Effect of Fluoride on Crystal Texture and Radiocalcium Uptake of Rat Bone. (28954)

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Recent studies suggest that fluoride may be of value in prevention and treatment of some important skeletal diseases affecting man. It has been reported that fluoride administration improves calcium retention in individuals with osteoporosis(1-3) and Paget's disease(2-4). Serial bone biopsies and Ca^{47} studies of some of these same subjects indicate increased mineralization and bone accretion after fluoride therapy(3). Clinical remission of pain associated with these disorders has been a consistent finding(1-4). Also noteworthy is the observation that the severity of periodontal disease is significantly less among adults using a drinking water containing 1.2 ppm F than in those whose water supply contains 0.1 ppm F(5). Finally, it has been suggested that increases in bone density associated with prolonged ingestion of elevated levels of fluoride may serve to counteract the effects of osteoporosis in the aged(6).

Of possible significance in these regards is the observation that fluoride improves the crystallinity of the mineral phase of bone in rats(7-9) and in man(10). This would act to reduce chemical reactivity and, conceivably, the susceptibility of bone to resorptive processes.

The purpose of the present study was to compare the effect of fluoride administration on the crystallinity (that is, crystal size and/or perfection) and calcium metabolism of rat femur, vertebrae, and alveolar bone.

Experimental. Twenty-one day old male Sprague-Dawley rats were given a diet containing less than 1 ppm fluoride(11) and drinking water containing either 0 ppm F (Group I) or 50 ppm F (Group II) for 14 months. Each was then administered intraperitoneally 1.0 ml of a solution containing 20 μc Ca^{45} and 10 μg Ca. Four hours after injection the mandible, one femur, and 3 lumbar vertebrae were removed. Approximately equal portions of the alveolar processes were obtained by dissection from individual rats and pooled by groups of three. All samples were extracted for 8 hours in alcohol and 4 hours in ether and powdered to pass a 200-mesh sieve. After X-ray diffraction line broadening analysis, the powdered samples were incinerated overnight at 550°C and the ash analyzed for F(12,13) and Ca^{45}(14).

X-ray diffraction patterns (Copper K-alpha radiation) were obtained on the powdered samples using a commercial geiger counter diffractometer with a high resolution slit system. The degree of resolution of the 4 principal reflections of the bone apatite X-ray patterns (Miller indices 211, 112, 300, 202) was used as a measure of the crystallinity of each specimen. Each diffraction pattern was assigned a "crystallinity" rating by matching it with one of a series of templates constructed to represent X-ray diagrams with different amounts of line broadening designated by a β_t value, the width at half maximum in degrees 2θ of each of the 4 Gaussian curves (representing the 4 principal apatite reflections) summed to form the template. As the value of β_t decreases, the crystal size and/or crystal perfection increases. These measurements have been described(15).

Results and discussion. The analytical data are shown in Table I. Concentration of fluoride in the bone ash was 20-30 times higher in the fluoride supplemented rats. In these same animals the femur contained significantly more fluoride than either the vertebra ($p<0.02$) or alveolar bone ($p<0.02$); the control bone samples did not differ ($p>0.05$) from each other in this respect. The finding that fluoride had little or no effect on bone ash content agrees with previous studies from this laboratory(8,9,16).

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TABLE I. Effect of Fluoride on Bone Ash, Crystallinity, and Radiocalcium Uptake in Rats.*

Bone sample	F in H_2O, ppm	F, % of ash	Ash, %†	β_t‡	Ca^{45}, % dose/mg ash $\times 10^{-2}$
Femur	0	.046 ± .0035	66.27 ± .56	1.05 ± .013	.506 ± .019
	50	1.098 ± .0413	65.68 ± .63	.93 ± .018	.472 ± .015
P value		<.01	>.05	<.01	>.05
		(11)	(12)	(12)	(12)
Vertebra	0	.049 ± .0034	58.57 ± .55	1.13 ± .022	.642 ± .021
	50	.917 ± .0557	59.90 ± .74	.95 ± .018	.618 ± .027
P value		<.01	>.05	<.01	>.05
		(12)	(12)	(12)	(12)
Alveolar	0	.032 ± .0098	67.63 ± .53	1.04 ± .037	.732 ± .069
	50	.895 ± .0290	68.34 ± .26	.93 ± .024	.711 ± .060
P value		<.01	>.05	<.05	>.05
		(4)	(4)	(4)	(4)

* All values expressed as mean ± S.E. No. of items comprising the mean given in parentheses.

