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

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Clinical and experimental observations tend to show that diet plays an important part in the etiology of rickets and much of the evidence obtained by the clinician goes further to demonstrate that the results obtained with certain oils and fats in the prophylactic and the curative treatment of the disease is quite marked. The recent experimental work of Mellanby [1918 and 1919] throws a very interesting light on the therapeutic value of fats and oils. Mellanby has shown that by keeping puppies on a certain basal diet, definite rickets could be induced in these animals and that the disease could be prevented or cured by the addition of certain substances, mostly oils and fats, to the basal diet. Of the substances which were capable of producing this beneficial effect the majority were found to be identical with those which contain the fat-soluble A factor. This important observation suggested the possibility that the fat-soluble factor or another accessory factor closely associated with it was concerned in the prophylaxis of rickets. However, investigations carried out clinically and experimentally in order to test this theory of the etiology of rickets have yielded results from which no definite conclusions can yet be drawn. Hess and Unger [1920] from observations made on groups of infants receiving diets some of which were rich, others deficient in the fat-soluble factor, could not obtain any definite evidence that the rickets which developed in some of their patients could be traced to an accessory factor deficiency. Harden and Zilva [1919] kept young monkeys on a diet deficient in the fat-soluble factor for several months. The animals declined, but no rickets developed. Mackay [1921] fed kittens on a diet deficient in this factor, but otherwise theoretically adequate, with the result that the animals ceased growing. No evidence of rickets however could be established at the post mortem examination. McCollum, Simmonds, Parsons, Shipley and Park [1921] recently analysed a series of diets on which rats developed rickets and although the

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results pointed to the possibility that a deficiency of the fat-soluble factor or calcium might be responsible for the production of the disease, the authors considered that with the experimental evidence so far at their disposal they were only justified in concluding that faulty nutrition was the cause of the observed rachitic changes.

We are at present engaged in studying various dietetic problems including the etiology of rickets in the pig. The pig is a very suitable animal for such a purpose as it is susceptible to rickets, is omnivorous and can be reared away from its mother on experimental diets.

The cause of rickets in the pig has often been attributed by agriculturists to a dietetic deficiency—calcium and phosphorus being the suspected elements. Thus one finds statements in various text-books on scientific feeding that pigs fed on potatoes, whey, maize and cereals develop rickets. If on the other hand such diets are supplemented by clover or meadow hay the disease does not occur. Although calcium is usually considered to be the limiting factor it will be noticed that the diets which are alleged to produce rickets in the pig are at the same time deficient in the fat-soluble factor, whilst clover and meadow hay besides supplying calcium also form a source for this accessory factor.

Herter [1898] on the assumption that fat deficiency might be responsible for the production of rickets placed young pigs two months old on a diet of skimmed cow's milk. The animals deteriorated after several months but in the author's opinion rickets did not develop. It is, however, of interest to note that Herter found that phosphates were imperfectly absorbed when this diet low in fat was used. The diets were of course also low in the fat-soluble factor.

Our primary object was to find out whether pigs, brought up from birth on a diet rigorously restricted in the fat-soluble factor only, would develop rickets with the regularity with which animals, which are susceptible to scurvy, develop this disease on a scorbutic diet. Owing to the exacting attention that such experiments require we could only deal at first with a limited number of animals. The results obtained concerning the original object in view were not definite, but other observations of interest were made which will be discussed in this communication.

Experimental.

The experiments were carried out at the Reading University College Farm, Shinfield. Four animals, *A*, *B*, *C* and *D*, which were divided into two groups, were employed in this experiment. Group I, consisting of *A* and *B*, were placed on a diet containing the fat-soluble factor, whilst Group II, consisting of *C* and *D*, were kept on a diet rigorously restricted in that factor. The animals, which weighed three pounds each at the commencement of the experiment, were young Berkshire boars farrowed on August 13th out of "Whitley Sensation" by second prize boar "Swinton Cogniac." On August 17th they were placed on the experimental diets. During the first three days a mixture resembling

in composition sow's colostrum was given to the animals. The diets were of the following composition:

Group I.		*Group II.*	
Extracted dried milk	7·75 g.	Extracted dried milk	7·75 g.
Cream (cow's milk)	20·2 ,,	Autoclaved olive oil	9·5 ,,
Purified caseinogen	12·6 ,,	Purified caseinogen	13·0 ,,
Salt mixture	0·28 ,,	Salt mixture	0·36 ,,

The above was made up to 100 cc.

