CXV. THE RELATION OF THE FAT-SOLUBLE FACTOR TO RICKETS AND GROWTH IN PIGS. III.

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WITH A REPORT ON THE PATHOLOGICAL HISTOLOGY OF THE BONES,
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SOME years ago we began this investigation with the object of studying the possibility of inducing rickets in pigs by dietetic means. We have already shown [Zilva, Golding, Drummond and Coward, 1921; Golding, Zilva, Drummond and Coward, 1922] that by depriving the animals of vitamin A growth was appreciably restricted, and was accompanied by osteoporosis. The histological examination of the ribs of such animals, which was kindly undertaken for us by Prof. Korenchevsky, did not reveal any significant increases of osteoid tissue, a condition which is considered to be pathognomonic of rickets.

In this communication we are bringing forward evidence which we believe demonstrates that under certain conditions it is possible to induce typical rickets in pigs by depriving them of the fat-soluble antirachitic vitamin. The systematic experiments described here were the outcome of an observation that pigs, fed on a diet which included dried separated milk in large quantities and which was otherwise almost free from the fat-soluble vitamins, grew well but eventually displayed rachitic symptoms.

EXPERIMENTAL.

The problem was investigated in two sets of experiments. In one, all the animals were studied in the absence of direct sunlight. The second was a repetition of the first, except that two of the animals were exposed to direct sunlight and given cod-liver oil in addition.

Exp. 1. In this experiment eleven pigs were employed; they consisted of selected Middle Whites and crosses between Berkshires and Middle Whites, and belonged to two litters farrowed on the same day (February 14th, 1923).

The mothers of these litters were kept under normal farm conditions at the time of service and during gestation. At the commencement of the experiment (April 17th, 1923), the pigs were 62 days old. The diet consisted of toppings, barley meal, dried separated milk, "animal charcoal," swedes and chalk. The average daily consumption and calcium and phosphorus content of the foodstuffs were as follows:

	CaO %	P ₂ O ₅ %	Average daily consumption in oz.
Toppings	0.113	2.06	27
Barley meal	0.189	0.930	2
Dried separated milk	2.16	2.39	6.5
Animal charcoal ...	40.00	33.1	1
Chalk	50.8	—	1
Swedes	—	—	3

The average intake of CaO per day was, therefore, 1.085 oz., and that of P₂O₅, 1.06 oz., giving a ratio of Ca/P = 1/0.6. The dried separated milk powder was about nine months old and was prepared from a summer milk. It was kindly supplied to us by Messrs J. Lyons and Co., Ltd.

One group of the animals received swedes from April 30th, as an anti-scorbutic. The other group was not given any vitamin C. The animals were arranged as follows:

No.	Sex	Breed	Weight at commencement of experiment in lbs.	
122	Sow	Middle White	30	Received swedes
124	Hog	" "	33	" "
126	"	" "	41	" "
128	"	Berkshire × Middle White	30.5	" "
130	"	" "	31.5	" "
121	"	Middle White	33	Received no swedes
123	"	" "	28.5	" "
125	"	" "	35	" "
127	"	Berkshire × Middle White	39	" "
129	"	" "	36.5	" "
131	"	" "	33	" "

All the pigs were kept in a roomy animal house with gauze blinds drawn and were allowed sufficient exercise. The following is the protocol of the animals:

Pig 122. This animal first showed lameness in all its limbs on June 8th. This condition became gradually worse. On July 9th the administration of cod-liver oil was begun, the basal diet and other conditions being kept the same. The initial dose was $\frac{1}{4}$ oz. of oil per day, which was eventually increased to $\frac{1}{2}$ oz. The animal gradually improved, and by the end of August its condition appeared to be normal. At the same time the rate of growth was greatly increased, the animal gaining at the rate of about 1.25 lbs. per day as against 0.77 lb. a day during the period before the cod-liver oil was given.

Pig 124. This animal grew at the rate of about 0.7 lb. per day until June 15th when it became very lame. On July 6th its condition was so bad that it was decided to kill it.

Professor Korenchevsky's report upon the histology of the rib junctions in this and the other pigs which came to autopsy is given below, p. 876.

Pig 126. This animal grew at the rate of about 0.7 lb. per day until June 6th, when its growth almost ceased. A few days previously (May 30th), the animal became very lame. This condition became worse and on July 6th it was slaughtered.

Pig 128. This animal gained at the rate of about 0.9 lb. per day. Lameness was first observed on June 8th and gradually became worse, the animal crawling on its knees. It was killed on June 20th.

Figures 8 and 9 portray the condition of the animal before being killed.

