XXXI. THE RELATION OF THE FAT SOLUBLE FACTOR TO RICKETS AND GROWTH IN PIGS. II.

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THE experiments described in this communication form a part of an inquiry now in progress, the main purpose of which is to study the part played by the accessory factors in the nutrition of agricultural stock and in the etiological factors concerned in the causation of rickets in pigs. In a previous communication [Zilva, Golding, Drummond and Coward, 1921] in which the etiology of rickets in pigs was investigated we have shown that a rigorous elimination of vitamin *A* from the diet of young pigs did not conduce to the production of well-defined rickets. We were, however, able to demonstrate in those experiments that such a dietetic deficiency has a very marked effect on the development of these animals. Indications were also obtained that the deprivation of this dietetic principle possibly has some bearing on the production of healthy young.

Having been unsuccessful in producing rickets in pigs experimentally by depriving them of vitamin *A* alone we next attempted to ascertain whether a dietetic deprivation of calcium and vitamin *A* would lead to the production of the disease. The results obtained by us form the subject of this communication.

EXPERIMENTAL.

Our animals were divided into four groups. Group I received a diet deficient in vitamin *A* and in calcium ($-A - Ca$), group II received a diet deficient in vitamin *A* ($-A + Ca$), group III received a diet deficient in calcium only ($+A - Ca$), group IV received a diet containing calcium and vitamin *A* ($+A + Ca$). Two pigs were placed in each group. Group I consisted of two boars, group II of one boar and one sow, group III of two sows and group IV of two boars. The young pigs, which belonged to the same

litter, were started on their special diets when they were 53 days old. They were young Berkshires, born of a sow 14 months old. The sow was kept for some time on a diet of toppings and whey deficient in vitamin *A* and manifested this deprivation by retarded growth [Drummond, Golding, Zilva and Coward, 1920]. On supplementing the above diet with lucerne the animal resumed growth and continued an apparently normal existence. After 74 days of correct feeding she was served by a pedigree boar and soon after was placed again on a diet of toppings, whey and swedes. This diet which was poor in vitamin *A* was purposely planned as we did not desire the young pigs to be born with a store of the vitamin. After 116 days nine pigs were born and at the age of 65 hours were divided into two sections. The chart below shows the history and subsequent allocation of the pigs. One lot received its supply of vitamin *A* entirely from the mother, the other received additional vitamin *A* in the form of cream ($\frac{1}{8}$ oz. per day) and eventually in the form of cod liver oil ($\frac{1}{2}$ rising to 1 oz. per day). Two of the animals in the former section received an equal supply of additional oil in the form of inactive olive oil, made into an artificial cream at first and later given to balance in nutrients the cod liver oil given to Section II.

The two remaining pigs in the first section received only the sow's milk; the sow being fed all the time on a diet shown to be poor in the vitamin *A*.

As soon as it was convenient the young pigs were given additional toppings which at the end of this preliminary period of 53 days (period I) reached a ration as high as 3 lbs. per day for the litter of eight pigs. The actual intake of food and the corresponding increase in weight for the entire experiment is summarised in Table I. The growth of the animals is graphically represented in Fig. 1. The average daily gains in pounds for period I were for pigs in section I receiving no oil 0.533 and 0.514, for those receiving olive oil 0.377 and 0.5, while the daily gains of the pigs in section II receiving cod liver oil were 0.481, 0.453, 0.344 and 0.509.

The agreement between the rates of growth of the pigs in the two sections is made more evident by employing the formula advised by R. A. Fisher,

Table I

[illegible]

Fig. 1.

Chief Statistician, Rothamsted Experimental Station, for calculating the relative growth rate per day per cent. using natural logarithms, viz.:

$$\frac{\log_e M_2 - \log_e M_1}{K_2 - K_1} 100$$

where M_2 = the weight at the end of the period,
 M_1 = „ „ commencement of the period,
 $K_2 - K_1$ = duration of period in days.

The following figures are obtained for Section I (– *A*) without oil 4.91 and 4.50, with olive oil 4.14 and 4.31, for Section II (+ *A*) 4.25, 4.45, 3.83 and 4.34.

It is evident from the above figures that a sow, even when fed on a diet deficient in the fat-soluble factor and having undergone a previous deprivation in this factor, is capable of rearing her young satisfactorily. This confirms further our earlier observation that the requirements of the pig for the fat-soluble factor are not of a high order.

