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Effects of Fluoride Intake on Disuse Atrophy of Bone in Rats. (31218)

ITZHAC GEDALIA, ARMIN SCHWARTZ, JONA SELA, AND EFRAIM GAZENFIELD
(Introduced by F. G. Sulman)

Laboratory of Oral Chemistry and Fluoride Research and Department of Diagnostic X-Ray,
Faculty of Dental Medicine,* Hadassah-University Hospital and Tel-Hashomer Hospital, Hebrew
University-Hadassah Medical School, Jerusalem, Israel

Disuse atrophy of bone is characterized by a reduction of the mass of bone tissue accompanied by hypercalcemia and increased excretion of calcium in the urine(1).

The influence of nutrition and various hormones on degree of bone atrophy has been studied in rats(2,3). It was found that increasing the calcium content of the diet resulted in an increase in the ash content of the humerus from a paralyzed extremity, whereas an increase of the phosphorus content of the diet had no demonstrable effect. Administration of estradiol propionate reduced or prevented bony atrophy, whereas testosterone propionate increased it(3).

It has been reported that negative calcium balances which occur in human skeletal diseases such as osteoporosis and Paget's disease (4), or in hypervitaminosis D in rats(5) can be improved by administration of fluoride. The purpose of this investigation was to study the effect of fluoride administration on atrophy of bones in rats.

Materials and methods. Forty rats of the Hebrew University strain Sabra weighing 150-170 g were used in this experiment. Rats of this size were expected to be able to withstand a nerve severance operation (4 rats died from hemorrhage during the operation).

In addition the bones of these animals were still in an actively growing metabolic state and able to incorporate fluoride rapidly(6). The rats were maintained on a standard mixed diet containing 3.2 ppm F and were provided with drinking water containing 0.55 ppm F until the beginning of the experiment. This diet is considered to be low in fluoride for rats(6).

The rats were divided into 2 main groups:

Group 1—15 rats received distilled water as drinking water.

Group 2—21 rats received distilled water containing 25 ppm F as drinking water.

In 6 rats from Group 1 and 9 rats from Group 2, the right sciatic and femoral nerves were severed to permit the development of disuse atrophy. Using aseptic technique, the femoral nerve was severed in the area of the femoral triangle. The sciatic nerve was severed in the area below the gluteal region. The nerve ends were separated from each other. Operated and non-operated rats were fed *ad libitum* and killed with ether 2 months after starting the experiment. Before sacrificing, blood samples were taken from each rat for calcium analysis. The left and right femora were dissected out. X-ray radiograms of the femora were made to detect bone rarefaction resulting from paralysis. After defatting and drying both femora, their specific gravity,

* Founded by the Alpha-Omega Fraternity.

ash content and the calcium and fluoride contents of the ash were determined as previously described(5).

The significance of the differences between groups ($p < 0.05$) was calculated by the *t* test, taking into account that the distribution in both groups was normal.

TABLE I. Mean Specific Gravity, g Ash, Calcium and Fluoride per 100 g Dry Defatted Right and Left Femurs of Normal Limbs.

| Drinking water | No. rats | Serum calcium mean | Right femur—normal limb | | | | Left femur—normal limb | | | |
|----------------------------|----------|--------------------|-------------------------|----------------------|-----------|----------|------------------------|----------------------|-----------|----------|
| | | | Specific gravity mean | g ash per 100 g mean | % Ca mean | % F mean | Specific gravity mean | g ash per 100 g mean | % Ca mean | % F mean |
| Distilled water | 9 | 9.07 | 1.063 | 56.6 | 34.8 | 18.0 | 1.085 | 56.9 | 36.2 | 20.2 |
| Standard deviation | | .240 | .816 | 2.82 | .816 | 8.93 | .380 | 2.92 | .942 | 10.7 |
| Fluoridated water (25 ppm) | 12 | 9.07 | 1.178 | 58.4 | 35.3 | 60.0 | 1.16 | 58.4 | 35.8 | 72.0 |
| Standard deviation | | .395 | .224 | 1.16 | .957 | 19.2 | .208 | 2.66 | .913 | 24.1 |