Pig 130. Lameness was first observed intermittently from May 3rd until June 29th, after which period the lameness became permanent. Considerable swelling at the joints was observed from June 8th. On July 6th the animal was down on its knees. On July 9th cod-liver oil was administered. The weight curve of the animal is given in Fig. 1. Figs. 2 and 3 portray the appear-

Fig. 1.

ance of the pig before and after the cod-liver oil treatment. It was killed on September 19th and the report of the histological examination of the ribs is given below.

Pig. 121. Slight signs of lameness were observed on June 15th. From this date the condition varied, sometimes the pig appeared to be better, but gradually became worse; the animal was very badly "off its feet" on July 12th. At this stage it was allowed access to direct sunlight for a number of hours daily. The diet remained the same. The condition of the animal improved and the rate of growth increased, although the change was not so marked as in the case of the animal cured by cod-liver oil. It was killed on November 19th. Prof. Korenchevsky reports that the histological examination of the ribs revealed almost a normal condition. The weight curve of this animal is given

in Fig. 1, and Figs. 4 and 5 show its appearance before and after its exposure to direct sunlight.

Pig 123. The first sign of lameness was observed on June 15th and this gradually developed. On July 12th this animal was exposed to direct sunlight in the same way as Pig 121, and as in the case of that animal the condition of the pig and its rate of growth improved. It was killed on November 19th, and, as will be seen from Prof. Korenchevsky's report below, the histological examination revealed osteoporosis but not rickets.

Pig 125. This animal grew at the rate of about 0.9 lb. per day. It first showed lameness on June 8th, from which date the condition varied until July 26th, when it became very bad and the animal was killed. The report on the histological examination of the ribs is given later.

Pig 127. This pig gained at the rate of about 0.8 lb. daily. First signs of lameness observed on June 8th, and by July 17th, the animal was "off its feet." It was killed on July 30th.

Pig 129. This pig gained at the rate of about 0.8 lb. daily. First signs of lameness observed on July 20th. On August 26th the dried separated milk was replaced by fresh full-cream cow's milk (1 quart per day). As will be seen from Fig. 1, the animal continued to grow at the same rate as before. The symptoms of lameness definitely improved after the treatment, but did not entirely disappear even after 80 days on the fresh milk diet which was evidently not good enough to effect a complete cure. Figs. 6 and 7 portray the appearance of the animal before and after the addition of the fresh full milk to the diet.

Pig 131. This pig gained at the rate of about 0.8 lb. daily. The first signs of lameness were observed on June 15th. This condition varied for some time and the pig was killed on September 19th. For the result of the histological examination of the ribs, see below.

Exp. 2. This experiment was devised for confirmatory purposes with the modification that two animals were exposed to sunlight.

They were arranged as follows:

No.	Sex	Breed			Weight at commencement of experiment in lbs.
133	Sow	Berkshire and Middle White			40
136	Hog	"	"	"	44½
138	"	"	"	"	38½
134	Sow	"	"	"	39
140	Hog	"	"	"	46
137	"	"	"	"	33
132	"	"	"	"	20
135	"	"	"	"	22½

The animals received the same basal diet as the pigs in Exp. 1. Pigs 134, 140 and 137 were given apples to supply the antiscorbutic factor. Control Pigs 132, 135 received apples and cod-liver oil and were allowed access to a pen outside the sty where they had the benefit of direct sunlight during the day. All the animals belonged to the same litter, the sow having been fed on a diet deficient in fat-soluble vitamins during gestation and the rearing period.

The experiment started on August 10th, 1923. The following is the protocol of the animals:

Pig 133. This animal gained at the rate of 0.6 lb. per day. The first sign of lameness was observed on October 9th and gradually increased; the animal was slaughtered on October 26th.

Pig 136. This animal gained at the rate of 0.4 lb. per day. It was down on October 2nd, and was unable to stand up. It was killed on October 11th. At the post-mortem scorbutic haemorrhages were observed in the left thigh muscle.

Pig 138. This animal gained at the rate of 0.6 lb. per day. It showed first signs of lameness on October 9th which gradually became worse. It was slaughtered on November 8th. At the time of slaughtering the animal manifested pseudo-paralysis of the hind limbs similar to that observed in scorbutic monkeys. At the post-mortem examination numerous large haemorrhages were observed. They were specially large in the left thigh muscle and in the knee joint. Haemorrhages were also observed in the stomach wall and liver.

Pig 134. This animal gained 0.6 lb. per day. On October 19th it was very weak on its hind legs and was killed on October 26th.

Pig 140. This animal gained at the rate of 0.7 lb. per day. Lameness was first observed on October 12th, which became gradually worse. It was killed on November 30th. No histological examination was made in this case.

Pig 137. This animal did not thrive owing to some intercurrent disease and was, therefore, eliminated from the experiment.