On August 20th the above diets were changed to mixtures approximating in composition to sow's milk, which were made up according to the following formulae:

Group I.		*Group II.*	
Fresh cow's milk	60 cc.	Extracted dried milk	6·7 g.
Crude caseinogen	5·0 g.	Purified caseinogen	4·9 ,,
Cream (cow's milk)	5·0 ,,	Autoclaved olive oil	4·6 ,,
Salt mixture	0·66 ,,	Salt mixture	0·63 ,,

The above was made up to 100 cc.

To insure the solution of the caseinogen 5 % sodium bicarbonate (20 cc. per 100 g. of caseinogen) was used.

Daily doses of decitrated lemon juice and "marmite" were administered to supply the antiscorbutic and the antineuritic requirements of the animals.

The dried separated milk was extracted with light petroleum for two to three days.

The cream was freshly separated and was obtained daily from the Reading College Dairy. Its average fat content, as determined by the Gerber method, was 42 %.

The caseinogen was rendered free from detectable traces of the fat-soluble factor by being heated for 24 hours in shallow layers at 120° C. and being subsequently extracted for one day with 90 % alcohol and for two days with light petroleum. The olive oil was autoclaved for ½ hour at a pressure of two atmospheres. The salt mixture was of the same composition as that employed by McCollum and his collaborators.

As will be seen, the chief difference between the diets in the two groups was in the content of the fat-soluble factor. In Group II the active cream was replaced by the inactive olive oil.

At the commencement of the experiment the little pigs were fed by bottle every two hours day and night. After August 23rd the intervals were extended to three hours. By the end of August the animals received six meals a day, which were further reduced by September 15th to five meals, with a night interval of six hours. The diets were given *ad lib.* and the quantities consumed were recorded.

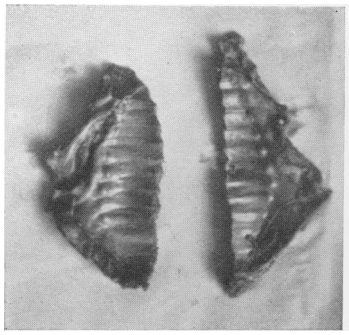
At first the intake of food and the growth of the two groups were approximately the same. After a few days, however, the animals in Group II commenced consuming less food than the animals in Group I and at the same

time ceased growing, whilst the control animals continued to gain in weight. See Photograph 2, Plate II.

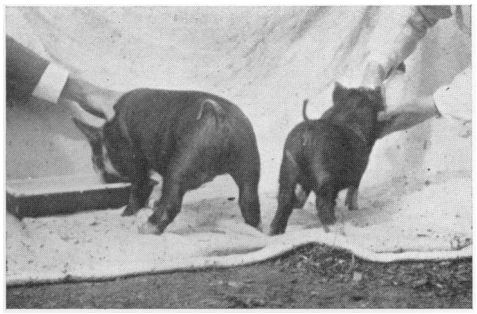
On September 14th pig *D* died during the night. A skiagraph was taken soon after death, but no abnormalities could be discerned. At the post mortem examination very pronounced "beading" at the costo-chondral junctions of the ribs was observed. Photograph 1, Plate II, shows the appearance of the costo-chondral junctions of the ribs of this animal. No other abnormal changes were established. The analysis of one of the long bones gave the following figures: moisture, 20·1 %; CaO, 29·3 % of the dry weight. We have so far been unable to obtain a really normal animal of the same age for comparative purposes. Histological preparations of the ribs, which will be discussed later, were kindly made for us by Miss F. M. Tozer, to whom we take this opportunity of expressing our best thanks.

