Chemical analyses of the bones of two women indicate that human bone may contain as much as 0.5–0.6 percent fluoride without being adversely affected.

Excessive Fluoride in Water and Bone Chemistry

Comparison of two cases

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CHEMICAL analyses of skeletal tissues of two women have provided new data on the effect on bone composition of excessive amounts of fluoride in drinking water. One of these women, subject A, 74 years old, lived for 24 years prior to death in Washington, D. C., where the drinking water contained 0.2 ppm fluoride. The other, subject B, died at 78 years after 34 years of residence in Bartlett, Tex., where the drinking water contained 8.0 ppm fluoride. Subject B, whose death was caused by a cerebral vascular accident, was a member of a population group previously studied (*1*). Subject A died of a heart attack.

The ingestion of fluoride and its concomitant occurrence in the animal body has been studied previously and extensively by the analysis of various tissues of cattle, sheep, swine, and small laboratory animals (*2–12*). Similar data for man, however, are limited and apply practically entirely to the bones and teeth.

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Roholm (*2*) tabulated the results of the fluoride analyses of human tissues available in the literature up to 1937. His fluoride data, expressed as a percentage of the bone ash, are considered normal for the bones of adults without unusual exposure to the mineral. Values ranged from 0.05 to 0.21 percent fluoride.

He also reported the fluoride content of bones of two men who had been employed in the handling of cryolite and were thereby exposed to excessive quantities of the dust of this fluoride-containing mineral. The ash of bone specimens from these two cryolite workers contained as much as 1.31 percent fluoride.

Boissevain and Drea (*13*) reported 0.12–0.35 percent fluoride in ash of human rib bones after 15–40 years of exposure to a domestic water containing 2.5 ppm fluoride. They found 0.07 percent fluoride in ash of one human rib with no known exposure to a fluoride drinking water. Linsman and Murray (*14*) attributed a case of so-called fluoride osteosclerosis to the use of drinking waters containing 4.4–12.0 ppm fluoride. The ash of the sternum and that of lumbar vertebral body were reported to contain 0.69 and 0.75 percent fluoride respectively.

In 1938 Wolff and Kerr (*15*) analyzed the skeletal tissues of a man, 48 years of age, exposed almost daily for 18 years to the dust of rock phosphate (3.88 percent fluoride). According to their data, based on 9 different bone specimens, 0.18–0.70 percent fluoride was present in the dry, fat-free bone.

Kilborn and co-workers (*16*) observed not only a high incidence of mottled enamel in the inhabitants of a remote province of China but also a chronic skeletal disease which they attributed to fluorosis. The drinking water used in two villages contained 6.3 and 5.9 ppm fluoride. A native of one of these villages, a 37-year-old man, was autopsied after accidental death. Sixteen different bones from this man averaged 1.27 percent fluoride in the dry bone and 1.91 percent fluoride in the bone ash. The results varied from 0.97 to 1.50 percent fluoride in dry bone and from 1.47 to 2.20 percent fluoride in bone ash.

The fluoride in the ribs of humans of different ages with no known abnormal exposure to fluoride was studied by Glock and associates (*17*). Although considerable variation occurred, there is evidence of an increase in skeletal fluoride concomitant with advancing age. Their results, which appear to be somewhat high, are:

Age (years)	*Percent fluoride in fat-free bone*	*Percent fluoride in bone ash*
Under 1	0.024	---------
Under 1	.033	---------
2	.06	0.115
15	.088	.174
22	.18	.397
25	.061	---------
27	.28	.591
32	.089	.194
37	.31	.687
37	.15	.280
54	.23	---------
59	.09	.186
60	.23	.473
68	.18	.380

This relation to age has been studied more recently by Smith and associates (*18*). In the age group 81–90 years, the fluoride in skeletal ash averaged a little more than 0.128 percent but ranged from 0.034 to 0.331 percent for 10 samples of rib and vertebra. Although marked variations thus occurred in the data for individuals of the same chronological age, these authors state that there is "a striking approximation to a linear relationship for the average bone fluoride concentrations when 10-year age groups are plotted against the logarithm of the age." These normal accumulations of skeletal fluoride were associated with the use of a drinking water containing 0.06 ppm of fluoride.

In a more recent study, the fluoride content of the iliac crest was found to vary from 0.0164 to 0.0505 percent on dry, fat-free bones in persons aged 32–84 years who had no known exposure to a fluoride water (*19*). Extensive data on the relation between fluoride in drinking water and fluoride in skeletal tissues are reported by Zipkin and associates (see pp. 732–740 of this issue of *Public Health Reports*).

