EXPERIMENTAL RICKETS IN RABBITS.

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Plates 21 to 23.

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INTRODUCTION.

Experimental rickets has been induced by dietetic means in dogs (Mellanby (1)), in rats (McCollum and collaborators (2); Sherman and Pappenheimer (3); and many others), and in swine (Zilva, Golding, and Drummond (4)). Although rickets is said to occur in rabbits (Jost and Koch (5)), up to the present time the experimental production of the disease in this animal has not been reported. One of us (Goldblatt), at the Lister Institute, London, attempted to study the effect of various deficient diets on the growth and on the bone development of rabbits and guinea pigs, but failed because the animals invariably refused to eat the synthetic diets and died.

For studies of metabolism and of blood changes in experimental rickets it is obviously an advantage to be able to produce the disease in a large yet relatively inexpensive laboratory animal. The purpose of the present investigation has been to determine whether or not it is possible to produce the disease in the rabbit by stomach tube feeding of diets which produce rickets in rats.

EXPERIMENTAL.

Series A.

Rabbits from 4 to 6 weeks of age and weighing from 250 to 500 gm. were employed in this investigation. They were kept in individual cages in a room shielded from direct sunlight. Every litter was divided into two groups, one receiving the rickets-producing diet and the other the same diet containing 2 per cent cod
liver oil. In some of the experiments the cod liver oil was administered separately, by pipette, and in others, mixed with the food. Two diets were used.

**Diet I.**—
- Egg albumin (Merck) ....................................... 20 gm.
- Wheat starch .............................................. 50 "
- Cottonseed oil ........................................... 15 "
- Salt mixture (No. 4, Korenchevsky (6)) ....................... 5 "
- CaCO₃ .................................................... 2 "
- Yeast (Fleischmann's) ..................................... 5 "
- Agar ..................................................... 5 "

**Diet II.**—This was No. 3143 of McCollum and collaborators (7), modified by the addition of 2 gm. of powdered agar per 100 gm. of the mixture.

Both diets are deficient in the antirachitic organic factor and have a low phosphorus but high calcium content. All of the solid ingredients were finely powdered and the diet mixtures passed through a fine flour sieve. To the ration, just before feeding, were added 2.5 cc. of decitrated lemon juice and an amount of distilled water only sufficient to make the ration fluid enough to pass with ease through a No. 12 catheter which was used as a stomach tube. No difficulty was experienced in the matter of passage of the stomach tube. In this investigation the tube has been passed about 1000 times. In the beginning, 5 gm. of the diet was given twice daily, and at the end of 3 days this was increased to 7½ gm. After 1 week the ration was increased to 10 gm. and at the end of 10 days to 15 gm. twice daily. This quantity was continued to the end of the experimental period.

One complication seriously interfered with the earlier experiments in this investigation. Within a week or two from the time artificial feeding was begun most of the animals, controls included, developed diarrhea, lost weight very rapidly, and died.

Of twelve rabbits that received Diet I, only two survived as long as 3 weeks. Of these two, at autopsy, one had severe rickets and the other moderate rickets. Those that died in 2 weeks or less showed only varying degrees of osteoporosis. Of eight control animals on Diet I containing 2 per cent cod liver oil, one lived as long as 3½ weeks. None of the controls showed any signs of rickets; but the bones were osteoporotic.

Only three out of twelve rabbits that received Diet II lived as long as 3 weeks and of these one had severe rickets and the other two moderate rickets. The remaining nine had merely varying degrees of osteoporosis. Seven control rabbits that received Diet II containing 2 per cent cod liver oil lived for varying periods up to 3½ weeks. All
finally developed severe diarrhea and died but showed no macroscopic or microscopic evidence of rickets.

The intestinal disturbance did not appear to be of infectious origin. At autopsy there was no evidence of acute enteritis or colitis, but the entire small and large intestine contained feces in a liquid or semi-solid state. There seemed to be a total inability to concentrate the intestinal contents.

Series B.

In order to supply the animals with more roughage than they were getting from the agar in the diets, advantage was taken of the work of Nelson and Lamb (8), who advised the administration of alfalfa to rabbits kept on synthetic diets. They used alfalfa meal, but we used whole alfalfa hay devitaminized by extraction for 36 hours with boiling alcohol in a flask with reflux condenser attached, and then by still extraction for 3 or more hours with ether. 5 gm. of the dried extracted alfalfa was allowed to every animal daily. They ate it readily, and from the time the administration of alfalfa began diarrhea ceased to develop.