In order to save the other declining animal in this group, cream was added to its diet in the same proportion as that in the diet of Group I. It was intended to supply a small amount of the fat-soluble factor sufficient to stop further decline. The animal soon resumed growth and the cream was accordingly discontinued on September 27th. From September 14th this animal received, besides the artificial milk mixture, an addendum of a basal mixture made up of 150 parts of starch, 40 parts of purified caseinogen and 10 parts of the salt mixture. After the cream was discontinued crude caseinogen was employed in this mixture instead of the purified substance in order to supply a further very limited amount of the fat-soluble factor. An average of about 250 g. of this mixture was consumed by the animal per day. From October 16th purified caseinogen was again employed, and on October 26th the artificial milk was replaced by a mixture consisting of olive oil, extracted dried milk, basal mixture and salt mixture. The olive oil and the caseinogen were discontinued on November 22nd. At this period the animal was consuming about 2 lb. of starch and $1\frac{3}{4}$ lb. of extracted fat-free dried milk per day. Towards the end of the experiment, owing to a temporary shortage of extracted dried milk, a mixture of starch and purified caseinogen was again resorted to.

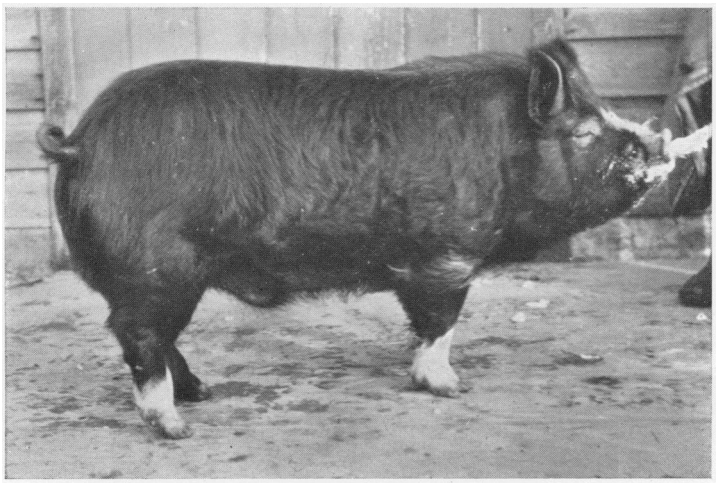
As will be seen from Fig. 1, which represents the weight curves of the animals, pig *C*, in spite of the restricted diet, kept on growing after the resumption of growth produced by the administration of the cream even better than the animals in the control group. No symptoms of rickets were observed during the experimental period. A skiagraph was taken on November 9th and a normal picture was obtained. The animal was slaughtered on January 19th. At the post mortem examination the following was found: Skin—good and clean. Thyroid glands—more connective tissue than in the control animal. Femur decidedly thicker and distinct enlargement of all bones at knee joint. Tarsal bones thicker than in control. Junctions between tarsals and metatarsals also thicker than in control. Spleen—a little heavier than in control. Liver—superficial foci of inflammation; weight of liver, $2\frac{1}{4}$ lb. More fat was found in this animal than in the control. Kidney normal. Adrenals



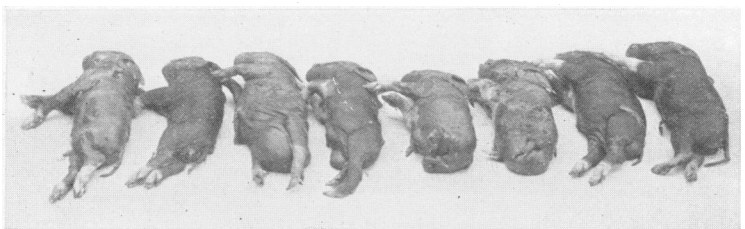
Photograph 1. Ribs from *D*.



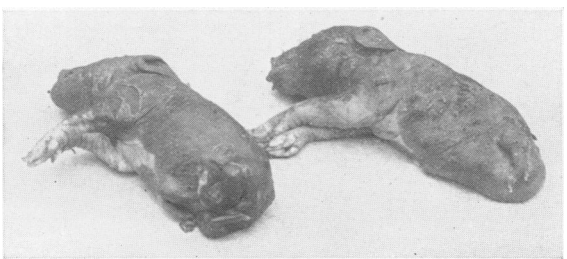
Photograph 2. *A* and *C* 28 days old.



Photograph 3. *C* before being slaughtered.



Photograph 4. Litter from Sow Lot I.



Photograph 5. Litter from Sow Lot I.

