Some effects of bone meal supplemented diet on bone and teeth of growing rats

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Bone meal (containing 500 ppm F) was added to the diet of growing rats in an amount corresponding to that recommended ingested by children. Addition of bone meal did not influence body weight increment, or growth and ash content of bone and teeth. Fluoride in bone and teeth increased as compared to control animals, but the increase was 2 – 4 times less than if fluoride had been given as NaF. Analyses of feces disclosed significantly increased values of Ca and F in animals fed the bone meal supplemented diet, indicating low absorption of these minerals from bone meal.

Key-words: Bone meal; fluoride; teeth; bone mineral

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Bone meal supplements to the diet have been proposed for nearly a century (13) to increase its content of calcium, phosphorus and trace elements, intending to obtain beneficial effects on teeth and other hard tissues. Furthermore, addition of bone meal to the diet has been claimed to reduce the incidence of dental caries in humans (10) and in animals (5, 9, 16, 19).

Bone meal is consumed either as bone meal tablets or added to food, in most cases bread. Ingestion of bone meal tablets of the amount recommended by the manufacturer, will increase the daily intake of calcium with about 50 %. Furthermore, bone meal tablets supply the body with an amount of fluoride corresponding approximately to that obtained through fluoridated water (1 ppm), or to that obtained through ingestion of NaF tablets.

Absorption of fluoride from NaF is almost complete (4), and its incorporation into hard tissue is well elucidated (2). The absorption of fluoride from bone meal, however, is considerably less (4), and few investigations have been performed to assess the incorporation of fluoride into bone and teeth after ingestion of bone meal (12).

The aim of the present investigation was to evaluate the incorporation of fluoride into bone and teeth of growing rats fed diets supplemented with bone meal in amounts comparable with those recommended to children, and

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compare it with the incorporation of fluoride from the same amount of fluoride ingested as NaF. Furthermore, the content of fluoride and calcium in serum and feces was estimated.

MATERIALS AND METHODS

Animals

Male and female Wistar rats were at an age of 33 d randomized according to weight into three groups, each consisting of 5 males and 8 females. One group was destined to be control group, one group for a bone meal containing diet, and one group for a NaF supplemented diet.

Diets

The pre-experimental diet was standard food pellets (F-content 19 ppm) and tap water *ad libitium.* During the experimental period the animals were fed a semi-synthetic diet (18) to which either bone meal or NaF was added:

1. Control group: Semi-synthetic diet containing 1.1 % Ca and 0.8 % P.
2. Bone meal group: Semi-synthetic diet supplemented with 16.2 g bone meal per kilo (1.6 %).
3. NaF group: Semi-synthetic diet supplemented with 17.9 mg NaF per kilo.

The content of calcium, phosphorus and fluoride in the different diets has been calculated to:

	Standard		Supplements		
	Ca	P	Ca	P	F
Control diet	1.1 %	0.8 %	0	0	0
Bone meal diet	1.1 %	0.8 %	0.6 %	0.3 %	8.1 ppm
NaF diet	1.1 %	0.8 %	0	0	8.1 ppm

The groups were fed the diet *ad libitum* together with tap water (containing less than 0.02 ppm F).

The experimental period lasted until the animals were 14 weeks (98 days) old. The animals were weighed at the start of the experimental period and at 8, 10, 12 and 14 weeks of age. At the end of the experimental period, the animals were killed by ether and bleeding. Blood was collected, femur and skull dissected free and stored frozen for later analyses. Furthermore, feces had been collected at an earlier occasion.

Analytical procedures

Blood was centrifuged and serum pipetted off for analyses of calcium and fluoride. Calcium was determined by atomic absorption spectrophotometry (AAS) after addition of 0.1 % lanthan, and fluoride was assessed with a fluoride selective electrode (Orion 96–09) attached to an Orion Research Model 701 A/Digital Ionalyzer after 1/10 part of TISAB III (Orion) had been added to the sample.

Hard tissues (femur and mandible) were cleaned for soft tissues and thereafter ashed at 600°C for 24 h. Furthermore, the length of femur was measured, and ash as percentage of dry weight calculated. After ashing the femur was divided into distal epiphysis (about 1/4 of total length) and diaphysis (about ½ of total length). Incisors and molars were dissected free from the mandible and their ash weight recorded.

