EFFECT OF STRONTIUM ON THE EPIPHYSEAL CARTILAGE PLATE OF RAT TIBIAE—HISTOLOGICAL AND RADIOGRAPHIC STUDIES

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Abstract—Following dietary administration of strontium carbonate, histological and radiographic changes in the epiphyseal cartilage plate of the rat tibiae were examined in the present study. The weight gain of the rat fed a strontium diet was less than that of the control rats. Longitudinal growth of tibiae and endochondral ossification were inhibited by strontium administration. The widths of both proximal and distal cartilage plates increased enormously as has also been shown by other investigators. Sizes of chondroblasts in columns of proximal cartilage plate in rats fed a strontium diet were smaller than those of the control rats and were not different between upper and lower parts. It is suggested that strontium inhibits bone growth through the inhibitory action on the maturation process of chondroblasts and the succeeding endochondral ossification.

Oral administration of strontium salts to animals induces "strontium rickets" (1, 2, 3). There have been many reports on the calcification lesions in the epiphyseal cartilage plate induced by administration of dietary stable strontium to animals (1, 3, 4, 5, 6). These calcification lesions have been postulated to be due to the adverse effects of strontium on intestinal absorption mechanisms (7).

In the present study, effect of dietary administration of strontium carbonate on the endochondral ossification in the epiphyseal cartilage plate of rat tibiae was examined by histological and radiographic methods to obtain basic data on the calcification lesions induced by this element. Cell size was also measured in order to determine the effect of strontium on proliferating cartilage cells or flattened cells in columns of the epiphyseal cartilage plate.

MATERIALS AND METHODS

Ninety male rats of Wistar strain, 4 weeks old with an initial body weight of 50 to 60 g were divided into three equal groups of 30. Each group was provided a normal well balanced diet (control group), a low Ca diet (low Ca group) or a strontium diet (Sr group) respectively, and given deionized water ad libitum for 4 weeks (8). Calcium content in the diets was 0.67% in the control group and 0.04% in both low Ca and Sr groups; strontium content, 1.50% in the Sr group; phosphorus content, 0.64% in all groups. During the experimental period, the body weight of the animals was recorded in order to observe the effect of oral administration of strontium carbonate on the growth of the animals.

The rats were decapitated under ether anaesthesia 4 weeks following the strontium
administration. Both left and right tibiae were dissected out immediately and adherent tissues were carefully removed. Tissues from 24 rats in each group were fixed in 10% neutral formalin and then radiographed using a Softex EMB type apparatus (Softex Co., Ltd., Tokyo). The length of the tibiae and the width of proximal and distal epiphyseal cartilage plates were recorded on the radiographs.

For histological examination, the right tibiae obtained from 24 rats in each group were decalcified in 10% EDTA solution (pH 7.2) for 3 weeks, embedded in paraffin, sectioned midsagittally at 6μm by using a sliding microtome and stained with haematoxylin and eosin.

The degree of endochondral ossification was examined microradiographically; here, the proximal heads of left tibiae from 6 rats in each group were used. After the fixation in 10% formalin, these tissues were dehydrated in ethanol, embedded in Rigolac (Riken Polyester Resin Co., Ltd., Tokyo) and polymerized (9). Midsagittal ground sections of 50μm in thickness were prepared. Contact microradiograms of these sections were taken (75 KV and 2 mA of current intensity) by using a Softex CSM type apparatus (Softex Co., Ltd., Tokyo). Eastman Kodak 649-0 spectroscopic film was used and the exposure time was 15 min. The films were developed for 3 min at 20°C in Microfine (Fuji Photo Film Co., Ltd., Tokyo), fixed for 10 min at 20°C in Fuji fix (Fuji Photo Film Co., Ltd., Tokyo), washed in running tap water for 30 min, dried and mounted. Linear distance between the proximal and distal ends along the central axis of the sagittal plane of a tibia was measured on the radiographs. Widths of both proximal and distal epiphyseal cartilage plates were measured along the central axis of the sagittal plane of the tibia.