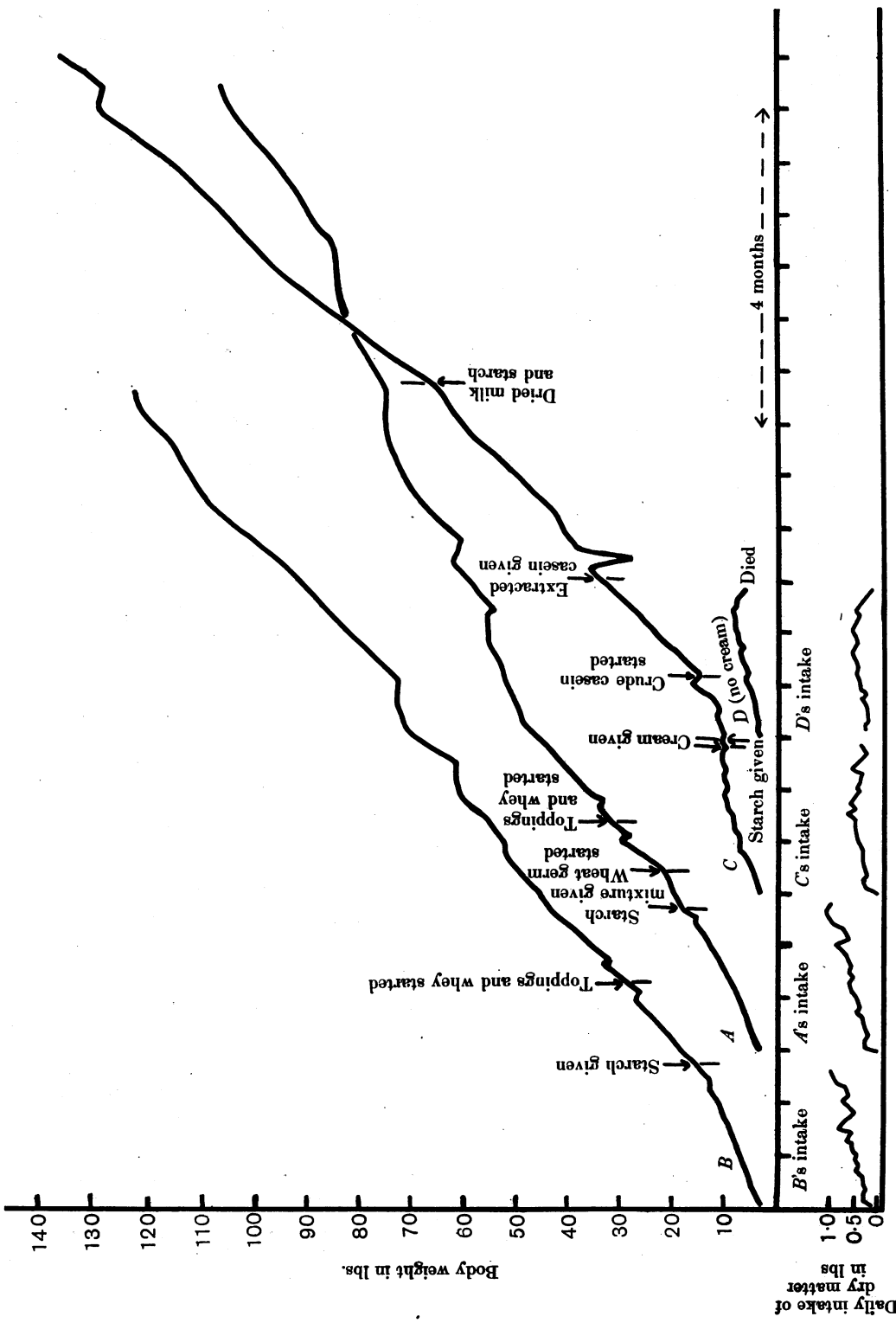

Body weight in lbs.
Daily intake of dry matter in lbs
B's intake
A's intake
C's intake
D's intake
4 months
Starch given
Toppings and whey started
Starch mixture given
Wheat germ started
Toppings and whey started
Cream given
Crude casein started
Extracted casein given
Dried milk and starch
Died
Starch given
A
B
C
D (no cream)

Fig. 1

normal. Ribs—angle of junction between bones and cartilage at costo-chondral junction markedly greater than in the control. In attempting to cut with a knife the same resistance was met as in the case of the control. The cartilage at the costo-chondral junctions strikingly thinner than the bony part as compared with the control animal. Longitudinal section at costo-chondral junction is decidedly wider than in the control and the line of active cartilage is also a little wider. Heart and lungs quite normal. Weight of leaf fat, 1 lb. 14½ oz. The analysis of one of the long bones gave the following figures: moisture, 14·7 %; CaO, 37·6 % of the dry weight. Photograph 3, Plate II, is a picture of pig *C* before being slaughtered.

