RICKETS IN DOGS.

METABOLISM OF CALCIUM AND PHOSPHORUS.

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For the production of experimental rickets, rats have been the animals most generally used. Mellanby (1, 2) and Jones (3) are the only recent investigators who have experimented extensively on dogs. Mellanby chose them because the course of rickets in puppies more closely resembles that in human beings. He maintains that this disease can be produced by the absence of vitamin alone, regardless of the calcium and phosphorus content of the diet.

It was our purpose to observe the metabolism of calcium and phosphorus in puppies on a rickets-producing diet, from an early stage until rickets developed. Diagnosis was established, as in Mellanby's study, by the appearance of the animal, radiographs, histological examination of the bones, and analysis of them for calcium. In addition, the calcium and inorganic phosphorus of the blood serum were measured in the food, urine, and feces, calcium and phosphorus were determined quantitatively, and the balances of these elements calculated.

Plan of Experiment.—Four mongrel puppies of a litter born September 20, 1925, were taken at the age of 1 week from the mother, which was a pet mongrel bitch of terrier type, weighing about 12 kilos. It was kept in a country place and allowed to run free. Its diet was mixed, principally table scraps. The young were not all of the same type. Three were medium sized long haired pups resembling their mother; the fourth, smaller and more dapper, was short haired.

The pups were raised on the bottle with cow's milk and 5 cc. of cod liver oil daily. 1 week before the experimental period three
of the puppies were given a diet consisting of 10 gm. of dried skim milk powder, 40 gm. of Quick Quaker Oats, cooked 5 minutes, 5 cc. of linseed oil, and 1 gm. of NaCl. This amount was given for each kilo of weight and equalled 250 calories per kilo. The other dog, the control, received cod liver oil instead of linseed oil. Each dog was given in addition 60 mg. of Harris yeast vitamin concentrate per kilo and daily 5 cc. of orange juice. The food was divided into two portions, one-half given in the morning and one-half in the evening.

Diets.—The diet of milk and oatmeal is relatively rich in calcium and poor in phosphorus. It was selected primarily because we desired to duplicate Mellanby's work (1, 2). Its Ca:P ratio is 0.66. Similar diets were used at the Lister Institute to produce rickets in rats, but the pathological condition which ensued was mild and the degree varied in the different animals. Mellanby does not claim that the diet produced the defect in every case, but it certainly produces severe rickets in dogs.

On November 8, when the puppies were 8 weeks old, they were placed in metabolism cages which permitted separation of urine and feces, and the excreta were saved from this date for the subsequent 10 weeks. Specimens from two of the dogs for the 1st week were lost; for these two dogs, the material studied represents the last 9 weeks. The experiment proceeded without other known errors. The same diet was given throughout, but only 225 calories per kilo were given during the 5th week and 200 calories per kilo for the last 5 weeks. All of the food was eaten. After the experimental period the dogs were given various additions of mineral salts to produce tetany. As the food was no longer eaten quantitatively the excreta were not analyzed. The animals were killed 2 months later, and the bones studied histologically and chemically. The diets had not been greatly altered, and the changes were not designed to cure the rickettotic process. Histological and chemical studies of the bones were made only as confirmation of the x-ray findings and blood studies.

Methods.—The feces were formed. They were dried on the steam bath with alcohol, ground, weighed, and sampled. The urine was preserved with thymol-chloroform, made to volume, and an aliquot was taken for analysis. Food, urine, and feces were analyzed for calcium and phosphorus. They were ashed
separately with HNO₃, and the ash was neutralized to methylred. The calcium was precipitated as oxalate and titrated with permanganate. The phosphate was determined by titration of the yellow precipitate,—a slight modification of Bang's method was used (4). The blood serum was analyzed for calcium by Clark and Collip's modification (5) of the Kramer-Tisdall method (6).

![Growth curves of dogs on a rickets-producing diet.](image)

Inorganic phosphorus in the serum was determined quantitatively, by Briggs' method (7). The x-ray pictures were made at the New Haven Hospital every week by courtesy of Mr. E. Furbish. Through the kindness of Miss D. Jackson, sections of the bones were decalcified in Müller's fluid and sectioned and stained with hematoxylin and eosin.
Results.

