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EDITORIAL

THE SKELETAL CHANGES IN CHRONIC FLUOROSIS

It is generally believed that the skeletal changes in chronic fluoride poisoning entail relatively little discomfort to the patient unless the bony appositions exert pressure on the spinal cord or on nerves at the points of exit from the spinal column. Of 20 cases with skeletal fluorosis in a U.S. southwestern Indian reservation, Morris (1) found "no demonstrable physiological adversities". In a more recent survey by Jolly (2), which covered 1300 patients with radiologically demonstrable skeletal fluorosis, 309 were free of symptoms. Roholm (3) described stiffness of the lumbar and thoracic spine which, in the more advanced cases of fluorosis, turned into marked rigidity, but in general the patients had "surprisingly little inconvenience."

Other accounts of skeletal sclerosis, however, especially those from India and North Africa, do not take such a light view on this problem. They noted that the disease is usually accompanied by stiffness and limitation of motion in the spine and in other joints. Teotia et al.(4) have elaborated extensively on the joint changes. They presented clinical and radiological evidence of arthritis in 187 out of 300 patients with endemic skeletal fluorosis in India (110 children, 5 to 14 years of age, and 77 adults). The spine, especially its cervical portion, appeared to be mainly involved; elbow, hip and knee joints followed next in order. This is in contrast to the former belief that skeletal fluorosis does not occur in early life.

Extensive destruction of joints has been described in detail by Soriano (5) in skeletal sclerosis in alcoholics due to intake of wine contaminated by fluoride. He demonstrated bony appositions in the articular capsules and in ligaments, osteomalacia in various parts of the bones and spontaneous fractures. Pinet (6) elaborated in detail on the arthritic changes of the spinal column consisting of fluoride-induced spondylitis i.e., lipping of the vertebral bodies and syndesmosis (ossification of ligaments connecting the individual vertebrae). Such changes are rather common in persons without excess fluoride intake, particularly in advancing age. No data are available on the question whether or not this so-called "normal" aging process might be related to fluoride.

During the 1960's, Jackson et al. (7) and Steyn et al (8) observed a peculiar endemic bone disease in children, from areas of South Africa where the fluoride content of the water ranged between 1.8 and 13 ppm, which they linked with skeletal fluorosis. This condition called Kenhardt disease was characterized by certain deformities in the lower extremities, especially by the occurrence of genu valgum. The children, however, did not present the features customarily linked with skeletal fluorosis. The authors concluded that fluoride together with some other environmental factors was etiologically related to Kenhardt disease because the bone changes differed from those characteristic of skeletal fluorosis.

Another variation of skeletal fluorosis was presumed to exist in the case of an infant boy reported by Klotz (9) who was born in close proximity to a fluoride-emitting fertilizer factory in Belgium. The principal feature in this case was periosteal calcification of long bones.

At the Seventh Conference of the ISFR, Krishnamachari presented a condition similar to or identical with Kenhardt disease which he encountered in an endemic fluorosis area in India. He too felt that this condition might be associated with agents in addition to fluoride. Whether or not the composition of drinking water, dietary habits, certain vitamin deficiencies, inhalation or ingestion of environmental pollutants or other factors contribute to such variations in the skeletal changes in fluorosis is of considerable interest to students of this disease. It seems reasonable to assume that the sclerosing of bones and ligaments may take on different forms under varying circumstances and give rise to manifestations the nature of which in the past has been a mystery to the medical profession.

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POPULATIONAL VARIATION OF FLUORIDE PARAMETERS IN WILD UNGULATES FROM THE WESTERN UNITED STATES

by

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SUMMARY: Assays of mandibular fluoride were utilized to evaluate sex, age, time, geographic, and species specific fluoride accumulation in control Rocky Mountain bighorn sheep, mule deer, elk, pronghorn antelope, Dall sheep, whitetail deer, and Rocky Mountain goats. Samples were collected from wild ungulates in the western United States and fluoride concentrations in the various species were compared and presented graphically. Fluoride was found to increase with age class, but geographically separated populations of the same species accumulated different values of fluoride concentrations as a function of age, as did samples from the same population collected at different times. Population density variables and habitat type preference may be responsible for these phenomena. In addition, the mandible, rib, femur, metatarsus, metacarpus, and tibia-fibia of normal mule and whitetail deer were analyzed for fluoride and intraskeletal variations established. Fluoride was generally highest in the mandible, and decreased in other bones.

Introduction

Normal fluoride levels of indigenous small animals have been studied (1) and control values for mule and whitetail deer have been described (2, 3). The literature suggests that intra- and inter-specific skeletal fluoride concentrations vary most among large mammals (2) but no previous reports on populational fluoride variation have appeared. While the variance within control animals is of minor importance when evaluating industrial-induced fluorosis (4), investigations were conducted to secure information on the distribution of fluoride in uncontaminated environments.

The objectives of this study were to assess variation in bone fluoride accumulations caused by sex, age, time, geographic, species, and intraskeletal considerations. These inquiries were conducted on

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ungulates, as an adequate sample size was available using previously collected specimens. Major emphasis was placed on Rocky Mountain bighorn sheep, not because fluorosis in that species had been documented but because several isolated bighorn sheep populations are to be found in western Montana. Wildlife biologists have monitored these herds for the past 25 years and there is a large selection of skeletal material in the University of Montana's Zoological Museum.

Ungulate habitat preferences and geographic distributions assign a low probability to the animals having had contact with agricultural lands. Thus, they were not exposed to excess amounts of vegetational fluoride from the application of commercial phosphate fertilizers (5). In fact, since most of the ungulates inhabited wilderness areas, they were all considered residents of nonfluoropolluted ecosystems.

Methods

Collection of Specimens: Bone samples were obtained from four sources: 1) University of Montana's Zoological Museum; Dr. P. L. Wright, museum curator and professor of zoology, permitted the removal of the angular apophysis from catalogued mandibles and data were acquired on Rocky Mountain bighorn sheep, Dall sheep, pronghorn antelope, and Rocky Mountain goats. 2) Sapphire Mountains Elk Study; Dr. R. Ream, University of Montana, School of Forestry, granted access to mule deer, elk and whitetail deer lower jaws that were gathered from hunter harvested cervids during research into human influences on elk ecology (6). 3) Personnel of the National Bison Range, Moise, Montana, provided mandibles and metacarpals of mule and whitetail deer after a herd reduction program. 4) Montana Fish and Game Department biologists and wardens furnished bones on a statewide basis from hunter, highway, and winter killed mule deer, pronghorn antelope, elk, and whitetail deer.

Type of Bone Tissue Assayed: Unless noted, fluoride analyses were performed on the angular apophysis of the mandible (7). This tissue was selected, since it was removable from museum specimens without ruining the scientific value of the mandible. In addition, the lower jaw is the skeletal structure most frequently retained during wildlife studies and adequate sample sizes were secured. The mandible is a sensitive indicator of fluoride concentrations (8) and ages were established with tooth replacement or wear techniques. To explore intraskeleton fluoride accumulation, rib, femur, metacarpal, metatarsal, or tibia-fibia bones were procured from individual deer when circumstances permitted. The method employed to acquire samples for fluoroassay have been recorded elsewhere (8).