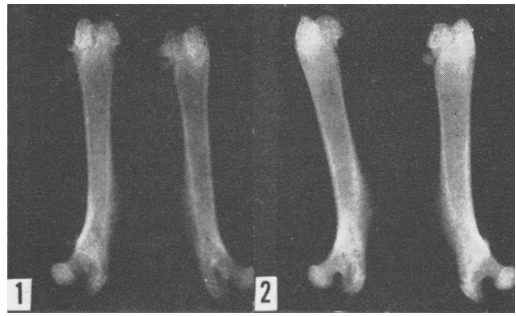


FIG. 1. Non-operated rats. Distilled water as drinking water. No difference was observed between radiograms of right and left femur.

FIG. 2. Fluoridated water as drinking water. No difference was observed between radiograms of right and left femur.

The specific gravity, X-ray radiograms, percentage of ash and calcium and fluoride contents of the left and right dried defatted femurs of the remaining non-operated rats, drinking distilled or fluoridated water, were similar to each other, $p > 0.05$ (Table I, Fig. 1 and 2). Furthermore, it is known that no hypertrophy or increase in total ash weight of the femora of the normal limbs resulted from an increased use of these limbs(3). It was considered justifiable, therefore, to study the effect of the atrophy by comparing the determinations of the femora of the right paralyzed limb and left normal non-paralyzed limb in the same operated group.

Results. The results are summarized in Tables I and II.

The mean calcium level of the serum in the paralyzed rat drinking distilled water was significantly higher than the mean serum calcium level of the paralyzed rat drinking fluoridated water (Table II) and also higher than the mean serum calcium level of the non-operated rats drinking distilled or fluoridated water (Table I). The mean specific gravity of the femur of the paralyzed right limbs was significantly lower than the mean specific gravity of the left femurs of rats drinking distilled water. The mean amount of femoral ash of the dry defatted femur of the paralyzed right limbs was significantly lower than that of the left femurs when expressed on a volume basis. The calcium content of the ash was significantly lower in the right

TABLE II. Effect of Fluoride Intake on Specific Gravity, Percentage Ash, Calcium and Fluoride of the Dry Defatted Rat Femurs of the Paralyzed and Non-Paralyzed Limb.

| Drinking water | No. rats | Serum calcium | Right femur—paralyzed limb | | | | Left femur—normal limb | | | | |
|--------------------|----------|---------------|----------------------------|-----------------------|-----------|-------------|------------------------|-----------------------|-----------|-------------|------|
| | | | Specific gravity mean | g Ash Per 100 ml mean | % Ca mean | mg % F mean | Specific gravity mean | g Ash Per 100 ml mean | % Ca mean | mg % F mean | |
| Distilled water | 6 | 9.77 * | 1.052 † | 57.28 | 60.2 † | 33.3 † | 25.6 | 57.02 | 67.25 | 35.3 | 20.2 |
| Standard deviation | | .258 | .0387 | 1.65 | 2.76 | .707 | 9.20 | 1.74 | 5.76 | .943 | 5.61 |
| Fluoridated water | 9 | 9.36 | 1.059 | 57.30 | 60.7 | 36.3 | 77.3 | 57.25 | 64.6 | 35.2 | 70.3 |
| Standard deviation | | .397 | .120 | 1.52 | 8.02 | .472 | 25.0 | 1.26 | 5.25 | 1.33 | 29.1 |

* Significantly different from paralyzed rat group drinking fluoridated water.

† Right femur—paralyzed limb significantly different from left femur—normal limb.

femurs as compared to the left femurs. The fluoride content of the ash was not significantly different between both femora. When the drinking water supplied to the paralyzed rat contained 25 ppm F, the fluoride content of the femoral ash increased significantly. The differences in specific gravity, femoral ash amount, expressed on weight or volume basis, and its calcium and fluoride contents, between both femora, were not significant.