To estimate the size of the cells in the zone of proliferating cartilage cells, one good section from each of 4 rats in each group was selected. Photomicrographs of the central part of the epiphyseal cartilage plate were taken at an original magnification of 200. The zone of proliferating cartilage cells, that is, chondroblasts, was defined at the region where cartilage cells multiply and form columns of flattened cells (10). Since the number of these flattened cells in a column was found to be approximately 26 in the preliminary observations, a column was arbitrarily divided into two parts, that is, upper (from the proximal end to the 13th cell) and lower (the rest of the cells) parts. Fifteen cells in each part in each section were randomly chosen on the printed pictures whose final magnification was 1700. The cells were cut out and weighed. Assuming that the thickness and density of the paper were uniform and, therefore, the weight of the paper was proportional to the area, the weight of the cutout of each cell was converted to the area.

RESULTS

Changes in the body weight

As seen in Fig. 1, average body weights in all groups of rats showed gradual increases during the experimental period but the weight gain was somewhat different among the groups. None of the rats died during the experimental period. In the group of rats fed a low Ca diet (low Ca group), the average body weights were not significantly different from those of the control group until the 5th day of the experiment. However, they were sig-
FIG. 1. Daily changes in the body weights in groups of rats. •—• control, ×—× low Ca, —— Sr groups. Each point represents the average value in each group. Vertical bars represent the standard deviations.

FIG. 2. Upper, middle and lower pictures represent the left tibiae from control, low Ca and Sr rats respectively. Note the differences in the lengths of the tibiae, and in the degrees of calcification among the groups. Arrows indicate the proximal cartilage plates. Magnification × 1.3.
significantly greater from the 6th to the 22nd day (p<0.05-0.01). The differences were not significant again from the 23rd to the 28th day. The weight gain in the group of rats fed a strontium diet (Sr group) was least among the three groups of animals throughout the experimental period. From the 3rd day of the experiment, the average body weight began to show significant decreases from the control values (p<0.05-0.001).

Radiographic findings

As seen in Fig. 2, calcification of the rat tibiae was poor in the group of rats fed a low calcium diet. In the group of rats fed a strontium diet, it was found that both the longitudinal growth of the tibiae and the calcification were inhibited. One of the most remarkable changes due to strontium administration was the marked increase of the width of the epi-

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<tr>
<th>TABLE 1. Changes in the length of the tibiae and in the width of proximal and distal epiphyseal cartilage plates</th>
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<tr>
<td>Tibial length (mm)</td>
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<tr>
<td>Control group</td>
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<td>Low Ca group</td>
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<td>Sr group</td>
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* Significant difference from the control value (p<0.001).

Mean±standard deviation of six left tibiae in each group are shown.

**Fig. 3.** Microradiographs of the proximal heads of rat tibiae.

a: Control group. Magnification ×18.
b: Low Ca group. Trabecular bones are thinner as compared with the control specimen. Magnification ×18.
c: Sr group. Mineralization was not observed at the site of the wide epiphyseal cartilage plate. Magnification ×18.
physeal cartilage plate.

Table 1 shows that tibial length and the widths of proximal and distal cartilage plates were significantly different between the control and Sr groups (p<0.001). But no significant differences of the lengths of the tibiae and the widths of cartilage plates were found between low Ca and the control groups. The differences were significant between low Ca and Sr groups (p<0.001).

**Microradiographic findings**

Fig. 3a-c shows microradiographs at the proximal heads of rat tibiae in the control, low

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**Fig. 4:** Photomicrographs of the proximal heads of rat tibiae.

a: Chondroblasts in control group. Haematoxylin and eosin. Magnification ×400.

b: Chondroblasts in low Ca group. No appreciable histological changes were found. Haematoxylin and eosin. Magnification ×400.

c: Chondroblasts in Sr group. Note the irregular arrangement of cells in the columns and small size of the cells as compared with the control specimen. Haematoxylin and eosin. Magnification ×400.

d: The zone of provisional calcification in control group. Lacunae of the hypertrophic cartilage cells are opened at the end of the columns. Haematoxylin and eosin. Magnification ×400.

e: The zone of provisional calcification in low Ca group. Opening of the lacunae of the hypertrophic cartilage cells are also seen at the end of the columns. A number of hypertrophied endosteal cells are visible around the trabecular bones. Haematoxylin and eosin. Magnification ×400.

f: The zone of provisional calcification in Sr group. Note that lacunae of the hypertrophic cartilage cells are not opened, that some cells are extremely flattened at the end of the columns and that osteoid tissue rather than trabecular bone is evident. Haematoxylin and eosin. Magnification ×400.
Ca and Sr groups, respectively. In the low Ca group, the trabecular bones were thinner than those in the control group. In the Sr group, mineralization was not observed at the site of the wide epiphyseal cartilage plate (Fig. 3c).