Sample Preparation and Analysis: A detailed description of the techniques for sample preparation and fluoride analysis used in this study have been presented earlier (2). Fluoride activity was determined with an ORION Fluoride Specific Ion Electrode (9). All bone tissues were assayed at two sample weights, 0.05 and 0.10 grams, and the single value assigned in the data is the average of the two trials. All results are expressed as ppm (parts per million) on a dry, defatted weight.

Aging of Ungulates: To evaluate specific fluoride accumulation in relation to longevity, various methods were utilized to establish the age of each ungulate. 1) The Dall sheep (*Ovis dalli dalli*) were the animals that Hemming (10, 11) studied to construct criteria of age in that species and his data were used after their accuracy was verified (12). 2) Tooth replacement, dental wear, and horn ring counts were employed to age male and female Rocky Mountain goats (*Oreamnos americanus*) (13). 3) In Rocky Mountain bighorn sheep (*Ovis canadensis*), tooth replacement sequences and wear patterns were applied to both sexes, whereas segment counts were valid only for adult males (14, 15). 4) Pronghorn antelope (*Antilocapra americana*) from the National Bison Range were the antelope Dow and Wright (16) collected to determine the changes in dentition associated with age in that species. These pronghorns were ear-tagged as day-old fawns and their precise ages were known. The longevity of other pronghorn antelope was estimated with the published criteria of Dow and Wright. 5) Elk (*Cervus canadensis*) were aged by tooth succession and wear (17, 18), as were mule deer (*Odocoileus hemionus*) (19 - 21) and whitetail deer (*O. virginianus*) (22).

Ungulates were aged under the supervision of P. L. Wright and because of the relative accuracy of the techniques, animals were assigned to yearly age classes. In some instances, individual categories were then combined to facilitate statistical analyses.

Results and Discussion

Study Areas: The majority of specimens were acquired from five geographic units and a brief description of each region will familiarize readers with these environments. Detailed discussions of geology, topography, vegetation, and climate are found in the literature.

1) Wildhorse Island is located approximately 70 airline miles north of Missoula, Montana, and bighorn sheep were introduced to that insular situation around 1940. Since that time, three studies (23, 24, 25) have been conducted on the buildup and decline of the bighorn population, with emphasis on the associated vegetational changes. Specimens in the University of Montana's Zoological Museum enabled appraisal of fluoride

concentrations in two time-differentiated groups of sheep, namely, 1953 - 1962 and 1971 - 1972.

2) Fifty airline miles southeast of Missoula, the Rock Creek area supports a remnant herd of Rocky Mountain bighorns that have experienced severe numerical fluctuations (26, 27, 28).

3) The National Bison Range, 30 miles north of Missoula, was created by an act of Congress in 1909 for the preservation of the American bison. In addition to buffalo, bighorn sheep, elk, whitetail deer, pronghorn antelope, mule deer, and Rocky Mountain goats are found within its enfenced confines (29-31). Wildhorse Island, Rock Creek, and the National Bison Range are characterized by Palouse prairie - bluebunch wheatgrass (Agropyron spicatum) type vegetation on the drier south and west facing exposures with open ponderosa pine (Pinus ponderosa) and Douglas fir (Pseudotsuga menziesii) associations at the higher elevations or on the moister sites.

4) The Sapphire Mountains, running from Missoula south, southeast for 50 miles, are mostly timbered with scattered ponderosa pine on the lower slopes, Douglas fir and lodgepole pine (Pinus contorta) found at middle elevations, and subalpine forests prevalent near the ridgecrests. This region, currently under intense logging and recreational pressure, supports sizeable populations of mule deer, elk, and whitetail deer (6).

5) The Ukakkminilagal Creek - Anaktuvak pass portions of the Brooks Range in northcentral Alaska are vegetated by alpine tundra communities (32). The mandibles of Dall sheep, shot by native subsistance hunters and collected by Alaska Fish and Game Department personnel, were assigned to two time-base populations; 1950 - 1954 and 1960 - 1965 (10, 11).

Lumpy Jaw: Several mandibles had abnormalities due presumably to lumpy jaw disease (33 - 36). While the highest instances of deformities occurred in bighorn and Dall sheep, skeletal symptoms were found in all species. As these lesions closely resemble callus deposits found in fluorotic deer (3, 8), it was probable that the diseased portions of the mandible had higher fluoride concentrations. To examine this hypothesis, samples of lumpy jaw calcifications and normal mandibular tissue were removed from adjacent sections of lower jaws and tested for fluoride. The fluoride content of the two substances showed no significant variation (Table 1): standard error of the paired difference t test was t = 1.362. Since the fluoride parameters were within the age specific confidence limits of the various ungulate populations, lumpy jaw abnormalities were not considered to affect mandibular fluoride levels.

TABLE 3

Effect of Silica upon Recovery of F^- from Vegetation by Extraction with Concentrated Mineral Acids †

Species	(1) Ashing/fusion/ potentiometry	(1) Coefficient** of variation	(1) Number of replicates	Acid extraction	Coefficient** of variation	(1) - (2) Mean difference
Yorkshire fog (Holcus lanatus, L)	1046 ± 52	5.0	8	1032 ± 42	4.1	+ 14
Annual Meadow-grass (Poa annua, L)	773 ± 37	4.8	5	782 ± 25	3.2	- 9
Timothy grass (Phleum pratense, L)	801 ± 24	3.0	7	797 ± 29	3.6	+ 4
Cocksfoot (Dactylis glomerata, L) *	562 ± 19	3.4	7	477 ± 48	10.1	+ 85
Tufted Hair-grass (Deschampsia caespitosa, L) *	577 ± 26	4.5	10	461 ± 37	8.0	+ 116
Common Horsetail (Equisetum arvense, L) *	1740 ± 105	6.0	10	1565 ± 167	10.7	+ 175
Stinging Nettle (Urtica dioica, L) *	588 ± 15	2.6	3	538 ± 45	8.4	+ 50

* Species known to contain abnormally high levels of silica (.27 - 11.3% SiO_2 on a dry weight basis)

** Standard deviation (σ) as a percentage of the mean. † Mean values (μgF/gm. d. wt) ± σ

Sexual Fluoride Variation: Mandibular fluoride levels were not correlatable with sex (Table 2). The observed age class distributions were caused by human management practices and were not species specific nor fluoride related patterns. For example, Wildhorse Island bighorn sheep were never legally hunted and the 1952 - 62 age pyramid, with a predominance of old sheep, reflected a stable population (24). The life expectancy of the 1971 - 72 bighorns had decreased, as the herd surpassed the island's carrying capacity and competition for forage was then partially limiting population growth (25).

Elk and mule deer from the Sapphire Mountains portray the effects of sex specific hunting seasons coupled with the rate of harvest. The Montana Fish and Game Department managed these elk by instituting an unconfined quota on antlered animals and a limited system for antlerless elk. Intense hunting pressure removed the male segment from the herd when the bulls were still young. The more restrictive harvest on cows enabled a segment of the females to gain older age. Conversely, mule deer were exploited under either sex regulations and longevity in these animals was not sex specific, as sportsmen were nonselective.