The ash of incisors, molars and epiphyseal/diaphyseal parts of femurs

was then dissolved in 1 N HCI for 24 h (about 1 ml acid per 30 mg ash). Fluoride concentration in the dissolved ash was determined with the fluoride electrode after dilution, neutralization and buffering (to 0.3 ml sample was added 2.0 ml dest. water, 0.27 ml TISAB III, 0.4 ml 1 N NaOH, which gave a final pH 5.0–5.2). Calcium content in the solution was assessed by AAS.

Feces were analyzed for their content of calcium and fluoride, after ashing at 500°C and dissolving in 1 N HCl, by the same methods as for hard tissues.

To determine the absorption of lead from bone meal (contains 7 ppm Pb), the epiphyseal parts of femurs from control animals and bone meal animals were analyzed according to the method of Fosse & Justesen (8).

Statistical treatment

Figures in tables are presented as mean and standard errors of the mean. Tests of significance have been performed according to Student's *t*-test.

RESULTS

Weight development during the experimental period is presented in Fig. 1. The final weight of males in the bone meal group and the NaF group was slightly, but not significantly ($0.3 < P < 0.4$), higher as compared to the controls, while in the female rats the final weight of the NaF group was significantly lower ($P < 0.001$) than that of the control group and the bone meal group. However, until an age of 8–9 weeks the weight development of these animals kept pace with the others.

Figures for calcium and fluoride content in serum are presented in Table 1. In males serum-Ca did not differ significantly from each other, while in females serum-Ca was significantly increased both in the bone meal group and the NaF group. Serum-F was low in the control groups of both sexes. In the males addition of bone meal or NaF to the diet increased serum-F significantly, highest in the NaF group. In female rats the increase in serum-F was slightly higher than in the males, but with no significant difference between the bone meal group and the NaF group. Measurements and analytical data for femurs are presented in Table 2. In males the lenght of femur was significantly increased in the NaF group ($P < 0.001$), while the ash weight was not significantly different in the three groups. The ash as percentage of dry femur weight showed a significant reduction in the NaF group ($0.01 > P > 0.001$). In female rats no significant differences were observed between the groups with regard to length and ash weight, but the ash as percent-

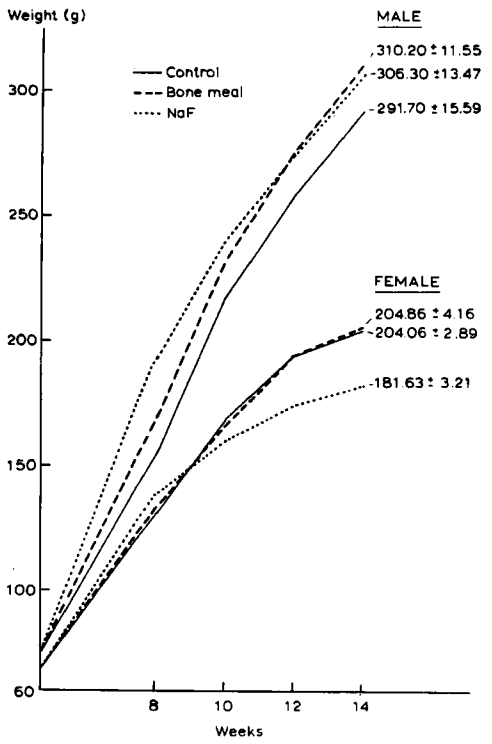

Fig. 1. Growth development during the experimental period

Table 1. *Content of calcium and fluoride in serum. Figures are means and SE*

	Serum-Ca mg/100 ml	Serum-F ppm
Control group males (5)	9.95 ± 0.18	0.009 ± 0.001
Bone meal group males (5)	10.15 ± 0.14	0.018 ± 0.001
NaF group males (5)	10.06 ± 0.12	0.023 ± 0.001
Control group females (8)	9.52 ± 0.08	0.011 ± 0.001
Bone meal group females (7)	10.10 ± 0.05	0.028 ± 0.003
NaF group females (8)	10.40 ± 0.09	0.025 ± 0.001

( ) = number of observations

Table 2. *Length, ash weight, ash as percentage of dry weight, and content of fluoride in femoral epiphysis and diaphysis. Figures are means and SE*