In the control group pigs *A* and *B* commenced showing weakness in their legs, animal *A* in the hind and animal *B* in the front limbs and afterwards also in the hind legs. The wooden floor was at this stage replaced by bricks and the animals somewhat improved soon after the change. This affection of the limbs was definitely established to be non-rachitic. On September 13th an addendum of basal mixture and wheat germ was made to both diets. From September 20th lucerne was given to pig *B* and from October 1st about 15 g. of crude cod-liver oil was administered to pig *A*. On October 6th the milk was discontinued in both cases, pig *A* receiving basal mixture, wheat germ, toppings and whey; pig *B* receiving crude caseinogen, toppings and whey. From November 22nd the diet of pig *A* consisted of toppings, whey and cod-liver oil, which was supplemented by maize gluten on December 23rd. The diet of pig *B* was supplemented by maize gluten on December 15th. Pig *B* was killed on January 19th and pig *A* on February 17th. At the post mortem examination no rickets could be established in either case. A piece of wire was found to have penetrated the stomach of pig *A* and to have caused local inflammation which most probably was responsible for the ill-health of the animal during the last few weeks of the experiment. The analyses of the long bones gave the following figures: pig *A*, moisture 16·8 %, CaO 35·5 % of dry weight.

On three occasions during the early part of the experiment the weights of our experimental animals were compared with those of the little pigs of the same litter which remained with the mother. On September 8th the pigs in the litter weighed 16½, 15, 13, 14½, 14¼ lb. whilst pig *A* weighed 14 lb., pig *B* 12¾ lb., pig *C* 8 lb. and pig *D* 9¾ lb. On September 24th the pigs in the litter weighed 21¾, 23¾, 26¼, 18½ lb., and pig *A* weighed 25½, pig *B* 22¾, pig *C* 12¼ lb. On November 26th the average weight of the litter pigs was 54·7 lb., whilst pig *A* weighed 65¼, *B* 72¼ and *C* 72 lb. It is therefore evident that the pigs brought up artificially gained more weight than the animals which remained with the mother.

The ribs of pigs *A*, *C* and *D* were examined histologically and the observations may be summed up as follows:

(1) A moderate increase in the number of proliferating and hypertrophic cartilage cells was found only in pig *C*.

(2) A deep penetration of blood vessels from the bone marrow into the cartilage was observed in all the three cases.

(3) The presence of fibro-cellular tissue instead of normal marrow was found in all cases. It was more pronounced in the case of pig *C*.

(4) The irregularity of the line at the costo-chondral junction was also recorded. This was most marked in the case of pig *D*.

The back and leaf (perinephritic) fats from pigs *A*, *B* and *C* were tested by our standardised technique for the presence of the fat-soluble factor on rats, and as will be seen from Fig. 2 which represents the weight curves of these rats, the fats derived from animals fed on a diet containing this factor were found to be more or less active; the reverse was the case with the fats from the animal fed on a deficient diet. There was one discrepancy in the case of the leaf fat of pig *B* which appears to have been due to an irregularity in the technique; unfortunately we did not have enough of this sample to repeat the test. These observations confirm the results we [1920] have previously obtained with pigs fed on diets of varying contents of the fat-soluble factor.

No marked difference was found in the water, connective tissue and nitrogen contents of the two fats. The refractive indices, the Polenske, iodine and potash absorption values were also almost identical.

The results of our experiments offer no definite information on the relation of the fat-soluble factor to the etiology of rickets. We are however of the opinion that if the deficiency of this factor alone bore the same relation to the etiology of rickets as that of the antiscorbutic and antineuritic factors to the etiology of scurvy and beri-beri, we should have obtained a better differentiation in the rachitic condition in the two groups, in spite of the limited number of animals employed. As it is, we find it impossible with the results so far at our disposal to explain the decided, although slight, rachitic changes observed histologically in our normally-fed animals.