Age Specific Fluoride Accumulation: Simple regression analyses were calculated correlating an ungulate's age with its ppm fluoride content (age, ppm). Generalized age class data were also plotted against average fluoride parameters ($\bar{x}$ age, $\bar{x}$ ppm). Strong positive correlations were exhibited in all species with coefficient values significant at either the 0.05 or 0.01 level (Table 3). Their values increased when samples within any particular population were averaged prior to regression computations, and by applying this technique perfect correlations were approached. The amounts of skeletal fluoride were near zero at birth (Fig. 1), and thereafter accumulation extended linearly as the ungulates aged.

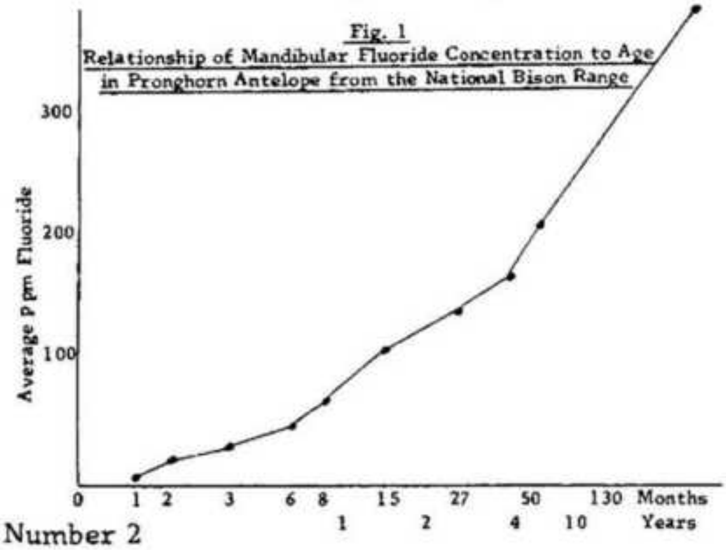


Fig. 1
Relationship of Mandibular Fluoride Concentration to Age in Pronghorn Antelope from the National Bison Range

TABLE 3

Age, Fluoride Regression Analysis

Population	Y[a]	r	SEE	SEB	N	t
BIGHORN SHEEP						
Wildhorse Island	20.747x + 204.898	0.495	131.478	6.152	37	3.373**
1953-1962	39.525x + 149.525	0.775	101.969	13.164	8	3.002*
(age, ppm)[b]	71.226x + 72.613	0.960	53.492	10.326	6	6.897**
($\bar{x}$ age, $\bar{x}$ ppm)[c]						
($\bar{x}$ age, $\bar{x}$ ppm) oldest deleted						
1971-1972						
(age, ppm)	78.488x + 251.213	0.814	111.377	9.320	38	8.422**
($\bar{x}$ age, $\bar{x}$ ppm)	65.428x + 2/8.539	0.898	90.706	12.097	9	5.409**
($\bar{x}$ age, $\bar{x}$ ppm) oldest deleted	90.393x + 184.482	0.989	31.631	5.978	7	15.122**
Rock Creek						
(age, ppm)	17.286x + 112.758	0.484	66.208	6.381	26	2.709*
($\bar{x}$ age, $\bar{x}$ ppm)	10.319x + 132.625	0.664	39.610	8.226	4	1.254
($\bar{x}$ age, $\bar{x}$ ppm) oldest deleted	23.433x + 79.908	0.998	4.943	1.550	3	15.115**
National Bison Range						
(age, ppm)	9.853x + 76.008	0.605	29.566	3.592	15	2.743*
($\bar{x}$ age, $\bar{x}$ ppm)	14.184x + 44.600	0.944	17.328	3.496	4	4.057*
PRONGHORN ANTELOPE						
National Bison Range						
(age, ppm)	27.456x + 58.189	0.899	44.625	2.628	28	10.456**
($\bar{x}$ age, $\bar{x}$ ppm)	30.286x + 38.198	0.970	27.991	2.520	10	11.292**
DALL SHEEP						
Brooks Range, Alaska						
1950-1954 (age, ppm)	68.098x + 492.414	0.707	285.379	10.132	48	6.787**
(x age, x ppm)	86.556x + 444.089	0.945	111.843	14.952	6	5.787**
1960-1965 (age, ppm)	53.724x + 412.552	0.532	196.461	14.436	37	3.722**
(x age, x ppm)	56.700x + 405.680	0.997	15.081	2.352	5	24.084**
MULE DEER						
National Bison Range (age, ppm)	41.225x + 85.482	0.826	70.282	9.937	10	4.149**
Sapphire Mountains (age, ppm)	23.142x + 4.137	0.791	33.537	2.638	48	8.774**
(x age, x ppm)	22.900x + 2.950	0.993	5.122	1.620	5	14.139**
WHITETAIL DEER						
National Bison Range (age, ppm)	58.947x + 31.342	0.639	69.165	25.089	10	2.349*
Sapphire Mountains(age, ppm)	15.365x + 18.889	0.699	25.031	4.205	16	3.654**
(x age, x ppm)	17.307x + 15.145	0.974	9.582	2.864	4	6.041**
ELK						
Sapphire Mountains(age, ppm)	33.595x - 2.182	0.825	41.842	3.836	38	8.758**
(x age, x ppm)	37.114x - 13.029	0.969	23.003	4.762	6	7.794**

[a] Y = regression estimate, r = correlation coefficient, SEE = standard error of the estimate, SEB = standard error of regression coefficient, N = sample size, t = sample t,
* = significant at t 05 level, ** = significant at t 01 level.

[b] Age in years. [c] $\bar{x}$ = mean.

TABLE 4

Age Specific ppm Fluoride Levels in Populations of Rocky Mountain Bighorn and Dall Sheep

Population	Age Class	N	$\overline{x}$	SD	CV	SE$\overline{x}$	EXE$\overline{x}$
BIGHORN SHEEP							
Wildhorse Island							
1953 - 1962	0.5 years	4	35.0	14.3	40.9	7.2	20.5
	1	3	205.0	102.8	50.1	59.3	28.9
	2	3	309.7	85.1	27.5	49.2	15.9
	3	4	347.2	25.4	7.3	12.7	3.7
	4	-	-	-	-	-	-
	5	3	463.7	57.0	12.3	32.9	7.1
	6	2	499.5	82.7	16.6	58.5	11.7
	7	6	405.2	62.8	15.5	25.6	6.3
	8+	11	353.8	129.3	36.5	38.9	11.0
	0.5-3.5	14	219.5	142.7	65.0	38.1	17.4
	4.5-5.5	4	398.8	137.9	34.6	68.9	17.3
	6.5-7.5	8	428.8	75.5	17.6	26.7	6.2
1971-1972	0.5	4	239.2	49.4	20.7	24.7	10.3
	1	1	352.0	-	-	-	-
	2	3	349.7	38.5	11.0	22.2	6.3
	3	13	501.0	153.8	30.7	42.7	8.5
	4	5	597.0	56.8	9.5	25.4	4.2
	5	4	696.0	81.3	11.6	40.6	5.8
	6	4	770.8	33.8	4.4	16.9	2.2
	7	3	750.3	27.5	3.7	15.9	2.1
	8+	1	639.0	-	-	-	-
	0.5-3.5	21	422.4	162.7	38.5	35.5	8.4
	4.5-5.5	9	641.0	82.5	12.8	27.5	4.3
	6.5-7.5	7	762.0	30.7	4.0	11.6	1.5
Rock Creek	0.5-3.5	7	136.7	96.6	70.6	36.5	26.7
	4.5-5.5	11	201.1	58.2	28.9	17.6	8.7
	6.5-7.5	6	241.7	34.8	14.4	14.2	5.8
	8+	2	193.5	65.8	33.9	46.5	24.0
National Bison Range	3.5	2	81.5	21.9	26.9	15.5	19.0
	4.5-5.5	7	132.4	29.5	22.3	11.1	8.4
	6.5-7.5	4	139.8	10.2	7.3	5.1	3.7
	8+	2	186.5	27.6	14.8	19.5	10.4
DALL SHEEP							
Brooks Range, Alaska							
1950 - 1954	6 months	5	488.4	302.7	62.0	135.4	27.7
	18	11	523.4	114.4	21.9	34.5	6.6
	30	11	644.8	212.9	33.0	64.2	10.0
	48	4	759.2	514.8	67.8	257.4	33.9
	72	7	1,156.6	373.4	32.3	141.1	12.2
	115	9	1,177.8	373.4	31.7	124.5	10.6
1960 - 1965	6	2	431.5	85.6	19.8	60.5	14.0
	24	10	530.9	144.3	27.2	45.6	8.6
	48	10	632.3	231.2	36.6	73.1	11.6
	72	11	726.4	213.3	29.4	64.3	8.8
	103	4	904.5	246.2	123.1	123.0	13.6