	Total femur			Femoral	
	length mm	ash weight mg	% ash	Epiphysis F/Ca in ppm	Diaphysis F/Ca in ppm
Control group Males (5)	34.72 ± 0.38	337.6 ± 16.4	62.82 ± 0.33	105.4 ± 19.8	114.1 ± 3.9
Bone meal group Males (5)	35.32 ± 0.35	358.0 ± 11.9	62.92 ± 0.20	356.5 ± 18.2	263.1 ± 28.8
NaF group Males (5)	37.08 ± 0.21	350.2 ± 12.5	60.84 ± 0.16	1691.5 ± 114.8	1109.2 ± 32.0
Control group Females (8)	32.24 ± 0.26	280.9 ± 5.4	64.14 ± 0.39	112.0 ± 15.4	135.1 ± 3.6
Bone meal group Females (8)	32.38 ± 0.23	283.1 ± 6.0	63.54 ± 0.18	433.9 ± 12.0	329.8 ± 21.4
NaF group Females (8)	32.31 ± 0.23	267.6 ± 5.7	62.64 ± 0.25	923.8 ± 63.6	650.1 ± 19.8

( ) = number of observations

Table 3. *Ash weights and content of fluoride in incisors and molars (as ratio F:Ca in ppm). Figures are means and SE*

	Incisors		Molars	
	Ash mg	F:Ca ppm	Ash mg	F: Ca ppm
Control group Males (5)	57.30 ± 1.19	51.98 ± 3.39	28.66 ± 0.90	70.64 ± 5.90
Bone meal group Males (5)	58.50 ± 2.24	101.36 ± 3.44	28.86 ± 0.85	177.34 ± 8.93
NaF group Males (5)	60.82 ± 1.91	337.82 ± 7.22	28.74 ± 0.74	560.38 ± 56.68
Control group Females (8)	47.88 ± 1.04	53.11 ± 4.18	28.00 ± 0.37	86.40 ± 7.08
Bone meal group Females (8)	50.25 ± 0.71	128.61 ± 5.83	28.14 ± 0.58	174.00 ± 13.30
NaF group Females (8)	48.80 ± 1.02	233.27 ± 7.06	26.89 ± 0.74	407.09 ± 8.82

( ) = number of observations

age of dry weight was significantly lower in the NaF group ($0.01 > P > 0.001$). The femurs of female rats showed in all groups a higher degree of mineralization than femurs in corresponding male rats.

As to the content of fluoride in femoral epihysis and diaphysis of the control group, no significant difference was found between the two parts of the bone neither in males nor in females. Addition of bone meal or NaF to the diet increased the amount of fluoride in the bones, but always to a greater extent in the epiphyseal part, and 2–4 times higher in the NaF group than in the bone meal group. In the bone meal group the incorporation of fluoride into the bone was highest in the female rats, while the opposite was true in the NaF groups. Particular high amounts of fluoride were found in the epiphysis of the male rats in the NaF group.

The figures for ash weights and fluoride content in incisors and molars are presented in Table 3. The ash weight of the incisors in male rats was considerably higher than for those of female rats. However, no significant differences were found between the three groups neither in males nor in females. The ash content of molars did not differ significantly from each other either between groups or between sexes. The F-content in the molars was 2–3 times higher in the NaF group than in the bone meal group. Significant sex differences were observed only between male and female animals in the NaF groups, males demonstrating the highest amount.

The results of the feces-analyses are presented in Table 4. A small amount of fluoride was found even in the control group. Addition of bone meal or NaF to the diet increased the amount of fluoride in feces considerably, however, mostly in the bone meal group. As to the calcium content in feces, no significant difference was observed between the control group and the NaF group, while feces-Ca in the bone meal group was increased by about 40 %.

Table 4. *Content of fluoride (as ppm of dry feces) and calcium content (as percentage of dry feces). Figures are means and SE*

	ppm F	% Ca
Control group	4.78 ± 0.61 (9)	3.69 ± 0.19 (10)
Bone meal group	28.59 ± 1.67 (10)	5.22 ± 0.26 (10)
NaF group	19.95 ± 2.52 (10)	3.51 ± 0.25 (10)

( ) = number of observations

The content of lead in the epiphysis of the bone meal group was 0.17 ppm, and significantly lower than that of the control group, which amounted to 0.30 ppm.