Pigs 132 and 135, although the worst animals of the litter at the beginning of the experiment, grew very well and by November 22nd showed no signs of lameness when the experiment was discontinued. At that time they were in excellent condition.

REPORT ON THE HISTOLOGY OF THE RIB-JUNCTIONS OF THOSE ANIMALS WHICH CAME TO AUTOPSY.

By V. KORENCHEVSKY.

The histological investigation was performed in the usual way, the rib-junction being previously partially decalcified in Müller's fluid.

Pig 124. The line of the zone of provisional calcification is uneven and lime deposition deficient. Protuberances of proliferating cartilage penetrate into the spongiosa. In many places in the centre of the trabeculae of the primary spongiosa, islands of cartilage cells are present. The trabeculae are curved and interlaced, and consist to a considerable extent of osteoid tissue. In the secondary spongiosa osteoid tissue surrounds only some of the trabeculae, and in a diminished degree. Under the periosteum rachitic osteoid proliferations are to be seen.

Diagnosis. Rickets.

Pig 126. The picture of the costochondral junction of the ribs of this animal is the same as that of *Pig 124*, with the exception that the amount of osteoid tissue is rather less.

Diagnosis. Rickets.

Pig 128. The histological examination of the ribs shows a convex and uneven line at the zone of provisional calcification with slightly deficient lime deposition, and protuberances of proliferating cartilage into the bone marrow. The primary spongiosa consists of interlaced thin trabeculae, the centre of which contains islands of calcified cartilage cells surrounded by an increased amount of osteoid tissue. The secondary spongiosa does not contain an abnormal amount of osteoid tissue, but the cortical bone does.

Diagnosis. Slight rickets.

Pig 130. Slight osteoporosis only.

Pig 121. Almost normal structure of bone.

Pig 123. Slight osteoporosis only. A photomicrograph of the costochondral junction of this pig is shown in *Fig. 10*.

Pig 125. The line of provisional calcification is convex, slightly uneven, and in some places calcification is deficient. The trabeculae are thin, but well calcified and do not show an abnormal amount of osteoid tissue.

Diagnosis. Osteoporosis.

Pig 127. The histological examination of the ribs shows the line of zone of provisional calcification to be convex and uneven in some places. Lime deposition is deficient. Here and there, protuberances of the proliferating cartilage penetrate into the bone marrow and the reverse. The primary spongiosa consists of curved interlaced trabeculae, in the centre of which are islands of calcified cartilage cells surrounded by abnormally increased osteoid tissue. The cortical bone and the secondary spongiosa in a lesser degree contain a small increased amount of osteoid tissue.

Diagnosis. Rickets.

A photomicrograph of a section of the costochondral junction from this pig is shown in *Fig. 11*.

Pig 133. The histological examination of the ribs reveals a picture similar to that of *Pig 124*.

Diagnosis. Rickets.

Pig 136. The picture of the costochondral junction of the ribs of this animal is similar to that of *Pig 127*.

Diagnosis. Rickets.

Pig 138. The line of the zone of provisional calcification is uneven, but there is no unusual proliferation of cartilage cells. The primary spongiosa is irregular and some of the trabeculae are surrounded by an increased layer of osteoid tissue. In the deeper spongiosa abnormal osteoid tissue is not seen. Under the periosteum the trabeculae consist of rough calcified bone tissue in the centre which is surrounded by osteoid tissue.

Diagnosis. Mild rickets.

Pig 134. The histological examination of the ribs revealed the line of zone of provisional calcification to be convex and slightly uneven; lime deposition is deficient. The proliferating cartilage is not increased. The primary spongiosa is scanty, its trabeculae irregular and interlaced. Osteoid tissue is slightly increased in the primary spongiosa. In the trabeculae islands of cartilage cells are present. The secondary spongiosa is osteoporotic.

Diagnosis. Very slight rickets with osteoporosis.

The above-mentioned changes in the pigs are very similar to those described by Korenchevsky [1922] on rats kept on a diet deficient only in the fat-soluble factor.

In conclusion I desire to thank Mr Dennis for the photomicrographs which accompany this report.

CONCLUSION.