**Histological findings**

Fig. 4a-f shows histological appearances of the flattened cells in the columns at the proximal head of the rat tibiae in the control, low Ca and Sr groups, respectively. No remarkable histological changes were found in the low Ca group as compared with the control group. In the Sr group, the arrangement of the columns seemed to be in disorder and the size of the cells appeared to be small. At the zones of provisional calcification, lacunae of the hypertrophic cartilage cells were opened at the end of the columns in the control specimen (Fig. 4d). Opening of the lacunae of the hypertrophic cartilage cells was also seen at the end of the column in the low Ca group, and a number of hypertrophied endosteal cells which may remove the trabecular bone as has been reported by other investigators (11, 12) could be seen around the trabecular bones (Fig. 4e). In the Sr group, no such opening of the lacunae was observed; some of the hypertrophic cartilage cells are extremely flattened at the end of the columns and osteoid tissue was observed instead of trabecular bone (Fig. 4f).

**Cell size**

Table 2 shows the average areas of the flattened cells in columns of the proximal epiphyseal cartilage plate in the rat tibiae. In both upper and lower parts of the columns, the average sizes of the cells in the low Ca group were not significantly different from those of the control group, but those in Sr group were only in the lower part significantly smaller than those in the control group (p<0.001). The average sizes of the cells in the lower part were significantly larger than those in the upper parts in both control and low Ca group (p<0.01), but no significant difference was found between lower and upper parts in the Sr group.

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<th>Table 2. Areas of the flattened cells in columns of the proximal epiphyseal cartilage plate in the rat tibia</th>
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<tr>
<td><strong>Area cell (μm²)</strong></td>
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<td><strong>Upper part</strong></td>
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<td>Control group</td>
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<td>Low Ca group</td>
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<td><strong>Lower part</strong></td>
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Mean : standard deviation of the means in each group are shown.
* Significant difference from the control value (p<0.001).
† Significant difference between the upper and lower parts (p<0.01).

**DISCUSSION**

Storey reported that the weight gain in Sr group was less than that in the control animals (6). Similar results were obtained in the present experiment. It is apparent that a dietary administration of strontium carbonate results in a retardation of growth in young animals.
Harrison and Fraser (13) reported that rats on a low Ca diet with complete vitamin supplements failed to grow. In the present experiment, however, the weight gain of the rats in the low Ca group was similar to, or greater than, that in the control group. This discrepancy would be due to the difference of the compositions of the diets; milk casein was not included in their diet. In the present experiment, the rats in the low Ca group apparently ingested more food to take up the small amount of calcium contained in milk casein in the diet and thus attained such a growth curve (Fig. 1).

Longitudinal growth of tibiae and endochondral ossification are apparently inhibited by strontium administration (7, 14). However, the zone of hypertrophic cartilage plate in Sr group became wider (Figs. 3c and 4c) as has already been shown by other investigators (1, 4, 5, 6, 15, 16). The average size of cells in columns of proximal cartilage plate in Sr group was smaller than seen in low Ca and control groups. In addition, cell sizes in Sr group were not different between upper and lower parts (Table 2). These results suggest that inhibition of the maturation process occurred in the chondroblasts in the Sr group.

It is also possible that strontium interfered with calcium metabolism locally or systemically, and such metabolism is considered to play an important role in cell function (2, 17, 18, 19). Further studies are under way to determine the process(es) initially inhibited after ingestion of strontium carbonate.

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REFERENCES

1) LEHNERDT, F.: Beitr. path. Anat. 46, 468 (1909)
3) FOLLIS, R.H.: Fedn Proc. 14, 403 (1955)
8) MATSUMOTO, A.: Tsurumi Univ. dent. J. 1, 21 (1975)