Antirachitic Potency of Vitamin D Sulfate in Human Milk

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Vitamin D activity of vitamin D sulfate in human milk was tested by prophylactic and curative methods using rats. The results were positive both in X-Ray and AOAC ash tests in agreement with the result of chemical assay.

In the foregoing papers, the authors have reported the relation between vitamin D and active sulfate metabolism (1-4), and the isolation of vitamin D sulfate from mammalian milk (5). Further tests for the antirachitic potency of human milk (containing 780 I.U. of total vitamin D in 2 ml as determined by the authors) by prophylactic and curative methods using rats previously fed on a vitamin D-deficient diet showed the positive results both in X-ray and AOAC ash test.

EXPERIMENTAL

1. Materials

The material used in the experiments were kindly supplied from the Red Cross Central Hospital of Japan, Tokyo. Fresh human milk secreted in 5-14 days after delivery was employed. The total vitamin D content was previously estimated by chemical assay to be 780 I.U., of which water soluble vitamin D sulfate was 750 I.U. in one liter.

Experiments were orally carried out with the amount corresponding to the human milk containing 780 I.U. of vitamin D per liter. At the same time, synthetic ammonium vitamin D₂ sulfate and pure vitamin D₂ were employed as the control experiment.

2. Experimental Animals

Twenty-four Wister strain male white rats, weighing about 35 g, were used. They were housed in the dark room kept under air condition at 24° and 40% humidity. They had been previously fed on the synthetic diet consisting, in percent, α-starch 37, sucrose 40, egg albumin 18, fat-free yeast and mineral matter 4 (NaCl 0.17, MgSO₄·7H₂O 0.27, iron citrate 0.12, NaHCO₃ 0.18, Ca(H₂PO₄)₂·H₂O 0.54, 

1 Metabolic activities of vitamin D in animals. VII.
2 佐橋佳一，鈴木隆雄，松田宮郎，浅野勉.
calcium lactate 1.00, CaCO₃ 1.10g. Ca : P is about 5) vitamin mixture (given daily per head, thiamine 40 μg, riboflavin 60 μg, calcium pantothenate 40 μg, nicotinic acid 40 μg, folic acid 10 μg, retinol acetate 20 I.U.).

3. Feeding of Animals for Prophylactic Test

Each four animals were divided at random into six groups: The first group was given daily 10 I.U. of vitamin D₂ in 0.02ml of ethyl palmitate, and served as the first positive control. The second group was orally given daily 10 I.U. of synthetic ammonium vitamin D₂ sulfate in 0.02ml of ethyl palmitate per head, and served as the second positive control. The third group was given daily 2ml of fresh human milk (containing about 1.5 I.U. of vitamin D). The fourth group was given daily the vitamin D-sulfate fraction (fractionated from 2ml of human milk). The fifth group was given daily the inorganic phosphate corresponding to the phosphate content in 2ml of fresh human milk. The sixth group was served as a negative control.

4. Feeding of Animals for Curative Test

The animals previously fed on the vitamin D-deficient ration, were subjected to the ordinary X-ray test. They were divided into four groups, each consisting of three animals. The first group was given daily 10 I.U. of vitamin D₂ and the second group 10 I.U. of synthetic ammonium vitamin D₂ sulfate in 0.02ml ethyl palmitate per head. The third group was given daily 10ml of fresh human milk containing about 8 I.U. of vitamin D (previously estimated). The fourth group was served as a negative control.

5. Measurement of Antirachitic Potency

In prophylactic experiments, the growth curves were recorded for 28 days. The antirachitic symptoms were preliminarily tested by the ordinary X-ray test and by the ash determination of left tibia according to the AOAC method. In curative experiments, the antirachitic potency of the compounds were measured by X-ray test and by the ash content of left tibia after 8 days.

RESULTS

1. Prophylactic Experiments

The growth curve for 28 days are shown in Figure 1. The antirachitic potency of vitamin D₂ sulfate in rats was seen in X-ray tests. Rachitic symptoms correspond to the Bourdillion’s scale No. 1 were detected both in the vitamin D-deficient rats and those supplemented with inorganic phosphate. When administered with vitamin D₂ or synthetic ammonium vitamin D₂...
**TABLE 1**
Ash Content of Left Tibia in Prophylactic Test

<table>
<thead>
<tr>
<th>Ash per cent</th>
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<tbody>
<tr>
<td>Group receiving vitamin D₂ (10 I.U./head/day)</td>
</tr>
<tr>
<td>Group receiving vitamin D₂ sulfate (10 I.U./head/day)</td>
</tr>
<tr>
<td>Group receiving 2 ml human milk (about 1.5 I.U. as vitamin D/head/day)</td>
</tr>
<tr>
<td>Group receiving vitamin D liberated from barium vitamin D sulfate (about 1.5 I.U. as vitamin D/head/day)</td>
</tr>
<tr>
<td>Group receiving phosphorus (1.675 mg phosphorus/head/day)</td>
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<tr>
<td>Vitamin D-deficient group</td>
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sulfate (10 I.U. as vitamin D₂), no rachitic symptoms were recognized in X-ray pattern indicating the Bourdillon's scale No. 10-12.

In the experiments with the human milk containing the presumed amount of 1.5 I.U. vitamin D, both the group receiving the human milk and that receiving the vitamin D fraction from the human milk indicated the X-ray patterns as shown in the scale No. 7-8. Although the response was slightly lower in the ash test than those receiving synthetic ammonium vitamin D₂ sulfate.

The appearance of rachitic symptoms has been reported to depend on the ratio of Ca: P, and the more the phosphorous content, the less deficiency symptoms appear. The prophylactic ability of milk may therefore be ascribed to the content of phosphate. However, in the present experiment supplied with inorganic phosphate.

FIG. 3  X-Ray Photographs for Curative Tests of Tibia in Rats Receiving Human-Milk Vitamin D Sulfate, as Compared with Bourdillon's Standard
corresponding to the amount contained in 2 ml of the milk, as shown in Figs. 1-2 and the above assumption was denied.

Finally, the rats were killed and the total ash of the left tibia was estimated by the AOAC method. The results are shown in Table 1 which suggests the presence of a considerable amount of water-soluble vitamin D in human milk.

2. Curative Experiments

Milk vitamin D sulfate was tested by the ordinary curative method using vitamin D-deficient rats. The animals were orally given the corresponding amount of milk containing 10 I. U. of vitamin D, and the same ability as vitamin D was confirmed by X-ray and AOAC ash tests as shown in Figure 3 and Table 2.

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