N = sample size, $\overline{x}$ = mean, SD = standard deviation, CV = coefficient of variation in %, SE$\overline{x}$ = standard error of the mean, and EXE$\overline{x}$ = experimental error of the mean in %.

Fig. 2
Relationship of Mandibular Fluoride Concentration to Age in Bighorn Sheep from Wildhorse Island

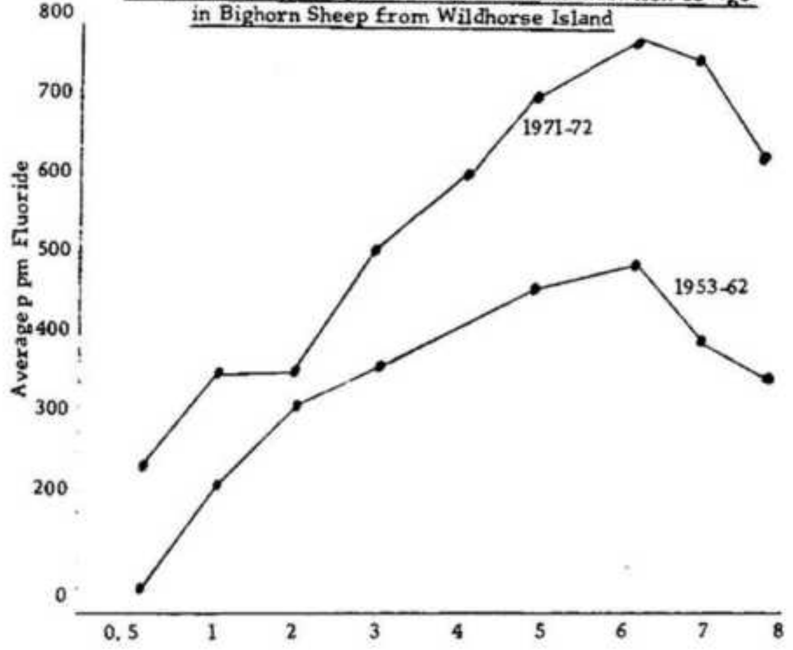
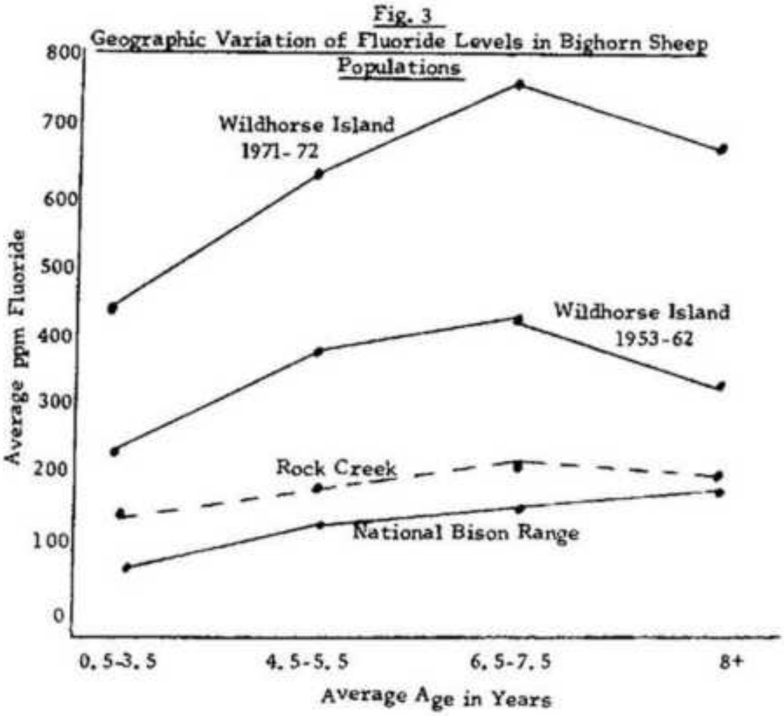


Fig. 3
Geographic Variation of Fluoride Levels in Bighorn Sheep Populations



The best individual age - fluoride content correlation was achieved in pronghorn antelope, $r = 0.899$. This degree of association was probably ascertained because the precise ages of these antelopes were known. In the other species, ages were established with the aid of tooth wear techniques which are subject to interpretative judgements. Consequently, in the latter cases an unknown percentage of the ungulates were assigned to incorrect longevity categories (37). Thus, intraregression variances were expanded and correlation coefficients were reduced. Inadvertent mistakes also magnified the variability of fluoride concentrations within any one cohort. Furthermore, the accuracy of the dental wear methods is inversely proportional to age, and therefore systematic errors were most frequent in the oldest classifications. The raw data support this contention, though there was a tendency for discrepancies in fluoride content to augment as the animals advanced in years, regardless of the age criteria that were applied.

Of the 12 ungulate populations, 3 of the bighorn sheep herds had fluoride levels that declined in the eldest age classes (Table 4, Fig. 2). When these decreasing cohorts were removed antecedent to regression analyses, their values rose; 0.775 to 0.960, 0.898 to 0.989, and 0.664 to 0.998 in the Wildhorse Island 1953-62, 1971-72, and the Rock Creek sheep. The mechanisms that curtailed fluoride accumulation in these senior age classes are probably related to population size and habitat capacity considerations, as this trend was only detected in underharvested herds.

Throughout North America, bighorn sheep are managed with a restricted removal of 3/4 curl rams or are totally unhunted, and all these isolated bands have experienced large numerical vacillations due, in part, to competition for a qualified range resource (38). These herds, including the Wildhorse Island and Rock Creek sheep, have a predominance of archaic individuals, especially females. Conversely, the bighorn sheep on the National Bison Range are kept below the environment's carrying capacity by live trapping and removal of both sexes under restocking programs. These bighorns have maintained a young, stable population and mandibular fluoride levels in these sheep increased through all cohorts (Fig. 3). Furthermore, the other ungulates on the National Bison Range are kept within the habitat's supportive ability for each species and in total (30, 31), while big game in Montana are so extensively exploited that forage is seldom a limiting factor. In Alaska, wolves, natives, and trophy hunters crop the Dall sheep.