The above experiments, however, bring into prominence the following points:

(*a*) That when pigs are "off their feet" it does not necessarily imply that it is due to rickets. The affection of the limbs of pigs *A* and *B* was decidedly not of a rachitic nature.

(*b*) That the requirements of the pig for the fat-soluble factor are of a low order. This has already been pointed out by us [1920] in a previous communication. In this investigation the comparatively small quantity of cream and crude caseinogen consumed by pig *C* was sufficient to act as a source of the fat-soluble factor for the animal during about four months.

(*c*) That the fat-soluble factor promotes growth in the pig. This was demonstrated by the resumption of growth of pig *C* on the addition of cream to the restricted diet. It was further confirmed by the following experiment, which is a continuation of that described in the previous communication already referred to and in which we kept pigs on diets consisting of toppings

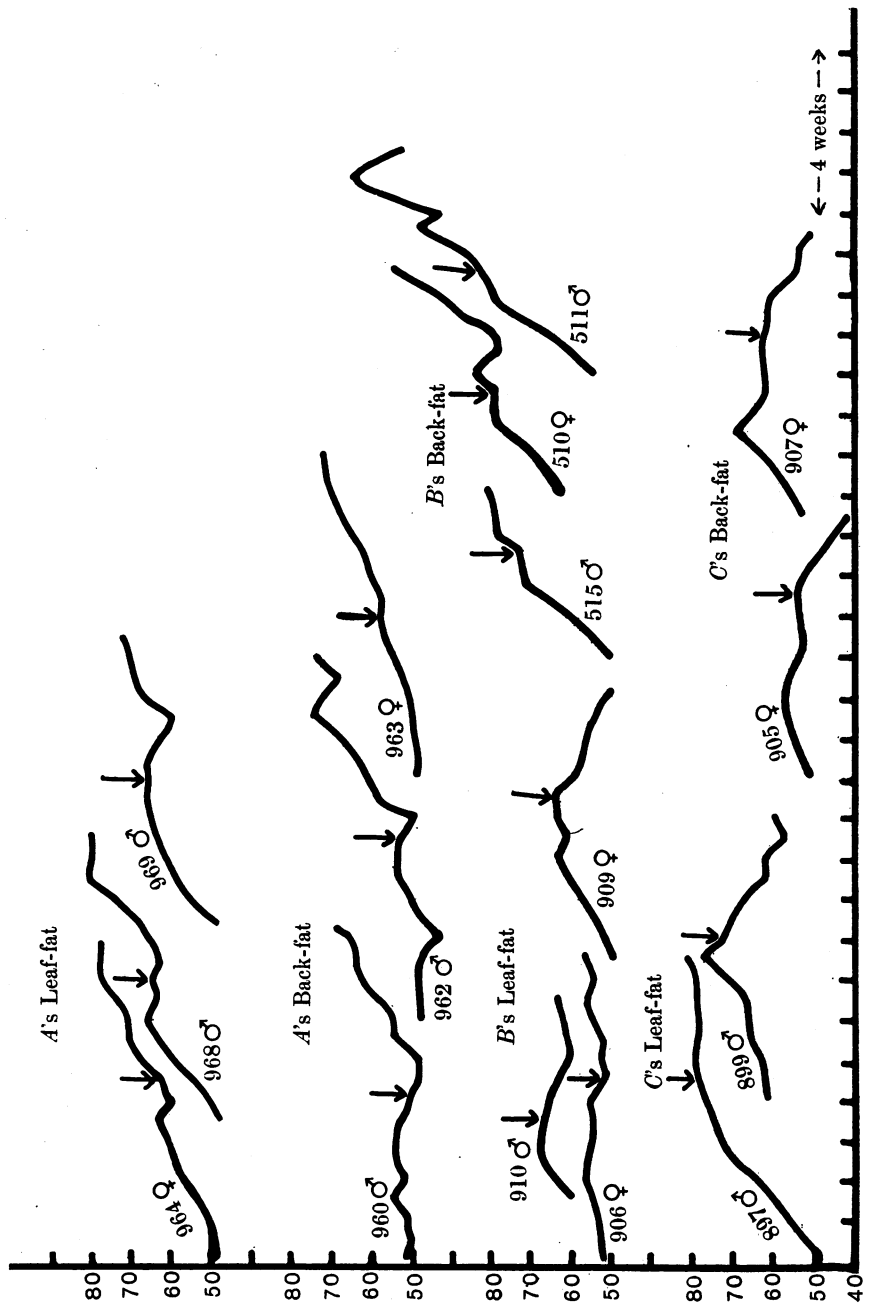

Body weight in grams.
80
70
60
50
40
A's Leaf-fat
964♀
968♂
999♂
A's Back-fat
960♂
962♂
963♀
B's Leaf-fat
910♂
906♀
909♀
B's Back-fat
515♂
510♀
511♂
C's Leaf-fat
898♂
899♂
C's Back-fat
905♀
907♀
← 4 weeks →

Fig. 2

and synthetic whey (Lot I) and toppings and ordinary whey (Lot II). Both these diets were highly deficient in the fat-soluble factor, the diet of Lot I being the more restricted. In the early stages of the experiments these animals showed a considerable growth which eventually slackened off. On September 23rd the sows belonging to Groups I and II had their restricted diets supplemented by an addendum of the fat-soluble factor. At this time the animal in Lot I had practically ceased growing, whilst the animal in Lot II was growing at a rate much below the normal. It was decided to administer the fat-soluble factor to the sow in Lot I in the form of crude cod-liver oil which was found to be very active in the case of rats and to the sow in Lot II in the form of lucerne. Lot I was given at first $\frac{1}{4}$ oz. of cod-liver