Diet I.

Of a litter of five rabbits (see Table I) three (Nos. 3, 4, and 5) were given Diet I and alfalfa and two control rabbits (Nos. 1 and 2) received Diet I containing 2 per cent cod liver oil and the same ration of alfalfa. For 2 weeks they were fed Diet I by stomach tube and then it was found by trial that these rabbits ate the diet voluntarily. For the remaining 2½ weeks the rabbits were allowed to eat of their own accord and consumed on an average 35 gm. of the diet and 5 gm. of alfalfa daily.

X-Ray Examination.—At the beginning of the experiment, and at intervals of 1 week thereafter, radiograms of the animals were made. In the three rabbits on Diet I and alfalfa, definite abnormalities of the skeleton suggestive of developing rickets were noticeable by x-ray as early as 1 week after the commencement of the experiment, and from week to week the radiograms showed progressive changes. In the radiograms taken immediately before the animals were killed the rachitic metaphysis was very wide and long, there was definite cupping and fuzziness of the end of the diaphysis and imperfect calcification of the epiphysis. In the radiogram of one of the controls (No. 1; Fig. 2)
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<tbody>
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<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>gm.</td>
<td>gm.</td>
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<tr>
<td>1</td>
<td>F.</td>
<td>No. I + cod liver oil.</td>
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<td>620</td>
<td>8.9</td>
<td>11.9</td>
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<td>&quot; I + &quot; &quot; &quot; &quot; &quot;</td>
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<td>640</td>
<td>8.17</td>
<td>10.5</td>
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<td>775</td>
<td>3.25</td>
<td>13.5</td>
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<td>3.1</td>
<td>13.0</td>
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<td>&quot; I &quot; I</td>
<td>395</td>
<td>660</td>
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### TABLE II.

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<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>gm.</td>
<td>gm.</td>
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<tr>
<td>6</td>
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<td>7</td>
<td>&quot; II + &quot; &quot; &quot; &quot; &quot;</td>
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<td>550</td>
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<td>12.5</td>
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<td>325</td>
<td>Died early.</td>
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<td>620</td>
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<td>13.08</td>
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<td>Died early.</td>
<td>3.8</td>
<td>13.08</td>
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<td>380</td>
<td>3.6</td>
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a slight irregularity appeared at the end of the 3rd week, in the zone of provisional calcification, which persisted to the end. Control Rabbit 2 remained normal throughout the experimental period.

Clinical Examination.—In Rabbits 3, 4, and 5, deformity of the chest and enlargement of the costochondral junctions and of the epiphyses were apparent at the end of 2 weeks, and at the end of the experimental period these changes were very pronounced. Pot belly and muscular weakness developed during the last 10 days.

Macroscopic Examination.—At autopsy all the signs of very severe rickets were present in Rabbits 3, 4, and 5, and there was no gross evidence of rickets in the two control animals.

Microscopic Examination.—Histologically, the diagnosis of very severe rickets in Rabbits 3, 4, and 5 was confirmed, while the controls (Nos. 1 and 2) showed only slightly deficient osteogenesis in the slender trabeculae of the primary spongiosa, but these were numerous and well calcified and there was no other abnormality (see Fig. 6).

Chemical Examination.—From every rabbit, at the end of the experimental period, about 25 cc. of blood was obtained from the carotid artery. The amount of calcium in the blood serum was determined by the Kramer-Tisdall (9) method, and the amount of inorganic phosphate by the Tisdall method (10). In the rachitic animals the calcium content of the serum was slightly greater than in the controls, but the inorganic phosphate of the serum was very much lower (see Table I).

Diet II.

A litter of seven rabbits was given Diet II and extracted alfalfa. Three rabbits were chosen as controls and received the same diet containing 2 per cent cod liver oil. One of the controls died 3 days after the beginning of the experiment but the cause was not determined at autopsy. One animal on Diet II died of bronchopneumonia 11 days after the commencement of feeding. All were fed by stomach tube for 3 days and from then on allowed to eat of their own accord. They consumed on an average 30 gm. of the diet and 5 gm. of extracted alfalfa daily. The litter were killed at the end of 2½ weeks. Grossly and histologically, the controls showed no evidence of rickets while the remaining three rabbits showed from slight to moderate rickets. Chemical examination showed only slight differences in the percentage of calcium in the blood serum of the rachitic and control rabbits but a great diminution in the amount of inorganic phosphate in the blood serum of the rachitic rabbits (see Table II).
SUMMARY.