The positive correlation of fluoride content with age explains a proportion of the variation that was reported in control mule and whitetail deer, since those studies did not account for the deer's ages (2, 3). In all probability, longevity specific fluoride accumulations also occur in normal small mammal and avian populations. Thus, data on fluoride concentrations in animals are more meaningful if age differential retention is defined.

Variation with Time: The two time-base populations of Wildhorse Island bighorn sheep (Table 4, Fig. 3) indicate that the bighorns which died during 1971-72 had the larger concentrations of mandibular fluoride. These statistically valid differences (Table 5) were presumably caused by density dependent factors and not time, per se, as there is no evidence in the literature to support the idea that a species' pace of metabolic fluoride detention changes over a period of time (5).

Other researchers proved that skeletal fluoride levels were directly linked to the amount of fluoride in the animal's diet (5). Assuming that similar mechanisms regulate fluoride deposition in wild ungulates, the diets of the 1971-72 bighorns contained more fluoride than those of the 1953-62 sheep. As vegetational accumulation of background fluoride was not involved, the bighorn sheep's food habits changed between 1953-63 and 1971-72 to subject the latter to larger aggregations of fluoride.

TABLE 5

t Test of Age Class Fluoride Levels in Bighorn Sheep Populations

	Age Class (in years)	Wildhorse Island 1971-72	Rock Creek	National Bison Range
Wildhorse Island 1952 - 63	0.5-3.5	3.90**	1.60	3.35**
	4.5-5.5	3.26**	2.78*	3.81**
	6.5-7.5	11.44**	6.19**	10.60**
	8+	7.31**	2.64*	3.80**
Wildhorse Island 1971 - 72	0.5-3.5		5.61**	8.80**
	4.5-5.5		13.49**	17.15**
	6.5-7.5		28.38**	49.10**
	8+		9.58**	23.20**
Rock Creek	0.5-3.5			1.39
	4.5-5.5			3.31**
	6.5-7.5			6.75**
	8+			0.14
Wildhorse Island 1952 - 63	0.5	7.94**		
	1	2.48		
	2	0.74		
	3	3.45**		
	4	-		
	5	4.44**		
	6	4.45**		
	7	11.45**		
	8	7.31**		

* Significant at t 05 **Significant at t 01

The Wildhorse Island bighorn sheep were relatively stable from 1953 to 1963 and, as such, were at or near that species' habitat potential (23, 24). The 1971 - 72 bighorns were more than twice as numerous as they were during the previous period and the harsh winter of 1971 - 72 decimated that population after the range had deteriorated considerably on preferred grazing areas (25). Even in the absence of major predators or human exploitation, as on Wildhorse Island, bighorn sheep do not venture far from escape cover (38, 39). The concentration of sheep on these limited, rough topographic areas caused all the animals to severely crop the forage resources. This probably increased the bighorns' ingestion of soil particulate contaminated vegetation which, in turn, enhanced their dietary fluoride intake (1).

Dall sheep from the Ukakkminilagal Creek - Anaktuvak Pass setments of the Brooks Range were also segregated into two time-orientated populations; 1950 to 1954 and 1960 to 1965 (10, 11). The disparities in mandibular fluoride levels from these two groups (Table 4, Fig. 4) were not statistically significant due to substantial intra-age class variation. Supplemental information on the numerical composition of either Dall sheep herd was not available.

Mule deer, elk, and whitetail deer were collected from the Sapphire Mountains in 1970, 1971, and 1972. Since intraspecific fluoride analyses did not demonstrate any yearly deviations, the data were combined into single sets of population measurements. As these deer and elk herds were sustained at analogous, sub-carrying capacity levels during the sample period (6), these data support the contention that population dynamic influences were responsible for the observed distribution of fluoride parameters in the two groups of bighorn sheep from Wildhorse Island.

Geographic Intraspecific Variation: Geographic variation of fluoride levels was evident in bighorn sheep (Fig 3) and these age-class differences were significant (Table 5). As previously discussed, the three bighorn populations inhabit similar vegetation types in western Montana; the National Bison Range and Wildhorse Island are situated only 40 airline miles apart. Forage and water samples were collected from these two areas to ascertain the possible association of background fluoride concentrations with the observed skeletal accumulations. Kay's methods (1, 4) were utilized for fluoroanalyses.

Unexpectedly, a negative correlation was documented as the fluoride levels were higher in the Bison Range flora (Table 6); bluebunch wheatgrass, $t = 4.58$, and total grasses, $t = 5.16$, were both significant at the 0.01 level. These species constitute a major portion of the bighorns' diets (23 - 25, 40 - 42), but forage specimens were not obtained from the preferred grazing areas on Wildhorse Island because heavy utilization of the vegetation prevented the acquisition of analytical material. Additionally, the actual plants selected by either population were not tested for fluoride and, thus, the bighorn's actual fluoride intakes were not assessed.

Fig. 4

Relationship of Mandibular Fluoride Concentration to Age in Dall Sheep from Ukakkminilagal Creek and Anaktuvak Pass, Brooks Range, Alaska

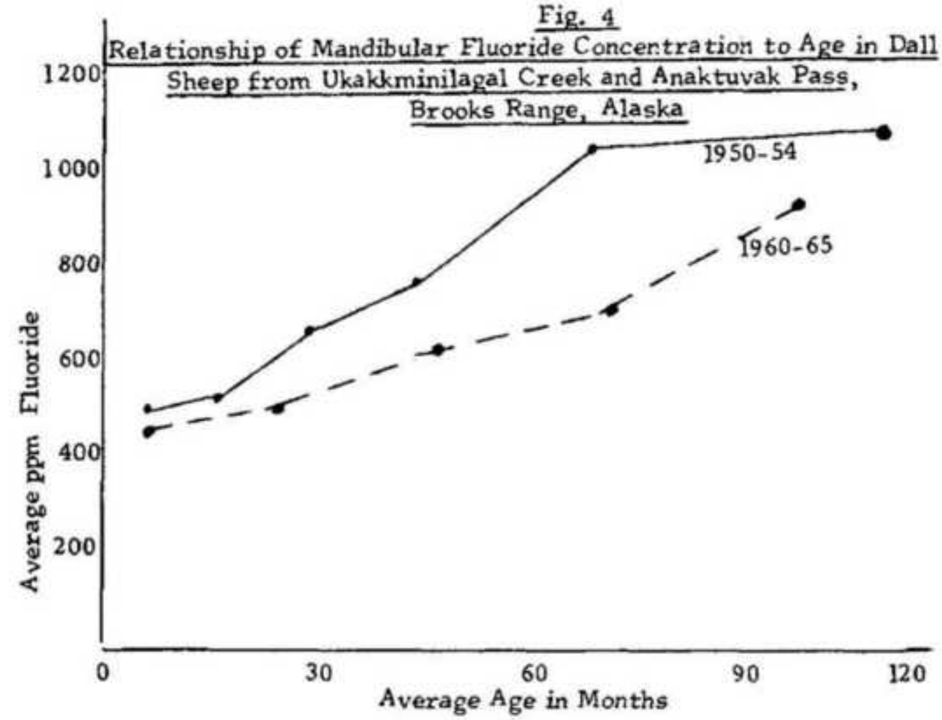


TABLE 6

ppm Fluoride Levels in Vegetation and Water Samples from the National Bison Range and Wildhorse Island

