

EFFECT OF FLUORIDE INGESTION ON CORTICAL AND CANCELLOUS BONE COMPOSITION

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Excessive ingestion of fluoride results in the accumulation of fluoride in calcified tissues. Fluoride accumulation in cancellous bone, which has a very high surface/mass ratio, is much greater than that in cortical bone (1). As fluoride interacts both with inorganic and organic constituents of bone, it was of interest to analyze the chemical composition of cancellous and cortical bone of rabbits treated with high doses of sodium fluoride, to evaluate, the exact nature of fluoride action.

Table 1. Organic constituents of bone in normal rabbits treated with sodium fluoride (means \pm SD, (n))

	Cortical bone		Cancellous bone	
	Hydroxyproline	Nitrogen	Hydroxyproline	Nitrogen
Control (8)	2.15 \pm 0.22	4.46 \pm 0.55	3.32 \pm 0.30	5.11 \pm 0.26
Organic constituents after fluoride ingestion				
3 months (5)	1.45 \pm 0.61*	3.14 \pm 0.46*	2.76 \pm 0.24*	4.98 \pm 0.03
6 months (5)	1.42 \pm 0.61*	3.15 \pm 0.45*	2.70 \pm 0.24*	4.95 \pm 0.06
8 months (5)	1.81 \pm 0.01*	2.78 \pm 0.03*	2.96 \pm 0.26*	5.20 \pm 0.10
10 months (5)	1.63 \pm 0.06*	2.87 \pm 0.21*	1.87 \pm 0.87*	4.90 \pm 0.10
12 months (5)	1.78 \pm 0.02*	2.68 \pm 0.10*	1.96 \pm 0.05*	5.05 \pm 0.17

Data expressed as mg% of dry defatted bone. *P < 0.05 (in comparison to control).

Materials and methods: Rabbits weighing 600 to 800 g were fed with a dose of sodium fluoride (10 mg/kg body weight), a dose which is widely used in the experimental induction of fluorosis. The NaF was administered daily through an intragastric route up to a period of 12 months and the animals were killed at varying intervals. Cortical (diaphyseal) bone from the femur and cancellous bone from the iliac crest region of the pelvic girdle were dissected out and freed from marrow. Fat free bone powder was prepared using an ether acetone mixture (1:1 v/v) and acetone. The bone powder was analyzed for total nitrogen (2) and hydroxyproline content (3). Calcium, magnesium and zinc were determined by atomic absorption spectroscopy (4). Control animals were given similar treatment but deprived of sodium fluoride.

Table 2. Inorganic constituents of bone in normal rabbits and rabbits treated with sodium fluoride (means \pm SD, (n))

	Cortical bone			Cancellous bone		
	Calcium	Magnesium	Zinc	Calcium	Magnesium	Zinc
Control (8)	256 195 \pm 9804	6626 \pm 527	149 \pm 36	178 221 \pm 32 409	3984 \pm 508	222 \pm 61
Inorganic constituents after fluoride ingestion						
3 months (5)	229 404 \pm 43 622	7188 \pm 544*	183 \pm 20	221 031 \pm 20 824*	5418 \pm 96*	227 \pm 75
6 months (5)	278 801 \pm 15 797*	6959 \pm 107*	223 \pm 29	226 054 \pm 16 520*	5751 \pm 413*	583 \pm 122*
8 months (5)	289 685 \pm 23 333*	5251 \pm 245*	233 \pm 28	284 662 \pm 11 601*	5147 \pm 675*	712 \pm 81*
10 months (5)	280 475 \pm 15 070*	5564 \pm 362*	248 \pm 59	272 940 \pm 11 107*	5751 \pm 379*	544 \pm 75*
12 months (5)	242 799 \pm 36 838	6168 \pm 215*	235 \pm 58	252 009 \pm 12 642*	4209 \pm 411	531 \pm 180*

Data expressed as ppm of dry defatted bone. *P < 0.05 (in comparison to control).

Results and discussion: From the results obtained, it is obvious that fluoride ingestion in excess adversely affects the hydroxyproline content both in cortical and cancellous bones which suggests reduced collagen biosynthesis. Decreased uptake of C¹⁴ proline in fluoride toxicity both *in vitro* (5) and *in vivo* (6) has also been reported, providing conclusive evidence that the bone matrix of both cortical and cancellous bones is laid down with inadequate collagen.

Table 3. Molar ratio of magnesium/calcium in cortical and cancellous bone of rabbits

	Cortical bone	Cancellous bone
Control	0.04	0.04
Molar ratio after fluoride ingestion		
3 months	0.05	0.04
6 months	0.04	0.04
8 months	0.03	0.03
10 months	0.03	0.03
12 months	0.04	0.03

The reduced nitrogen content in cortical bone is possibly due to the reduction in protein biosynthesis and/or collagen biosynthesis (5, 6). However, the unaltered nitrogen content of cancellous bone may be due to increased non-collagenous

proteins *viz.* proteoglycans, known to occur due to fluoride ingestion (1). The cancellous bone shows increased zinc, calcium and magnesium contents following fluoride ingestion. Increased zinc content in cancellous bone may be an index of increased metabolic activity (7) which is evident from increased osteoblastic activity (8). The decreased molar ratio of Mg/Ca indicates that the rate of conversion of amorphous calcium phosphate (ACP) to crystalline hydroxyapatite (HA) has been enhanced (9). Our observations in this regard correspond with the results reported by Baud and his collaborators (10). However, there is considerable variation in calcium and magnesium contents of cortical bone during different phases of fluoride ingestion and this possibly reveals a fundamental aspect of the physiological status of cortical bone.

From the above discussion, it is evident that fluoride poisoning produces significant changes in both organic and inorganic constituents of bone. However, the effects of fluoride on cortical and cancellous bone are different. The differential response is possible due to the intrinsic variations in biochemical characteristics of bone (11).

1. Susheela, A.K. and Jha Mohan (1981) *Experientia*, in press
2. Minari, O. and Zitversmit, O.B. (1963) *Anal. Biochem.*, 6, 320
3. Kivirikko, K.L., Laitinen, O. and Prockop, D.J. (1967) *Anal. Biochem.*, 19, 249
4. Parker, H.E. (1963) *Atomic Absorption News Letter*, 13, 1
5. Proffit, W.R. and Ackerman, J.L. (1964) *Science*, 145, 932
6. Susheela, A.K. and Mukherjee, D. (1981) *Toxicol. Eur. Res.*, 3, 99
7. Hammernt, S. and Mclean, F.C. (1966) in *Zinc Metabolism*, (Prasad, A.S. ed.), p. 14, C.C. Thomas, Springfield.
8. Jowsey, J., *et al.*, (1972) *Am. J. Med.*, 53, 151
9. Eans, E.D. and Posner, A.S. (1965) *Trans. N.Y. Acad. Sci.*, 28, 233
10. Baud, C.A., Ponezot, J.A. and Tochan-Danghy, H.J. (1976) *Cal. Tissue Res.*, 21 (Suppl.), 452
11. Susheela, A.K. and Jha Mohan (1981) *IRCS Med. Sci.*, 9, 640

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