oil per day; this was after five days increased to $\frac{1}{2}$ oz., and after 42 days this was again increased to 1 oz. per day for 20 days. The animal consumed the oil with great alacrity and resumed growth immediately after the commencement of the treatment. Lot II was started at first on $\frac{1}{2}$ lb. of lucerne per day; this after five days was increased to 1 lb., and after two weeks to 4 lb. per day. On December 3rd both sows were taken to the boar and became pregnant. They were then placed again on their original restricted diets. Fig. 3 represents the weight curves of these animals as well as that of the sow belonging to Lot III, which was fed all the time on toppings, whey and grass, a diet rich in the fat-soluble factor. On March 30th the sow in Lot I gave birth to eight pigs all of which were born dead or died very soon after birth.

One of the young lived for five hours and on post mortem examination was found to be normal except for a slight general oedema. Of the remaining seven pigs four showed a marked abnormality in the development of the hind limbs to a varying degree. This malformation was particularly pronounced in two cases (see Photographs 4 and 5, Plate II) in which the hind limbs were represented by thin tail-like appendages.

Oedema was generally present in all the eight bodies to a varying degree; four cases showing very marked ascites, hydropericardium and hydrocele, but there was no association between the severity of the oedema and the malformation of the limbs. The large hydroceles in these cases may be seen from the photographs. In nearly all cases there was more or less severe hydronephrosis.

The weights of the eight young were respectively 950, 1100, 1250, 900, 950, 1050, 1100 and 1350 g.

Analyses of the bones of three of the pigs were made, but their value is limited by the fact that only one analysis from a normal pig of the same age was available.

	Pigs from litter Lot I.			Normal control
	1	2	3	
	g.	g.	g.	g.
Weight of humerus	5·2	5·68	4·27	4·5
% moisture	41·6	46·3	44·5	40·9
% CaO on dry weight	34·45	33·71	32·80	37·24