Species	N	$\bar{x}$	SD	CV	SE$\bar{x}$	EXE$\bar{x}$
National Bison Range 11/9/72						
Bluebunch wheatgrass *Agropyron spicatum*	15	6.0	2.8	46.7	0.7	11.7
Rough Fescue *Festuca scabrella*	1	1.8	-	-	-	-
Crested wheatgrass *Agropyron cristatum*	1	5.7	-	-	-	-
Grass—various species	5	4.8	2.4	50.0	1.0	20.8
Total Grasses	22	5.5	2.6	47.3	0.5	9.1
Water	11	0.31	0.07	24.8	0.02	6.5
Wildhorse Island 9/9/73						
Bluebunch wheatgrass	10	2.2	1.2	55.6	0.4	18.2
Water	1	0.38	-	-	-	-

Earlier it was suggested that the amount of dietary fluoride was directly related to ungulate density through overgrazing and the associated ingestion of soil-contaminated forage. This may also explain a proportion of the observed geographic fluoride variation, since the most crowded bighorn sheep had the greatest concentrations of skeletal fluoride (23 - 28). However, other parameters allied with aggregate fluoride composition and utilization of natural mineral licks, for example, should be investigated before a definitive conclusion is formulated.

Interspecific Variation: Fluoride concentrations in ungulates collected on the National Bison Range (Table 7) established a degree of species variability, but insufficient sample sizes precluded statistical evaluations. The specific differences appear to be minimal when the animals are young and to disappear with age. An identifical tendency was observed in cervids from the Sapphire Mountains (Table 8). In these instances, species specific fluoride accumulations were not definable which, in all likelihood, was due to these ungulates' submaximal population densities or their parallel forage preferences (29, 43 - 45).

TABLE 7

ppm Fluoride Parameters of Ungulates from the National Bison Range

Species	Age Class	N	$\bar{x}$	SD	CV	SE$\bar{x}$	EXE$\bar{x}$
Bighorn sheep	3.5 years	2	81.5	21.9	26.9	15.5	19.0
	4.5-5.5	7	132.4	29.5	22.3	11.1	8.4
	6.5-7.5	4	139.8	10.2	7.3	5.1	3.7
	8+	2	186.5	27.2	14.8	19.5	10.4
Mule deer	1.5 years	1	124.0	-	-	-	-
	2.5	3	183.0	46.1	25.2	26.6	14.6
	3.5	3	187.7	49.4	26.3	28.5	15.2
	4.5	2	375.0	32.5	8.7	23.0	6.1
	10+	1	456.0	-	-	-	-
Whitetail deer	1.5 years	3	136.0	21.8	16.0	12.6	9.2
	2.5	2	130.0	43.8	33.7	31.0	23.8
	3.5	5	247.4	85.7	34.6	38.3	15.5
Pronghorn antelope	1 month	1	5.0	-	-	-	-
	2	1	14.0	-	-	-	-
	3	1	29.0	-	-	-	-
	6	1	53.0	-	-	-	-
	8	1	81.0	-	-	-	-
	15	12	104.1	29.9	28.8	8.6	8.3
	27	3	154.0	43.9	28.5	25.3	16.5
	3.5-4.5 yrs.	2	162.0	7.1	4.3	5.0	3.1
	5.5-6.5	3	190.0	34.7	18.3	20.0	10.5
	9.5-12.5	3	363.7	103.2	28.4	59.6	16.4
Rocky Mountain goat	4 years	1	253.0	-	-	-	-
	5.5	1	212.4	-	-	-	-
	6.5	1	277.1	-	-	-	-
	9	1	288.9	-	-	-	-

TABLE 8

ppm Fluoride Parameters of Ungulates from the Sapphire Mountains

Species	Age Class	N	$\bar{x}$	SD	CV	SE$\bar{x}$	EXE$\bar{x}$
Mule deer	0.5 years	6	13.3	2.7	20.5	1.1	8.4
	1.5	19	42.2	17.9	42.3	4.1	9.7
	2.5	12	53.7	22.4	41.8	6.5	12.1
	3.5	6	87.7	41.9	47.8	17.1	19.5
	4.5	2	105.0	32.5	30.9	23.0	21.9
	6.5-10.5	3	207.0	109.8	53.1	63.4	30.6
Whitetail deer	0.5	2	18.5	0.7	3.8	0.5	2.7
	1.5	8	37.9	15.6	41.2	5.5	14.6
	2.5	3	69.7	4.2	6.0	2.4	3.4
	3.5-6.5	3	97.0	47.7	49.2	27.5	28.4
Elk	0.5	8	18.6	8.1	43.3	2.8	15.3
	1.5	12	56.1	28.7	51.2	8.2	14.8
	2.5	5	60.2	12.9	21.4	5.8	9.6
	3.5	5	111.8	73.8	66.0	33.0	29.5
	4.5	5	126.8	23.3	18.4	10.4	8.2
	5.5-7.5	3	253.0	96.0	38.0	55.4	21.9

TABLE 9

Average ppm Fluoride Parameters of Mule and Whitetail Deer from Montana

Species and Bone	N	$\bar{x}$	RF	SD	CV	SE$\bar{x}$	EXE$\bar{x}$
85 Mule Deer							
Mandible	50	189.9	1.000	137.2	72.3	19.4	14.1
Rib	2	168.0	0.884	31.1	18.5	22.0	13.1
Femur	29	152.9	0.805	77.2	50.5	14.3	9.4
Metatarsus	19	110.1	0.578	53.6	48.6	12.3	11.2
Metacarpus	21	137.1	0.721	80.3	58.6	17.5	12.8
Tibia-fibia	14	129.7	0.684	71.9	55.4	19.3	26.8
45 Whitetail Deer							
Mandible	23	160.0	1.000	77.8	49.3	16.4	10.3
Rib	2	116.0	0.724	11.3	9.7	8.0	6.9
Femur	20	114.5	0.715	60.7	53.0	14.7	12.9
Metatarsus	8	114.6	0.716	55.8	48.7	19.7	17.2
Metacarpus	16	78.2	0.488	25.4	32.5	19.6	25.1
Tibia-fibia	6	76.1	0.474	30.9	40.6	12.6	16.6

RF = Ratio of the average bone fluoride divided by the average mandibular fluoride.

A review of the data presented discloses that disparities in interspecific mandibular fluoride intensified when major geographic boundaries were crossed. Year-of-birth Sapphire Mountain elk, National Bison Range pronghorn antelope, 1971 - 1972 Wildhorse Island bighorns, and 1950 - 1954 Dall sheep had ppm fluoride values of 18.6, 53.0, 239.2, and 488.4, respectively, whereas 4.5-year-old animals had parameters of 126.8, 162.0, 597.0, and 759.2 ppm. Direct cause and effect comparisons are impossible to assess since, for example, no fluoride determinations have ever been conducted on materials from the Dall sheeps' Alaskan environment. The observed differences probably mirror variations in dietary fluoride exposures that ensue from species specific food habits, discrepancies in background fluoride concentrations, or density dependent vegetation utilization patterns. Additional research is necessary to qualify the interrelationships among these casual mechanisms.