It is evident from these bone analyses that fluoride may accumulate in skeletal tissues concomitant with the ingestion of fluoride. The presence of relatively large quantities of fluoride in drinking water would be expected to cause marked increases in skeletal fluoride after a prolonged exposure. This was found to have occurred in the woman of this study exposed to high-level fluoride water.

While a major interest in the chemistry of these human skeletal tissues is the extent of fluoride accumulations, it was our purpose also to throw some light on the relation of fluoride to other bone elements, particularly those concerned with calcification. Accordingly, the ash, calcium, phosphorus, magnesium, and carbon dioxide contents of the bones were determined.

Methods and Results of Analyses

For the chemical analyses, the bones were reduced to small pieces; the fat was extracted with alcohol and ether; and the sample was ground to pass a 60-mesh sieve and then dried at 110° C. A temperature of 600° C. was maintained in the electric muffle furnace for ashing. Fluoride was determined on the ashed samples by standard procedures (*20*, *21*), which require steam distillation using perchloric acid and titration of the fluoride evolved in the distillate with standard thorium nitrate. In addition to fluoride, calcium was determined on the ashed samples by triple precipitation of the oxalate from acid solution by slow addition of NH_4OH to avoid contamination with phosphate and to assure good separation of mag-

Table 1. Composition of skeletal tissues (dry, fat-free) of two adults with extremes of fluoride exposure

Bone specimen	Percent ash		Percent calcium		Percent phosphorus		Calcium/phosphorus		Percent magnesium		Percent carbon dioxide		Percent fluoride	
	A[1]	B[2]	A	B	A	B	A	B	A	B	A	B	A	B
Femur	68.73	70.94	26.46	26.71	11.70	11.71	2.26	2.28	0.37	0.35	3.93	3.95	0.062	0.551
Tibia	65.14	-----	24.92	-----	11.11	-----	2.24	-----	.38	-----	3.44	-----	.067	-----
Fibula	57.43	71.65	20.92	26.97	9.24	11.81	2.26	2.28	.49	.34	-----	4.00	.080	.512
Calvarium	68.10	71.44	26.12	26.79	11.61	11.87	2.25	2.26	.36	.39	3.70	3.96	.092	.653
Lumbar vertebra	43.39	60.24	15.69	22.38	7.46	9.45	2.10	2.37	.48	.34	1.48	3.42	.077	.550
Thoracic vertebra	-----	60.84	-----	22.66	-----	10.01	-----	2.26	-----	.33	-----	3.41	-----	.550
Dorsal vertebra	46.38	-----	16.72	-----	7.50	-----	2.33	-----	.31	-----	2.39	-----	.086	-----
Miscellaneous vertebra	-----	58.82	-----	21.90	-----	9.53	-----	2.30	-----	.33	-----	3.15	-----	.530
Rib	54.78	66.92	21.10	25.16	9.50	11.01	2.22	2.29	.35	.35	2.92	3.83	.089	.630
Right pelvis	-----	67.53	-----	24.56	-----	10.79	-----	2.28	-----	.32	-----	3.88	-----	.577
Acromioclavicular joint	-----	55.86	-----	20.27	-----	8.84	-----	2.29	-----	.32	-----	3.19	-----	.540
Crest of ilium	57.77	-----	21.90	-----	9.81	-----	2.23	-----	.36	-----	2.93	-----	.078	-----
Intervertebral cartilage	6.72	5.19	1.72	.61	.63	.29	2.73	2.10	.13	.15	.00	.20	.006	.011

[1] Washington, D. C., resident; 0.2 ppm fluoride in drinking water.

[2] Bartlett, Tex., resident; 8.0 ppm fluoride in drinking water.

nesium (*22*). Final weighing was as CaF_2. Magnesium was determined in the filtrate by double precipitation as magnesium ammonium phosphate. Phosphorus was determined on the unashed sample by the molybdovanadophosphate differential spectrophotometric method (*23*). Carbon dioxide was determined on the unashed samples by evolution with $HClO_4$ and absorption of the dried gas in a weighing bulb, a modification of the standard procedure (*22*).