By all diagnostic standards, marked rickets developed in two of the three dogs which received no cod liver oil. The third dog, Dog 3, the runt of the litter, was the most active, grew the least, and had bones that showed only slight rickets. The control, which received cod liver oil, had practically normal bones. It showed laxity of the tendons, mentioned by Mellanby (1, 2), and the wrists bent under its weight. The two ricketic dogs were less active than the control and the small dog which escaped marked rickets. They all grew well (see Fig. 1) and were in excellent condition at the end of the experiment.

The appearance of the two animals diagnosed as ricketic was similar to that described by Mellanby and figured by him ((1) Fig. 2). The ricketic rosary was marked, and the wrists were enlarged. On section, the fresh long bones showed marked ricketic metaphyses, over $\frac{1}{2}$ inch long and 1 inch wide. These appeared to be composed of cartilage and shelled out in one piece. The
photographs of the fresh bones, the analysis of the bones for calcium, and photomicrographs of the bone sections are not offered as evidence because they were obtained 2 months later and have no quantitative significance for the condition at the time of the experiment. They show that although some slight healing had taken place, severe rickets was still present in the two dogs. The third showed slight rickets, and the control had practically normal bones.

The radiograph taken at the end of the experiment (see Fig. 2) brings out very clearly a condition of severe rickets in Dog 4. There were similar changes in Dog 5, slight changes in Dog 3, and good calcification in the control. The condition shown by x-ray is rickets, as advanced as any shown by Mellanby, and resembles closely his radiographs of the dogs after 9 weeks on a diet which contained a liberal supply of oatmeal ((2), Fig. 2, Experiment 509).

Blood examinations were made in the third period only, to avoid introducing a factor of anemia. The findings, which are given in Table I, are typical of low calcium rickets for Dogs 4 and 5 and are normal for the other two. The blood findings, the gross appearance of the animals, the bones, the bone analyses, and the radiographs, clearly establish the diagnosis of rickets.

### TABLE I.

**Blood Serum Analyses of Dogs on Rickets-Producing Diet of Skim Milk, Oatmeal, Linseed Oil, and NaCl.**

<table>
<thead>
<tr>
<th>Days of experiment</th>
<th>Dog No.</th>
<th>Ca (mg. per cent)</th>
<th>P (mg. per cent)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan., 1986</td>
<td>4</td>
<td>6.0</td>
<td>4.7</td>
<td>Advanced rickets.</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>7.7</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>6.7</td>
<td>3.9</td>
<td>Advanced rickets.</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>6.1</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>11.0</td>
<td>5.1</td>
<td>Mild, healing rickets.</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>9.0</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>10.1</td>
<td>5.4</td>
<td>Control, normal.*</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>10.2</td>
<td>5.3</td>
<td></td>
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</table>

* The control dog received cod liver oil instead of linseed oil.

*The control dog received cod liver oil instead of linseed oil.*
Metabolism of Calcium and Phosphorus.—The calcium and phosphorus balances which accompanied these changes, measure the effect of rickets upon the mineral metabolism. The periods during which metabolism studies were conducted are indicated in Fig. 1. Growth was uniform and satisfactory throughout. Dog 3 grew less rapidly than its litter mates, but was apparently a smaller type. The weights of the dry feces for the experimental periods are given in Table II. The data show that those dogs (Nos. 4 and 5) which became ricketetic, produced larger amounts of feces.

Calcium and phosphorus values of the food, urine, and feces, and the balances are given in Table III. They represent three periods of 3 weeks each (except for Dogs 4 and 5, which had 4 weeks in the first period) calculated in terms of 1 day. The weight of the dogs for each period was taken as the average of the initial and final weight. The dogs weighed slightly differing amounts. The values have been reduced to represent the metabolism per kilo of weight in order to afford a comparison of the separate periods and of the individual dogs.

Paths of Excretion.—Calcium was present in the urine in such small amounts that it could not be determined accurately and is negligible. Phosphorus in the urine averages 35 per cent of the excretion for all four dogs. The control does not differ markedly. This percentage differs from those previously reported for two normal infants (8) on a milk diet, who excreted two-
thirds of the phosphorus in the urine. The urinary phosphorus of rats on a high calcium-low phosphorus diet was, however, only 5 per cent (9). The amounts of calcium and phosphorus excreted in the urine and stool respectively are practically the same in the normal and ricketic subject. The proportions excreted by way of the urine and stool depend primarily upon the composition of the diet.