At the end of this period, namely 53 days after birth, the animals were weaned and placed on their respective experimental diets. Each group was placed in a separate sty. The sties faced south and were divided by wooden partitions, the floor being partly wood and partly concrete. The bedding consisted of wood shavings or sawdust.

The basal diet for all groups consisted of toppings or wheat offal having the following average composition:

Moisture	11.51 %	Mucilage	59.60 %
Oil	4.29	Woody fibre	5.86
Protein	14.81	Ash	3.93

The dry matter of the above contained 0.338 % calcium. Besides the basal diet the pigs received supplementary protein in the form of caseinogen, which in the case of the vitamin-free diets was previously inactivated by being heated for 24 hours at 120° C. This inactivation was carried out for us by Dr R. T. Colgate in Messrs. Huntley and Palmer's Laboratory, for which we wish to express our thanks.

The food was weighed out three times a day at 8 a.m., 12 noon and 4.30 p.m.; it was mixed with cold water just before feeding and given in the form of a thin cream. The food was given on a live weight basis, being regulated to the quantity which the pigs would clean up.

The other two accessory factors, namely the antiscorbutic and the anti-neuritic factors, were supplied in the form of freshly prepared lemon juice and marmite (Commerical Yeast extract), about 7 cc. of the former being the daily dose, whilst $\frac{1}{8}$ oz. of the latter was given at intervals. Groups III (+ *A* – Ca) and IV (+ *A* + Ca) received a daily dose of cod liver oil as a source of the fat-soluble factor whilst the other two groups received an equivalent dose of inactive olive oil. Groups II and IV also received a daily dose of 1 oz. each of precipitated chalk and 1 oz. animal charcoal containing

67.3 per cent. of calcium phosphate as a source of additional calcium and phosphate; the other two groups received only the small amounts of calcium from the basal diet.

During the following 54 days (period II) the intake in all the four groups was the same. The increase in weight in the animals of the various groups however showed a marked disparity. The two animals in group I gained 41.88 lbs., in group II 49 lbs., in group III 62.7 lbs., and group IV 74.6 lbs. The relative growth rates, as calculated from the formula given above, were group I 0.99 and 0.98, group II 1.32 and 1.13, group III 1.47 and 1.34, and group IV 1.80 and 1.57. The average dry matter in the food consumed per pig for each pound of gain in live weight was: group I 4.4 lbs., group II 3.8 lbs., group III 3.0 lbs. and group IV, 2.5 lbs. By this time very marked differences in the general appearance of the animals in the respective groups could be discerned. The animals in group I developed a scurfy skin and saddle back, weak legs and joints painful to pressure. They were easily tired and were not playful. Those in group II also showed lack of vitality and a saddle back, those in group III were in good condition possessing glossy coat, whilst the animals in group IV were decidedly in the best condition. One animal in group I received an injury from a fall and died 87 days after the commencement of the experiment. At the post mortem examination it was found that the vertebral column was broken.

During period III, *i.e.* 35 days following period II, the increase in weight was as follows: group I 4.62 lbs. (one animal), group II 28.75 lbs., group III 42 lbs., and group IV 58.4 lbs., and the average dry matter in the food consumed per pig for each pound of gain in live weight was: group I (one animal) 14.6 lbs., group II 4.5 lbs., group III 4.0 lbs. and group IV 3.02 lbs.

Owing to the low condition of the remaining animal in group I it was decided to administer a small amount of the fat-soluble factor in order to save the pig. This was done by introducing caseinogen, which had not been previously inactivated by heating, in the diet during period IV. This addendum had its desired effect and the animal responded after about five days by resuming growth.

During this period of 22 days (period IV) the animals gained in weight as follows: group I (one animal) 10.25 lbs., group II 19 lbs., group III 41.7 lbs., and group IV 61.7 lbs. In the last period (period V) the inactivated caseinogen was alternated with crude caseinogen in order to keep the weight of the animals in group I and group II in check.

The experiment was terminated 145 days after its commencement. The animals were slaughtered with the exception of one sow in each of the groups II and III. The condition of the animals before slaughter was as follows: groups I and II wrinkled skin, ears carried forward, down on hind legs; group III skin rather rough, lack of size and bloom, flesh not quite firm, otherwise normal; group IV skin healthy and animals perfectly normal.

The photographs of the pigs before slaughtering are shown in Plate II,

two photographs having been taken of the pig in group I. The photographs were taken to scale.