† Based on dry, fat-free weight.

‡ Avg values calculated from composite template β values.

Comparison of the β values for the control samples show that the vertebra is less well-crystallized than the femur ($p<0.05$) but not alveolar bone. This corroborates previous studies which showed that the apatite of more highly mineralized tissues, *i.e.*, those with higher ash content, are generally more crystalline than apatites of tissues of lower mineral content. Thus enamel is better crystallized than dentin(17) and the more compact bone of the tibia shaft is more crystalline than the tibia ends(8,17).

Fluoride administration increased the crystallinity of each of the bone samples and offers further proof of its effect on crystal texture(7,10). This improvement was most pronounced in the case of vertebra where the β_t value decreased 16% as compared to 11% for femur and alveolar bone. These differences are admittedly small and must therefore be interpreted with caution. It is possible that the change in crystal texture attributable to fluoride may be related to initial crystallinity. Supporting this is a previous finding that, in contrast to bone, rat incisor apatite crystallinity did not change with an increase in fluoride content(8). It was suggested that since the incisor samples were initially more crytalline, as evidenced by a β_t value 35-40% lower than those for bone, the incisor apatite may be less susceptible than bone apatite to further improvement in crystallinity through fluoride incorporation.

Preliminary findings indicate that in Paget's disease the affected bone mineral is less crystalline than normal bone mineral(18). It is possible then that the crystallinity of Paget's bone would improve more than normal bone with fluoride treatment.

It is apparent that fluoride did not affect the skeletal uptake of radiocalcium. Previous studies also indicate that fluoride has little or no effect on Ca^{45} deposition in bone (8,9,16). The vertebra contained significantly more Ca^{45} than did the femur in both control ($p<0.01$) and fluoride supplemented animals ($p<0.02$). This was also true for alveolar bone ($p<0.01$) although these samples did not differ significantly from vertebra with respect to radiocalcium uptake. Of interest in this regard is a recent study which showed that the alveolar bone of rabbits took up 2 to 3 times more Ca^{45} and P^{32} than did the compact bone of the mandibular body and femur(19).

The reason for the different skeletal uptake of radiocalcium is not apparent. Certainly ash content was not a factor since the femur and alveolar bone with similar percents of ash contained markedly different concentrations of the radioisotope. It also seems clear from a comparison of the β_t values, particularly in fluoride-treated rats,

that Ca^{45} uptake was unrelated to bone crystallinity.

It is recognized that the deposition of radiocalcium in the mineral apatite of bone is the result of incorporation in newly formed crystals and surface exchange on existing crystals. There is also general agreement that the early fixation of circulating Ca^{45} is largely due to surface exchange. We have suggested, however, that the contribution of new crystal formation to isotope uptake under these conditions is of biological significance. Thus new crystal formation and not surface exchange was found to be primarily responsible for the short-term differential skeletal uptake of Sr^{89} and Ca^{45}(17).

The present results offer further support for this concept. It was recently found that improvement in bone crystal size and not in crystal perfection accounted for changes in β_t attributable to fluoride(20). Moreover, these same data indicated a nearly 1:1 correlation between percentage change in β_t and surface area. It follows, then, that if surface exchange dominated Ca^{45} uptake, the high fluoride samples with smaller specific surface areas should contain less of the radioisotope. This was not the case. The observation that the fluorosed bone contain as much Ca^{45} as the control samples could be due to an increased rate of bone accretion. This may possibly explain the beneficial effect of fluoride administration on the metabolic bone diseases previously noted.

Summary. Uptake of Ca^{45} and hydroxyapatite crystallinity as measured by degree of resolution of its X-ray diffraction pattern was compared in the femur, vertebra, and alveolar bone of rats given a drinking water containing either no fluoride or 50 ppm F for 14 months. Vertebra and alveolar bone contained significantly more Ca^{45} than femur. Fluoride administration improved bone crystallinity but did not affect radiocalcium uptake. The possible effect of fluoride in bone accretion is discussed in relation to the above findings.

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