Rickets has been produced experimentally in rabbits by the administration of diets deficient in the antirachitic organic factor and phosphorus but with a high calcium content.

Cod liver oil added to these rickets-producing diets prevented the development of rickets in rabbits.

We are indebted to Professor H. T. Karsner for constant encouragement and valuable criticism. It is with great pleasure that we acknowledge sincere thanks to Professor T. W. Todd whose deep interest in the work and continued aid greatly facilitated the investigation, and to Miss W. Kuenzel for valuable technical assistance in the radiographic work. All the radiograms were taken in the Department of Anatomy.

BIBLIOGRAPHY.


EXPLANATION OF PLATES.

**PLATE 21.**

FIG. 1. A series of x-ray photographs of part of the right fore limb of Rabbit 3 that received the rickets-producing Diet I and extracted alfalfa. (a) to (e) were taken at intervals of 1 week, (f) was taken 3 days after (e). Note the progressive separation between epiphysis and diaphysis of radius and ulna caused by the development of the rachitic metaphysis, the cupping and imperfect calcification of the end of the diaphysis, and the imperfect calcification of the epiphysis.

FIG. 2. A series of x-ray photographs of part of the right fore limb of Control Rabbit 1 that received Diet I containing 2 per cent cod liver oil, and extracted alfalfa. The photographs were taken at the same time as those represented in Fig. 1. At the end of the 3rd week (d) a slight irregularity appeared at the junction of epiphysis and diaphysis of the ulna and this persisted. Histologically, there was no evidence of rickets in this zone, but the hypertrophic zone of proliferating cartilage showed a localized increase in the number of layers of cells. The zone of provisional calcification was very densely calcified.

FIG. 3. X-ray photographs of part of right hind limb of Rabbit 3 showing progressive changes at the epiphyseodiaphyseal junction in the lower end of the femur and the upper end of the tibia, similar to those described for the radius and ulna of the same rabbit (see Fig. 1).

FIG. 4. Right hind limb of Control Rabbit 1. Normal throughout the experimental period.

**PLATE 22.**

FIG. 5. Rabbit 3. Photomicrograph of part of the lower end of the radius. Section stained with hematoxylin and eosin. Note the great number of layers of hypertrophic cartilage cells in the zone of proliferating cartilage, and the partial disorganization of this zone by the ingrowth of blood vessels and bone marrow. The zone of provisional calcification is absent. The trabeculae of the primary spongiosa have a slender densely calcified core which is surrounded by a relatively large amount of osteoid tissue; and the cortical bone also shows excessive subperiosteal as well as endosteal osteoid. Diagnosis: severe rickets.

FIG. 6. Control Rabbit 1. Photomicrograph of part of lower end of radius. Section stained with hematoxylin and eosin. The trabeculae of the primary spongiosa are short and slender but numerous and very well calcified. The zone of provisional calcification is well calcified. There are only from four to six layers of hypertrophic cartilage cells in the zone of proliferating cartilage. Diagnosis: slight osteoporosis.
Fig. 7. Rabbit 3. Photomicrograph of costochondral junction. Section stained with hematoxylin and eosin. Note the great deformity; the excessive proliferation of hypertrophic cartilage cells; the absence of a zone of provisional calcification; and the excessive amount of osteoid tissue in the region of the primary spongiosa as well as in the cortex. Diagnosis: severe rickets.

Fig. 8. Control Rabbit 1. Photomicrograph of costochondral junction. Section stained with hematoxylin and eosin. The trabeculae of the primary spongiosa are slender but well calcified. There is a definite zone of provisional calcification. The zone of hypertrophic cartilage cells consists of only four layers. The cortical bone is thin and rarefied, but what is left is well calcified. There is less than the normal amount of osteoid surrounding the trabeculae and in the cortex. Diagnosis: moderate osteoporosis.
(Goldblatt and Moritz: Experimental rickets in rabbits.)
FIG. 5.

FIG. 6.

(Goldblatt and Moritz: Experimental rickets in rabbits.)
FIG. 7.

FIG. 8.

(Goldblatt and Moritz: Experimental rickets in rabbits.)