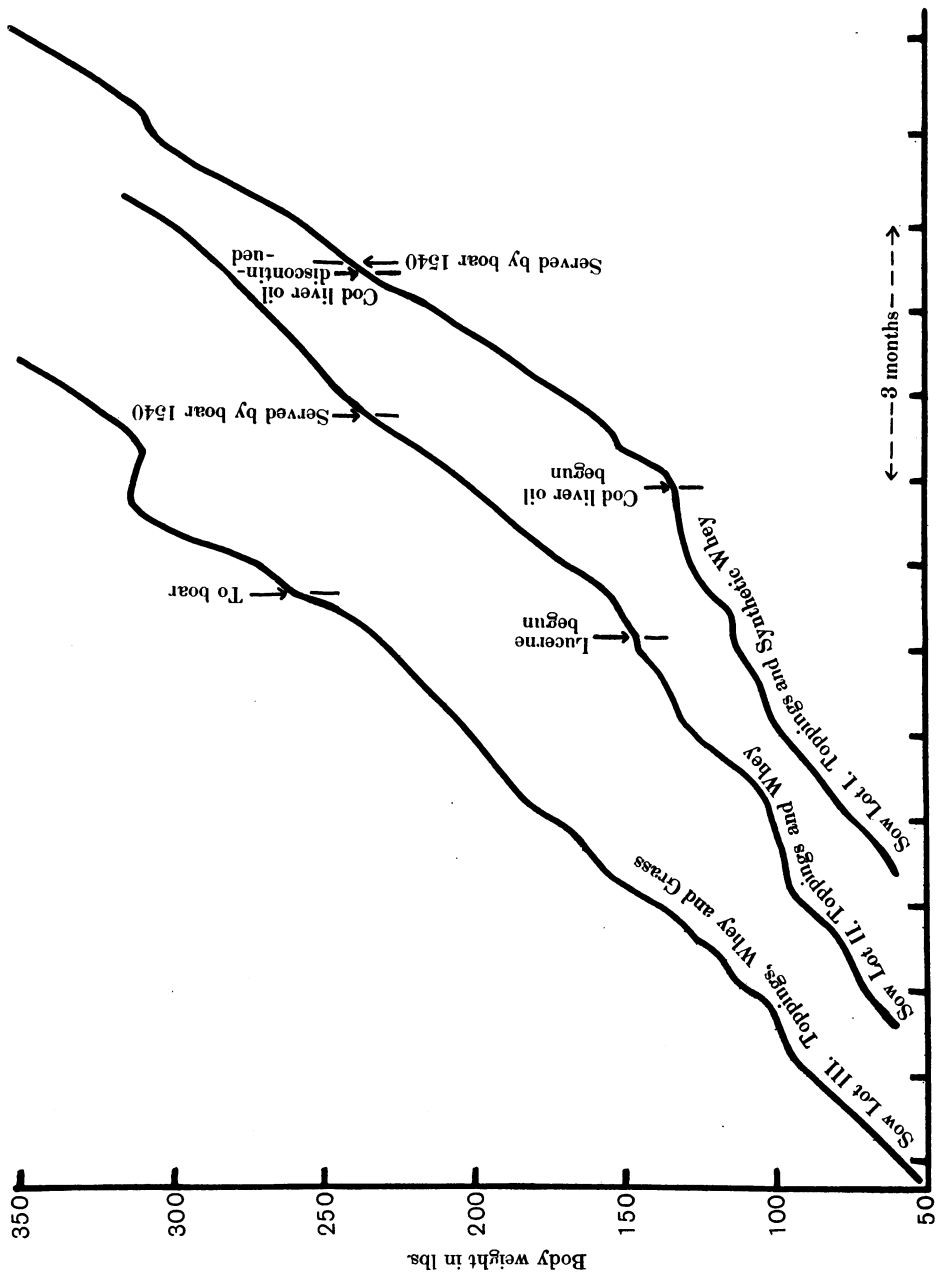

Body weight in lbs.
350
300
250
200
150
100
50
Sow Lot III. Toppings, Whey and Grass
Sow Lot II. Toppings and Whey
Sow Lot I. Toppings and Synthetic Whey
3 months
Cod liver oil begun
Lucerne begun
To boar
Served by boar 1540
Cod liver oil discontinued
Served by boar 1540

Fig. 3

We are not at present in a position to say whether this abnormal litter is to be ascribed to the drastically restricted diet which the mother had received, but further experiments are in progress.

The sow in Lot II farrowed down nine pigs on April 8th of which only one died.

In conclusion we wish to express our indebtedness to Mrs John Golding for the indefatigable help she rendered us during this inquiry.

The expenses of this research were defrayed from a grant made by the Medical Research Council, to whom our thanks are due.

Summary.

No definite rickets was induced in sucking-pigs fed from birth on a diet rigorously restricted in the fat-soluble factor.

The addition of the fat-soluble factor in the form of cream, cod-liver oil, and lucerne to a deficient diet stimulated growth in pigs declining in weight.

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