Intraskeletal Variation: The majority of mule and whitetail deer skeletal material was collected before the nature of age specific fluoride accumulation was discovered and, therefore, fluoride determinations on the various bones cannot be segregated into age categories. As geographic or populational variations may also exist within the data, the deer were individually tabulated by county, and the average values which were calculated are presented in Table 9. The mandible, rib, femur, metatarsus, metacarpus, or tibia-fibia were not removed from each deer, and while no direct correlations between the average skeletal concentrations can be formulated, the mandible had the largest amounts of fluoride followed by the femur or rib with the metatarsal, metacarpal, or tibia-fibia bones having the lowest fluoride levels.

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SKELETAL FLUOROSIS: ROENTGENOLOGICAL AND HISTOPATHOLOGICAL STÚDY

by

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SUMMARY: Detailed radiological and morphometrical studies have been reported in 100 patients of proven endemic skeletal fluorosis all of whom were symptomatic. Though the radiological features such as osteopetrosis, periosteal bone formation, osteophytosis and calcification of the interosseous membrane and ligaments remain the mainstay in the diagnosis of skeletal fluorosis, in increasing instances radiological findings have been variable and uncommon suggesting the presence of other metabolic bone disease such as rachitis, osteomalacia, osteoporosis, neo-osseous-porosis, coarsened trabecular pattern, cystic expansion of the bones of hands and feet, subperiosteal resorption of the phalanges, widened metaphyses with rarefaction and irregular erosions, hypercementosis at the roots of the teeth and erosions of the lamina dura. Morphometrical measurements of the iliac crest biopsies revealed osteoclastic resorption of the trabecular bone, increased numbers of resorption lacunae, periosteocytic osteolysis and increased osteoid covered surfaces. The calcification front had a normal distribution except in four patients with associated osteomalacia. Findings were similar in children suffering from endemic fluorosis. Our studies suggest that the stabilizing effect of fluoride on bone due to the conversion of hydroxyapatite to fluorapatite produces a compensatory hyperplasia of the parathyroid glands. Thus, the possibility of a beneficial effect of fluoride in osteoporosis has been challenged in the light of this parathyroid compensation.

In recent years there has been a revival of interest in fluoride metabolism (1). The use of sodium fluoride in the treatment of osteoporosis and the production of dialysis bone disease in patients who have been exposed to fluoridated dialysate for several years, has sparked further research in skeletal fluorosis and related bone disease (2, 3). It is apparent that industrial fluorosis may occur either from the deposition of flucride particles on the ground and on herbage, or from the presence of fluoride in the atmosphere where they may be inhaled by man. As a result of the rapid industrial proliferation, fluoride research is becoming of increasing significance in our country.

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This vast increase in the amount and scope of research in fluoride metabolism has led to the further discovery of endemic fluorosis areas in India with a high natural content of fluoride in the drinking water. Our interest shifted to the problems on "Fluoride and bone" in 1968 and since then we have been able to discover several zones of endemic skeletal fluorosis in the State of Uttar Pradesh. The most severely affected population is located in the district of Rai Barelli where the drinking water contained 24-26 ppm of fluoride. The growing children in this area have also exhibited severe degrees of skeletal fluorosis (4, 5). To the authors' knowledge these are the only reports in the literature on skeletal fluorosis in children.

In our metabolic clinic, where all bone problems are referred for a radiological opinion, we have seen increasing numbers of patients with skeletal fluorosis where the radiological findings have been variable and uncommon. Whereas the radiological features of skeletal fluorosis have been well described, reports on the morphometric study of the bone are sparse.

In our opinion, the radiological advice can be best formulated on a background knowledge of the histopathology of the bone. Therefore, the purpose of the current communication is to review the radiological findings in 100 patients with skeletal fluorosis in correlation with the morphometrical measurements of their bones.

Material and Methods

Of 100 patients studied, 78 were male and 22 female. All were symptomatic ranging in age from 10 to 62 years. Only twelve were less than 15 years of age. They belonged to a poor socio-economic strata and had resided in the endemic area in District Rai Barelli, U. P. since birth. The diagnosis of skeletal fluorosis was based on the following: 1. the endemic nature of the disease, 2. the characteristic clinical picture, 3. the high fluoride content of drinking water, 4. increased urinary excretion of fluoride, 5. excess content of fluoride in bone ash, 6. characteristic radiological and histological changes. None of the patients had vitamin D deficiency. Renal disease and intestinal malabsorption were excluded in each case by appropriate investigations. All patients were untreated at the time this study was undertaken.

The skeletal and dental radiographs were taken in each case with a standard exposure. Morphometrical measurements of the undecalcified sections of the bone obtained from the iliac crest by open biopsy were studied in 44 patients (six children). Without previous decalcification, sections 5 μ thick were cut with a Jung microtome and stained by Von Kossa technique, with solochrome cyanine, toludino blue and thionin. Quantitative observations on mineralized and non-mineralized bone were made using Zeiss I and II integrating eye pieces and also with a Whipple's grid eye piece. The fluoride content of the drinking water was determined by specific fluoride ion electrode as detailed by Taves (6). Thirty samples of drinking water were analyzed; they contained 24-26 ppm of fluoride.

Results

The characteristic radiological findings which were encountered in 45% of our cases are summarized in Table 1. All showed abnormal calcification, poorly formed Haversian systems and disordered lamellar orientation. In four patients who had concurrent osteomalacia osteoid comprised 46.5 ± 3.7% of the total area of trabecular bone and 55 ± 9.1% of the calcification front.

TABLE 1

Characteristic Radiological Findings in 55% of the cases

1. Osteosclerosis (Spine, pelvis, thorax).
2. Irregular periosteal bone formation.
3. Ectopic calcification (ligaments, capsules, interosseous membrane, muscular attachments, tendons).
4. Dense and thick cortex with reduction of marrow cavity.
5. Irregular osteophytosis.
6. Irregular exostoses.
7. Rough and accentuated muscular attachments.
8. Hypercementosis of the root of teeth.

Bone is a physiologically active and vital body tissue and the excess deposition of fluoride produces alterations in its structure and the radiological appearance as indicated in Tables 1 to 3.

In our series, the generalized osteosclerosis was the characteristic radiological finding of skeletal fluorosis due to the deposition of excess of fluoride and secondarily of calcium in the bones. In our earlier reports (4, 5) we have also demonstrated that the severity of skeletal fluorosis depends upon the concentration of fluoride in drinking water, duration of stay in the endemic area, the occupation and the nutritional state of the individual. The sclerosis has been more pronounced in laborers and farmers who had worked in the fields and ingested more water.

The cervical and lumbar spine, pelvis and the thorax were the parts affected earliest and most severely, presumably because of the greater retention of fluoride at these sites of maximum physical and mechanical stress. Calcification of the interosseous membrane in the

Fig. 3
Radiograph of Hands in a 10 Year Old Child

Increased density of the bones and the epiphyses, microcystic trabeculation and sub-cortical erosions in phalanges due to hyperparathyroidism secondary to the fluorosis.

Fig. 1
Radiograph of

Periosteal new bone formation and calcification on the interosseous membrane, a diagnostic feature of skeletal fluorosis.

Fig 2
Histopathology of iliac bone

Irregular distribution of osteoid tissue among the calcified trabeculae of the bone (von Koss x 100).