From the preceding results it is seen that out of ten animals which went "off their feet," the bones of which have been examined histologically by Prof. Korenchevsky, eight, namely Pigs 124, 126, 127, 128, 133, 134, 136 and 138, showed abnormal amounts of osteoid tissue and some changes in the proliferating cartilage, and in his opinion had been suffering from rickets. Only two failed to show significant amounts of osteoid tissue. The histological picture in these cases was that of osteoporosis. That the rachitic condition was brought about by the consumption of a deficient diet is shown by the fact that on rectifying the ration by the addition of cod-liver oil and fresh full-cream milk (Pigs 122, 129 and 130), the animals were restored to normal health. The constitution of the diet leaves little doubt that it was the deficiency of the antirachitic vitamin which was responsible for the production of the disease, as the diet was complete in all essentials, with the exception of this factor. The striking feature in this investigation is that pigs which developed rickets grew well in the early part of the experiment. In our previous experiments [Golding, Zilva, Drummond and Coward, 1922], the fat-soluble vitamins were more rigorously excluded from the diet with the result that growth was soon greatly impeded, and although a faulty deposition of calcium was demonstrated and a condition of osteoporosis resulted, rickets did not develop. In the present experiments sufficient fat-soluble vitamin A was introduced through the medium of the dried separated milk to induce growth. As has been shown [Zilva, Golding, Drummond and Coward, 1921], very little of the growth-promoting vitamin is necessary for pigs, and the amount supplied was sufficient to produce good growth. When the gain in weight of these animals is compared with that of our previous sets as tabulated below it is seen that not only did these pigs grow much better than the animals on the diets more restricted in the fat-soluble factors (- Ca and - Vitamin A), but that they thrived almost as well as the control animals (+ Ca + Vitamin A). We are, therefore, forced to conclude that the presence of a small amount of

the fat-soluble vitamins or vitamin, sufficient to induce growth but not the deposition of calcium commensurate with this rate of development, is a favourable condition for the production of rickets in pigs. This view has been urged by Mellanby in his pioneer work on experimental rickets, by Korenchevsky [1922], and by many other workers since. The presence of large quantities of dried separated milk in a diet fulfils this condition admirably. This is one more instance to add to those brought forward by Mellanby [1921], Korenchevsky [1921, 1922] and Goldblatt [1923] in which rickets is produced by fat-soluble vitamin deficiency alone.

Diet	Weight in lbs. on June 3, 1921 (53 days old)	Gain in lbs. in 54 days
- Vit. A - Ca	29.5	20.8
- Vit. A + Ca	30.0	21.0
	22.5	23.5
	30.5	25.5
+ Vit. A - Ca	28.5	34.6
	26.5	28.0
+ Vit. A + Ca	21.0	30.0
	34.6	40.0

No. of pig	Weight in lbs. on April 21, 1923 (62 days old)	Gain in lbs. in 56 days
124	33.0	38.0
126	41.0	37.5
128	30.5	51.0
127	39.0	44.5
133	40.0	34.0
136	44.5	22.7
138	38.5	32.5
134	39.0	34.5

The results are of interest from the point of view of the practical agriculturist, since pigs are sometimes brought up on rations with a content of the fat-soluble vitamins similar to that of our rachitic diet, and it is therefore not surprising that when apparently thriving they suddenly go "off their feet."

On the assumption that these observations hold true for human beings, the danger of using dried separated milk "fortified" with inactive fats for infant feeding becomes evident.

In this connection we wish to stress the point that the production of rickets in our animals can in no way be associated with a deficiency of mineral constituents in the diet especially of calcium and phosphorus. As will be seen above, these elements were consumed in abundance in the form of inorganic compounds. The ratio of Ca/P ingested was 1/0.6 which, according to the investigations of McCollum, Simmonds, Shipley, and Park [1922], Goldblatt [1924] and Boas and Chick [1924], is not favourable to the production of rickets. We cannot, therefore, support the view of Orr and his collaborators that rickets in pigs is necessarily due to a deficiency of mineral constituents in the diet.

It is of interest to note that two pigs on the diet free from the anti-scorbutic factor developed scurvy. This was fully confirmed by the post-mortem examination of the animals. Plimmer [1920] recorded a case of four

young pigs fed on a diet of meal, sharps, and boiled turnips which developed symptoms conjectured by him to be those of scurvy. On giving raw swedes to these animals the symptoms improved. No post-mortem evidence was, however, produced to show that the animals were really suffering from scurvy. We have made several attempts to induce this disease in pigs by dietetic means and, with the exception of the above mentioned cases, have failed.

In conclusion we wish to express our best thanks to Professor V. Korenchevsky for his valuable help in the preparation and the interpretation of the histological specimens of the ribs.

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Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9

Fig. 10.
Photomicrograph of section of costochondral junction of rib of pig 123
partially cured by sunlight.

Fig. 11.
Photomicrograph of section of costochondral junction of rib of pig 127 showing rickets.

Sp. = Spongiosa.	BM = Bone marrow.
ZCC = Zone of columns of proliferous cartilage cells.	
ZHC = Zone of hypertrophic cartilage cells.	
ZPC = Zone of provisional calcification of proliferous cartilage.	
CB = Calcified bone.	P = Periosteum.
Ost. = Osteoid tissue.	L = Lime deposition.