The post mortem examination revealed no abnormalities of the organs beyond that the ribs of the animals in group I were easily fractured. It is also to be reported that the fat of the pigs of groups I and II was, in the butcher's opinion, softer and not of such good quality as the fat of the other pigs. This was confirmed by the estimation of the melting and solidifying points of the fats.

Group		Melting points		Solidifying points
		21°	22·9°	19°
	II	21°	23·8°	19·3°
	III	25°	25·20°	22°
	IV	31·5°	32·8°	22·5°

Table II gives the size, weight, breaking points, and calcium content of the bones.

Table II.

		Weight of humerus in g.	Distance of bearing points in inches	Breaking weight in tons	Same corrected to 3½" length	CaO percentage of dry matter
Group	I	103	3½	0·210	0·210	29·52
	II	127	3½	0·345	0·345	29·05
	III	117	4	0·292	0·336	33·52
	IV	152	4	0·466	0·532	38·08

No abnormal flavour or taste was reported by a number of people who consumed the joints derived from the animals fed on the cod liver oil.

The fifth, sixth and seventh ribs of one animal in each group were examined for us histologically by Prof. V. Korenchevsky to whom we are also indebted for the interpretation of the results. The following is a summary of the observations made:

(1) Group IV (+ A + Ca) showed a somewhat abnormal picture with very slight osteoporosis and a belated deposition of lime salts in the newly formed bone.

(2) In all cases the bone marrow especially in the region of the secondary spongiosa consisted of a fine fibrous reticulum with but few bone marrow cells.

(3) The histological condition in animals belonging to groups II (− A + Ca) and III (+ A − Ca) was not very different from that of group IV (+ A + Ca). Only a higher degree of osteoporosis resulting from a diminished activity of osteoblasts could be seen.

(4) In group I (− A − Ca) the condition of osteoporosis was more pronounced. Moreover a more frequent incursion of the proliferating cartilage into the bone marrow was in evidence. In these places was also noticed defective calcification in the zone of provisional calcification.

It is quite evident that in spite of the very marked changes which have been effected by our restricted diets, no rickets in the pathological sense of the word has been induced. The animals in groups I and II have on various

occasions during the experiment displayed a condition which would have been described by the practical man as the pigs being "off their feet." No doubt such a condition has been before now loosely referred to as "rickets." Although defective calcification was found in the zone of provisional calcification in the case of group I ($-A - Ca$) no increase in the amount of osteoid tissue could be established and therefore no faulty deposition of calcium in the newly formed bone in the sense of rickets can be asserted.

A remarkable feature in our experiments is that even in the case of group IV ($+A + Ca$) which acted as our control, a normal histological picture was not obtained. This requires further investigation. Possibly the restricted diet of the mother may be responsible for this. Another point to be considered is that the animals consuming the calcium-deficient diet received 0.338 % of calcium in their food. With animals, such as pigs, which consume large bulks of food it was difficult from a technical point of view to reduce this calcium intake. However the calcium deficiency was definite enough to impair the growth and diminish the calcium content in the bones of the animals in group III ($-Ca + A$) and it is very doubtful whether pigs which develop rickets under farm conditions, receive a diet much poorer in this element. We refrain from reviewing the literature which has appeared during the last few months in connection with the etiology of rickets. Most of the experimental work was done on rats and the results and conclusions of the various investigations are conspicuously contradictory. We cannot however conclude without briefly referring to the results obtained by Korenchevsky [1921]. This investigator, working with rats, obtained a definite condition of rickets on a diet free from the fat-soluble factor and calcium. Whether our apparently different results were due to the higher content of calcium in our experimental diets, or whether it was due to the different character of the experimental animal will most probably be decided by future investigation. We are continuing our experiments and although the dietetic hypothesis of the etiology of rickets forms our main line of work, we are not excluding such a hypothetical factor as light, especially in view of the latest work of Hess and Unger [1921], of Powers, Park, Shipley, McCollum and Simmonds [1922].

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PHOTOGRAPHS OF PIGS TAKEN ON OCT. 10TH, 1921

Group I - Vitamin A.

Group II - Vitamin A + Calcium and Phosphate.

Group III + Vitamin A.

Group IV + Vitamin A + Calcium and Phosphate.

Pigs from Groups IV, III, II, I.

Pigs from Groups IV, III, II, I.

Cross sections of pigs from Groups IV, III, II, I.

Group I - Vitamin A.

Group II - Vitamin A + Additional Calcium and Phosphate.

Group III + Vitamin A.

Group IV + Vitamin A + Additional Calcium and Phosphate.