DISCUSSION

In the present study addition of bone meal to male rats did not influence their weight development to any significant extent, while NaF addition reduced weight increment in female rats. The growth curve of the female rats left its growth channel at an age of 8 weeks and obtained a final value about 11 % below that of the control group. The reason for this growth reduction remains unexplained. Previous investigation with doses of fluoride 10 times the present did not influence the growth (unpublished results).

The effect of fluoride ingestion on serum-Ca is conflicting, as both unchanged (1,17) and reduced (3,7) values are reported. The reason for this discrepancy is thought to be differences in calcium intake (6). In the present investigation the Ca-content of the diet was adequate in the control diet and the NaF diet, and high in the bone meal diet. The small, but significant, increase in serum-Ca observed in the fe-

male bone meal group may be due to the additional calcium given to animals close to their growth plateau, and the further increased serum-Ca in the female NaF group by the reduced growth activity in these animals.

Small, but detectable amounts of fluoride were found even in the serum from the control groups. The origin of this fluoride may be small quantities from the diet, or fluoride released by the constant remodelling of the skeleton (14), which has incorporated F from the pre-experimental diet, containing a relatively high amount of fluoride. Serum-F increased both in the bone meal group and in the NaF group. The difference between the two groups were, however, negligible, supporting former observations that serum-F is fairly well regulated if ingestion of F is held within reasonable limits (2).

Femur length was significantly increased only in male rats fed the NaF supplemented diet. However, as no concomitant increase is ash content was found, the femur was left slightly hypomineralized as compared to the controls. A smaller reduction in ash percentage was also found in female rats fed the NaF diet. Further investigation with morphological techniques is needed to disclose the reason for this hypomineralization. The F-content of femur was estimated separately in ephiphysis and diaphysis, because the two parts of the bone behave differently with respect to growth. Growth activity and remodelling is highest in the epiphysis, and thus a greater part of bone tissue formed during the experimental period is expected to be found in the epiphysis, while more pre-experimentally formed bone is expected to be found in the diyaphysis. As expected, the content of fluoride in the diaphysis of control animals was higher than that of the epiphysis, while the reverse was true in both test groups. Furthermore, the incorporation of fluoride into bones from the bone meal supplemented diet was considerably less than from the NaF supplemented diet, an observation fully in accordance with that of Hübner (12). In rats the incisors are completely renewed in 7–8 weeks (15), and consequently the whole incisors were in the present investigation formed during the experimental period. Neither addition of bone meal nor NaF to the diet influenced the ash content of the incisors. As to the incorporation of fluoride into the incisors, the values were 2–3 times less when F was given as bone meal compared to NaF, findings in order of magnitude with those of Hübner (12).

The molars were erupted and most of their substance formed before the experimental period started. The F-content of the molars in the control group was therefore, as expected, higher than in the incisors, but surprisingly the same was true also in the bone meal group and in the NaF group. However, these findings are in full accordance with those of Hübner (12), and may reflect incorporation of fluoride into primary dentin, secondary dentin and cementum, which in rats are formed in great amounts at the apex of the tooth. The difference between animals of the bone meal group and the NaF group in F-content of molars was of the same order (2–3 times) as in F-content of incisors.

Small amounts of fluoride were found in feces even in the control group. The origin of that feces-F may be fluoride released by remodelling of pre-experimentally formed bone tissue, and secreted through the gastro-intestinal secretions. Ingestion of fluoride as bone meal or NaF increased the amount of feces-F considerably. However, the feces-F in the bone meal group was elevated above the control level by 40 % as compared to the NaF

group. Feces-Ca in the NaF group remained at control level, but increased with about 40 % in the bone meal group. As the bone meal added about 50 % Ca to the control diet, it may be understood that only a negligible part of the additional calcium supplied by the bone meal has been absorbed. Bone meal contains lead and other toxic trace elements. It may be feared that ingestion of bone meal may increase the body burden of unwanted trace minerals. The present investigation indicates that this fear may be unfounded, at least with respect to lead, as incorporation of lead into bone tissue was reduced in the bone meal group. This effect may be caused by the increased intake of calcium, which may inhibit the absorption of lead from the dietary sources (11).

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