In table 1 the analytical data are presented on the dry, fat-free bone. As might be expected, the most remarkable difference in the chemical content of the bones of these two aged adults lies in the fluoride. As much as 0.653 percent fluoride was present in the calvarium of the Bartlett woman, subject B, with a minimum of 0.512 percent fluoride present in the fibula. In striking contrast, the maximum percentage of fluoride in the skeletal tissues of the control, the nonfluoride subject A, was 0.092 percent in the calvarium. The fluoride concen-

Table 2. Composition of bone ash of two adults with extremes of fluoride exposure

Bone specimen	Percent calcium		Percent phosphorus		Calcium/phosphorus		Percent magnesium		Percent carbon dioxide		Percent fluoride	
	A[1]	B[2]	A	B	A	B	A	B	A	B	A	B
Femur	38.49	37.65	17.02	16.51	2.26	2.28	0.53	0.49	5.72	5.57	0.090	0.771
Tibia	38.25	------	17.06	------	2.24	------	.58	------	5.28	------	.103	------
Fibula	36.43	37.64	16.09	16.48	2.26	2.28	.85	.47	------	5.58	.139	.715
Calvarium	38.36	37.50	17.05	16.62	2.25	2.26	.53	.55	5.43	5.54	.135	.914
Lumbar vertebra	36.16	37.15	17.19	15.69	2.10	2.37	1.11	.56	3.41	5.68	.177	.913
Thoracic vertebra	------	37.25	------	16.45	------	2.26	------	.54	------	5.60	------	.904
Dorsal vertebra	36.05	------	16.17	------	2.23	------	.67	------	5.15	------	.185	------
Miscellaneous vertebra	------	37.23	------	16.20	------	2.30	------	.56	------	5.36	------	.901
Rib	38.52	37.60	17.34	16.45	2.22	2.29	.64	.52	5.33	5.72	.162	.941
Right pelvis	------	36.37	------	15.98	------	2.28	------	.47	------	5.75	------	.855
Acromioclavicular joint	------	36.29	------	15.83	------	2.29	------	.61	------	5.71	------	.966
Crest of ilium	37.91	------	16.98	------	2.23	------	.62	------	5.07	------	.135	------

[1] Washington, D. C., resident; 0.2 ppm fluoride in drinking water.

[2] Bartlett, Tex., resident; 8.0 ppm fluoride in drinking water.

trations in all the different dry, fat-free bone specimens were relatively constant.

Table 2 shows the percentage composition of the bone ash of the two women. It will be noted that the femur and fibula ash of B contained somewhat less fluoride, 0.771 percent and 0.715 percent respectively, than did the other specimens of bone ash of B, which were quite uniform and ranged from 0.855 to 0.966 percent fluoride. The femur ash of A also contained less fluoride than did the ash of other bones. One explanation of this observation may be the higher ash content of these bones.

Some differences in the ash, calcium, and perhaps phosphorus, in addition to fluoride, in the dry, fat-free bones of these two particular women occurred (table 1). In 5 possible comparisons of the same bones from these 2 similar individuals, more ash, calcium, and phosphorus were contained in the bone specimens of B than in those of A. These differences are most pronounced with respect to ash and calcium. There is a suggestion of some elevation in percentage of carbon dioxide in the bones of B compared with A. The calcium-phosphorus ratio and the magnesium content of the two subjects were comparable.

Discussion

The results of the fluoride analyses of these human skeletal tissues agree with previous experimental and clinical evidence that skeletal tissues become an extensive depository of fluoride. The fluoride accumulations found in B resulted from the prolonged use of a drinking water containing an excessively high concentration of fluoride. As indicated by comparison of the data for these two subjects, this accumulation of fluoride increased slightly the calcification of skeletal tissue.

The ash and perhaps the calcium of dry, fat-free bones of B were slightly higher than the ash and calcium in normal human bones, as shown in table 3. Fluoride analyses of the bone specimens from normal subjects, previously studied by Illinois investigators (*24–26*), were made at the National Institute of Dental Research. Analytical data for the individual bones of A and B have been averaged and are presented for comparison with the other findings.

There is a remarkably close agreement among the data obtained on the normal men and on our own normal subject A. Whereas the ash values of the bones from these subjects varied from 56.67 to 57.85 percent, the ash content of the bones of subject B averaged 64.91 percent. The calcium averaged 24.16 percent compared with a variation of 21.26 to 22.84 percent in adult bone exposed to low-fluoride water. There is no indication of an effect of fluoride on the calcium, phosphorus, and calcium-phosphorus ratio of the bone ash.

It seems evident, considering the data in table 3, that a slight increase in calcification did occur

Table 3. Comparison of composition of human skeletal tissue exposed to normal and high-fluoride water.

