light appeared to be without effect on the results of the Ca deficiency and it is surmised that the food being grown under conditions of abundant sunshine, contains sufficient vitamin D for the requirements of the pigs.

10. Neither serum phosphatase nor serum Ca was significantly affected by the low Ca of the ration.

11. The ash of the bones was considerably reduced.

12. Bone atrophy or osteoporosis was present but no rickets. In a third experiment in which the ration of a pair of pigs contained ·5 gms. CaO and 10·5 gm. P_2O_5 the bones of the surviving pig showed incipient osteodystrophia fibrosa and bone atrophy after 105 days but no indications of rickets. The suggestion is made that Ca deficiency per se will not produce rickets, but might produce osteodystrophia fibrosa in pigs and further work along these lines is being carried out.

Ca and P Deficiency, Normal Ratio.

13. 0·1 gm. Ca and ·6 gm. P were contained in the daily ration in one experiment and ·11 per cent. of Ca and P respectively in another experiment.

14. The weight increase of the pigs was unaffected for the first twenty weeks of the experiment when the animals on the Ca and P deficient ration began to increase more slowly in weight which differed significantly from that of control pigs at the end of the experiment after approximately another twenty weeks.

15. The detrimental effect of Ca and P deficiency was by no means as severe as that of a deficiency of either Ca or P together with an excess of P or calcium respectively; the abnormal ratio of Ca : P in the latter case is believed to be responsible for the enhanced effect of the deficiency.

16. Food consumption was poorer than in the control group, percentage ash in the bones low and the inorganic P of the serum not as low as that of the pigs on P deficiency but Ca excess.

17. It would seem that the absence of light affected the health of the pigs detrimentally. The pig in the light showed at its worst only slight stiffness while that in semi-darkness could hardly stand and walked only with the greatest difficulty; its legs were very stiff and almost out of control.

18. Microscopically the ribs of all four pigs showed marked bone atrophy and a suggestion of rickets in two of the four animals. The suggestion is made that the deficiency of P and Ca was not acute enough or that the experiment was not conducted for a sufficiently long period to produce rickets or ostitis fibrosa or both. Work along these lines is being continued.

Ca and P Sufficiency with Abnormal Ratio.

19. A ration containing 6 gms. Ca and 8·2 gms. P was given to one pair of pigs while in the case of a second pair of animals the ration contained 13·7 gms. Ca and 3·0 gms. P.

20. Body weight, food consumption, blood analysis and bone composition, chemical and histological did not alter significantly when Ca and P were present in the ration in the proportions given above instead of that contained in the ration of the control group.

Erdalkali-Alkalizität (E.A.).

21. Rations whose average E.A. for the 120 days experimental period were 6·5 mgm. equivalents and approximately 62 mgm. equivalents respectively were fed to eleven weeks old pigs. Sufficient Ca and P were present for normal growth.

22. All the pigs grew normally and remained healthy, nor was there any significant difference in body weight, food consumption and bone development as judged radiographically and histologically.

23. Neither rickets nor osteodystrophia fibrosa developed as was reported by Marek on this ration.

24. It is suggested that the explanation of the apparently anomalous results lies in the fact that abundant vitamin D was present in our experiments while this was a limiting factor in Marek's experiments.

25. A strongly negative E.A. (– 25 mgm. equivalents) in a ration consisting of maize produced bone atrophy and no rickets. As maize protein is admittedly deficient in protein—qualitatively and quantitatively—the results obtained cannot be ascribed exclusively to the abnormal E.A. of the ration.

Marek produced rickets and osteofibrosis with this ration and the difference between his results and those obtained here is again ascribed to the difference in the Vitamin D content of the ration made up at the two Institutes.

26. It is suggested that the Erdalkali-Alkalizität of a ration when sufficient P and Ca are present is not exclusively responsible for the development or not of osteodystrophic diseases in pigs.

The writers wish to acknowledge gratefully the collaboration of Dr. Thomas, Mr. van der Wath, B.V.Sc., and Mr. B. A. du Toit, M.Sc. during the course of the investigation.

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