Discussion. Allison and Brooks(7) produced atrophic changes in the bones of dogs by section of nerves using roentgenographic and histologic techniques for observing the gross and minute changes in the atrophic bone. According to Armstrong *et al*(3), the ash percentage and total ash content is lower in the humerus of rats in which a significant degree of bone atrophy is present owing to paralysis of the limb. In our experiment paralysis of the right limb of rats was found to produce a number of effects on the right femur. The specific gravity, the ash content calculated on a volume basis and its calcium content were reduced. The disuse atrophy of bone did not change the natural fluoride content of the ash. A similar effect was observed in PTH-treated rats(8). During the resorption of bone the mobilized fluoride is probably excreted in the urine(9).

The atrophic humeri in Armstrong *et al's* (3) experiment also exhibited a greater net decrease in total minerals than in proteins. It seems that the change in specific gravity was produced by the mineral release(5,8). The reduction in size of the right femur (Fig. 3) could have resulted from an increase in the rate of bone resorption or from a decrease in the rate of bone formation. Increased bone resorption and decalcification is characterized by a calcium increase in the serum(1,5). A rise of 0.8-2.1 mg% in calcium serum level was observed in human disuse bone atrophy(10). The disuse atrophy of the right femur over the period of 2 months brought about hypercalcemia (Tables I and II). A loss of 10-30% of bone salt is necessary to produce an appreciable change in roentgenograms of the bone(1,10). X-ray of femur of paralyzed limb showed thinning of the cortex which is evidence of osteoporosis

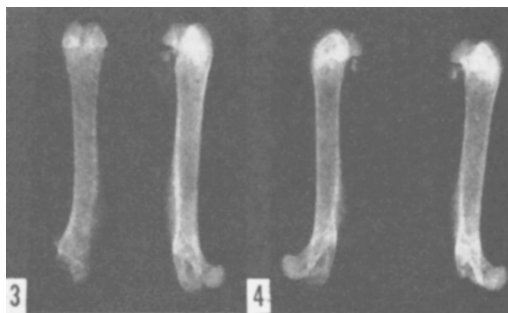


FIG. 3. Operated rats. Paralyzed femur on left-hand side. Distilled water as drinking water. Overall density of the paralyzed femur is reduced. The compacta is thinner, both absolutely and in relation to width of the bone.

FIG. 4. Fluoridated water as drinking water. Reduction in density of paralyzed femur is minimal.

(1,11) (Fig. 3). Our experiment shows that fluoride given at a concentration of 25 ppm in the drinking water to rats produces a number of effects. Fluoride seems to counteract the typical reactions of the bone atrophy syndrome, reducing the calcium content of the serum. A decrease in bone resorption in the femur of the paralyzed limb was prevented probably by a reprecipitation of calcium as fluorapatite of low solubility on the surface of the bone crystals by ion exchange(5,12). Osteoporosis could hardly be detected by X-ray (Fig. 4). It has been shown previously that physiological doses of fluoride given in vitamin D-intoxicated rats convert negative calcium balances into positive ones(5). All these findings are in accord with the opinion of most investigators that fluoride increases the mineralization rate of bones(4,5).

Summary. The effects of fluoride intake on disuse atrophy of the femoral bone were investigated in rats after severing the right sciatic and femoral nerves. X-ray radiograms of

the femora were made. Calcium content of the blood, specific gravity, ash percentage and calcium and fluoride content of both femora were determined in operated and non-operated rats drinking distilled or fluoridated (25 ppm) water for 2 months after starting the experiment. The results showed that fluoride counteracts the typical bone atrophy syndrome; it reduces the calcium content of the serum and prevents bone resorption. It is suggested that increased bone resorption is prevented by a reprecipitation of calcium as fluorapatite of low solubility on the surface of the bone crystals.

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