Subject and exposure	Dry, fat-free bone					Bone ash	
	Percent fluoride	Percent ash	Percent calcium	Percent phosphorus	Calcium/ phosphorus	Percent calcium	Percent phosphorus
Normal water fluoride:							
Male, 35 years [1]	----------	56. 67	21. 61	9. 48	2. 28	38. 13	16. 72
Male, 46 years [2]	0. 037	56. 89	22. 81	9. 86	2. 31	40. 09	17. 33
Male, 60 years [3]	. 038	56. 93	21. 26	10. 08	2. 11	37. 34	17. 70
Male, 48 years [3]	. 077	57. 85	22. 84	10. 30	2. 22	39. 48	17. 80
Female A, 74 years	. 079	57. 71	21. 72	9. 74	2. 23	37. 63	16. 87
Average of normal exposures	. 058	57. 21	22. 04	9. 89	2. 23	38. 53	17. 28
8.0 ppm water fluoride:							
Female B, 78 years	. 556	64. 91	24. 16	10. 55	2. 27	37. 22	16. 25

[1] Reference 24. [2] Reference 25. [3] Reference 26.

in the skeletal tissues of B. This is regarded as the result of exposure to excessive waterborne fluoride.

Bone Pathology and Fluoride Content

For previous evidence bearing on the relationship between fluoride content and pathological changes in human bone tissues, we must refer to the early classic studies of Roholm (*2*). The two autopsy cases he studied represent the characteristic skeletal pathology produced by excessive fluoride. The abnormalities were concomitant with 0.76 to 1.319 percent fluoride present in the ash of the affected bones. An unusually low value of 0.31 percent fluoride is reported for the frontal bone of 1 of these 2 cryolite workers.

The deleterious effects of fluoride, observed in experimental animal studies, have resulted consistently from excessive fluoride exposures, and the observations supply substantial information concerning the relationship between bone fluoride and bone pathology. Swine bones, according to Kick and associates (*3*), retained upwards of 0.30–0.40 percent fluoride before any toxic effects of fluoride were discernible. In the bones of dairy cattle suffering from extreme fluorosis, the severity of bone exostosis increased directly with the fluoride content, a mild form of exostosis being associated with 0.53 percent fluoride in the bone tissue (*9*).

Recently Suttie and associates (*27*) reported that dairy cows tolerated the ingestion of 30 ppm of fluoride in the diet, with a concomitant accumulation of 0.46 percent fluoride in dry, fat-free rib bones. On the basis of the evidence that 0.01–0.15 percent fluorine is present normally in the bone ash of animals, Peirce (*6*) suggested that the "ingestion of quantities of fluorine which apparently exert no untoward effects on the general health of animals or which bring about no obvious morphological change in its skeleton, may nevertheless increase ten- to fifteen-fold the fluorine content of bones and teeth."

The data available through the present study provide additional evidence regarding the threshold level of fluoride which may be tolerated by human skeletal tissues. As much as 0.5–0.6 percent fluoride in the bones of B did not prove to be a physiological hazard. This is about 10 times the quantity of fluoride regarded as normal. X-ray examination, medical, and clinical studies made prior to the death of B (*1, 28*) do not reveal any skeletal abnormalities or systemic conditions of consequence to health or well-being which could be directly associated with the remarkable increases in the skeletal fluoride content. Other reports contain extensive information on the relation between fluoride content and bone pathology (see pp. 721–731 and 732–740 of this issue).

It must be concluded in the light of the available evidence that human skeletal tissue may have a very high degree of physiological tolerance to accumulations of fluoride.

Summary

Analytic chemical studies of similar human skeletal tissues obtained at autopsy from two comparable women were conducted to determine the effect of a prolonged exposure to drinking water containing 8.0 ppm of fluoride on the chemistry of human bones.

As a result of the prolonged use of this fluoride drinking water, the fluoride in dry, fat-free skeletal tissues ranged from 0.512 to 0.653 percent, as compared with 0.062 to 0.092 percent fluoride in the skeletal tissues of a subject, comparable in age, height, weight, and sex, with no unusual water-fluoride exposure.

There was some indication that the prolonged use of drinking water containing 8.0 ppm fluoride accounted for an increase in the ash and a slight increase in the calcium content of the skeletal tissues.

The absence of any gross or systemic findings, or of any impairment of health or well-being, malformation of the skeletal tissues, or malfunction generally in the one subject studied, indicates that human bone may not be affected by as much as 0.5 to 0.6 percent fluoride. These findings compare favorably with other previous evidence pertinent to human bone as well as fluorosed animal bones.

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