**TABLE III.**

*Metabolism of Calcium and Phosphorus on Rickets-Producing Diet.*

Mg per day; calculated per kilo of dog.

<table>
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<tr>
<td></td>
<td></td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>4</td>
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<td>36</td>
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<td></td>
<td>II</td>
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<td>55</td>
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<td></td>
<td>III</td>
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<td>57</td>
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<td>24</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>102</td>
<td>24</td>
</tr>
</tbody>
</table>

* The control dog received cod liver oil instead of linseed oil. For time of periods see Fig 1.

**Balance of Calcium and Phosphorus.—**The balance of calcium of all dogs was positive in all periods. The two ricketic dogs retained 53 and 50 per cent of the calcium intake; the dog which failed to develop rickets, on the same diet, retained 62 per cent and the control 77 per cent of the intake. The dogs received cod liver oil before the experimental period and some of the vitamin was probably stored, as is indicated by the difference between the
first period and the two subsequent periods in which the balances are less favorable. If, therefore, the first period is omitted, the values of retention become reduced to 45 and 42 per cent for the two ricketic dogs and remain practically the same for the two other puppies. This shows strikingly that the development of rickets, even on a low calcium diet, is associated with diminished, but not negative balances of calcium.

The balances show a greater deficiency of phosphorus than of calcium. One of the two ricketic dogs showed a negative balance in the third period. The average retentions corresponding to the calcium values given above are 16.2 and 8.5 per cent for the two ricketic dogs, 22.6 for the third puppy, and 34.5 per cent for the control. The averages for the last two periods are 7.8, -4, 19.2, and 31.2 per cent respectively. Thus the balances of calcium and especially of phosphorus are larger for the control than for the ricketic dogs.

The deficiency of phosphorus is further corroborated by the ratio of the retentions of calcium and phosphorus. The normal proportion of calcium to phosphorus retained was shown to be 1.58 for the rat (10). For normal infants, the value calculated from many published data closely approximates this. It may be assumed for our purpose to be similar for the dog also. Higher ratios represent excess calcium retention; lower ratios represent excess phosphorus retention. As shown in Table III, the two ricketic dogs, except for the first periods, showed an increased proportion of calcium retained. The third dog and the control represent calcium and phosphorus retention in normal proportions.

**Discussion.**

The metabolism data show marked differences between the control which received cod liver oil and the animals on the same diet, which received a fat without vitamin. Two of the experimental animals developed marked rickets. That the third did not develop severe rickets as measured by x-ray and blood findings may be due to the preliminary treatment with cod liver oil, or to the animal's failure to gain as rapidly as its litter mates, or to species difference. The bones, however, showed some evidence of rickets when examined histologically. The study of the metabolism indicates a condition midway between that of the ricketic
animals and that of the control. These data do not measure a qualitative difference but indicate a quantitative measurement of the defect in calcium and phosphorus metabolism. They are preferable, even, to an analysis of the bones, for these may show calcification at the expense of the rest of the body, but the metabolism of calcium and phosphorus measures this defect in the whole organism.

The study of the metabolism shows that development of severe rickets is associated not with a negative balance, but with a diminished positive balance of both calcium and phosphorus, as has been previously shown with the rat (9).

These data show that it is important to measure not only the calcium, but also the phosphorus, and to calculate the relationship between the amounts of these two elements retained. Although in this case the phosphorus in the food exceeded the calcium, the phosphorus balance was smaller or even negative. This indicates an exception to the law that the element of which least is consumed is the limiting factor in nutrition. Here the limiting essential lies in the unknown constituents of the diet. The action of vitamin D thus indicates a new mechanism in the stabilization of calcium and phosphorus metabolism.

Our data also point strongly to the undue and incorrect emphasis laid upon calcium in contradistinction to phosphorus in ossification. The process is universally referred to as calcification. The more important defect in rickets lies in the inadequate retention of phosphorus.

**SUMMARY.**

1. The study of the metabolism of calcium and phosphorus in puppies on a rickets-producing diet gives a quantitative measure of the degree of abnormality.

2. The calcium balances are positive but less than normal. The phosphorus balances are still more deficient and may be negative.

3. Even on a diet high in phosphorus and low in calcium the most marked deficiency lies in the phosphorus retention.
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