TABLE 2

Unusual Radiological Manifestations Observed in 45% of the Cases

1. Osteosclerosis with coarse trabecular pattern.
2. Microcystic expansion of the bones of hands and feet.
3. Expansion of the ends of long bones.
4. Thin cortex and osteoporosis.
5. Neo-osseous-porosis.
6. Subperiosteal phalangeal resorption.
7. Osteomalacia.
8. Neo-osseous-malacia.
9. Para-Haversian canal resorption
10. White and dense epiphyses and metaphyses.
11. Modelling deformites (phalanges and metacarpals).
12. Irregular and rickety metaphyses.
13. Growth arrest lines.
14. Sclerosis and bridging of sella turcica.
15. Irregular narrowing of intervertebral foramen, spinal canal and foramen magnum.
16. Erosions of lamina dura and resorption of alveolar bone.

TABLE 3

Quantitative Observations on Mineralized and Non-Mineralized Bone in the Iliac Crest of 44 Patients with Endemic Skeletal Fluorosis

	Percentage of Trabecular bone surface covered with osteoid	Maximum width of osteoid seams (u)	Percentage of total bone surface with resorption lacunae	Periosteocytic surface resorption area (μ^2)	Percentage of osteoid surface with a calcification front
Mean	22.54	108.19	16.18	77.33	74.2
S.D.	5.95	22.89	4.29	10.25	14.25
S.E.	1.72	6.61	1.24	2.96	3.45
Normal	10.3	80.	5.0	46.	95.

Fig. 4
Dental Radiograph

Hypercementosis around roots of teeth with loss of lamina dura and resorption of alveolar bone due to secondary hyperparathyroidism.

Fig. 5
Histopathology of Iliac Bone

Irregular osteoclastic resorption of the calcified trabecular and increased periosteocytic osteolysis suggestive of hyperparathyroidism (x100).

forearms, irregular periosteal bone formation, calcification of ligaments, muscular attachments and tendons and exostoses were the other common findings (Fig. 1). Quantitative histological studies in such cases have shown that fluoride stimulates rapid new bone formation with irregular mineralization (Fig. 2).

The variable and unusual radiological findings observed in this series (Table 2) suggest the complex toxic and irritant effects of fluoride on bone. In four female patients with skeletal fluorosis, concurrent radiological manifestations of osteomalacia which included triradiate pelvis and biconcave vertebrae were observed. Morphometric measurement of the iliac bone in these cases showed that osteoid comprised 46.5 ± 3.7% of the total area of trabecular bone with the calcification front of 55 ± 9.1 (Table 3). On the other hand, in patients who only had radiological skeletal fluorosis, the histopathological studies of the bone showed irregular islands of osteoid tissue lying in the deeper layers of the calcified trabeculae. All showed abnormal calcification, poorly formed Haversian systems and disordered lamellar orientation.

Neo-osseous malacia is the term we have preferred to use for the newly formed bone which remains uncalcified and suggests that the rate of new bone formation is greater than its accompanying mineralization. Normally, the osteoid formed is rapidly mineralized by the deposition of microcrystalline hydroxyapatite crystals. The coarse trabecular pattern, the microcystic expansion of the bone of hands and feet, para-Haversian canal resorption, subperiosteal resorption in the phalanges, erosions of the lamina dura and resorption of the alveolar bone were the uncommon radiological manifestations presumably caused by hyperparathyroidism secondary to the fluorosis (Fig. 3 and 4).

It has been suggested that the substitution of the hydroxy ion with fluorine makes the apatite crystals more stable and less reactive in surface exchange reactions. It can surely be assumed that this stabilizing effect of fluoride on the skeleton will eventually be counterbalanced by a compensatory increase in parathyroid function for the maintenance of normal ionized serum calcium concentration. Thus we feel that the radiological changes of osteoporosis and those suggestive of hyperparathyroidism are secondary to homeostatic elevation of the calcium removal rate from bone. This has also been suggested in histopathological examination of the biopsied bone when tennelling resorption of the calcified trabeculae and increased periosteocytic osteolysis suggestive of secondary hyperparathyroidism were observed (Fig. 5).

The porotic metaphyses (neo-osseous-porosis) has been the most interesting finding in younger cases and suggested defective collagen synthesis and remodeling of the bone secondary to the fluoride. This has been indicated by our observations of poorly formed Haversian systems and disordered lamellar orientation of the bone on histopathological studies. Multiple horizontal lines of trabeculae (Harri's lines) suggested repeated arrest of osteoblastic activity and were best seen in femoral and tibial radiographs. The expansion of the ends of the long bones has probably been caused by the mechanical strain at the sites of rapidly growing and poorly mineralized bone. The increased density of the epiphyses of the bones represented greater affinity of the fluoride to the areas of active growth.

Similar radiological and histological findings were observed in children. Histopathological evidence of parathyroid overactivity and metabolic bone disease observed in iliac crest biopsies correlated positively with the radiological features in all cases. The stabilizing effect of fluoride on bone due to conversion of hydroxyapatite to fluorapatite produces a compensatory hyperplasia of the parathyroid glands. This has been confirmed in our previous reports (9) by the demonstration of high levels of circulating plasma immunoreactive parathyroid hormone (IPTH) in patients with skeletal fluorosis. Recently it has been reported that fluoride has a beneficial effect on osteoporotics if it is combined with vitamin D and calcium supplimentation (10). This possibility of a beneficial effect of fluoride in osteoporosis has been challenged in the light of the compensatory parathyroid hyperfunction observed in this series. The overall effect of fluoride on bone is still not fully understood and further investigations of bone lesions in skeletal fluorosis are needed.

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TREATMENT OF ENDEMIC FLUOROSIS IN HUMAN BEINGS: AN EXPERIMENTAL STUDY

by

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SUMMARY: Short term in vitro studies showed that bone meal, Serpentine, calcium chloride, egg-shell powder, dowex and gelusil were effective in removing fluoride from water high in fluoride naturally. Serpentine, when administered orally in 25 mg dosage twice a day to normal controls and 50 mg twice a day to patients with endemic skeletal fluorosis inhibited absorption of fluoride from the bowels. The urinary excretion of fluoride was increased in a patient with endemic skeletal fluorosis on a fluoride-free diet following the administration of Serpentine. This increase suggested that Serpentine had a direct effect on bone. The exact mechanism of this action and that of bone meal is not known. Further work on this aspect is in progress.

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The prevalence of crippling endemic skeletal fluorosis in some of the states of India is a serious health problem facing the country. The tremendous increase in the annual use of fluorides compared with that of thirty years ago, together with the increased diversity of their use, has correspondingly increased the hazard associated with these materials. Whereas in India, skeletal fluorosis is due to excess ingestion of fluoride through drinking water whose fluoride level is high naturally, in western countries human fluorosis is mostly due to the fluoride emitted by industries concerned with aluminum, cryolite, phosphate, clay, glass, fertilizers and hydrofluoric acid. However, as a result of the rapid industrial proliferation in our country as well, fluoride research is gaining increasing significance. The problems of skeletal fluorosis attracted our attention in 1969 (1) when we discovered the disease in children and adults who resided since their birth in the endemic fluorosis areas in the district Rai Bareli of the State of Uttar Pradesh. The drinking water from these areas contained 24-26 ppm of fluoride.

In spite of the vast literature on fluoride and human health surprisingly little attention has been given to preventive measures and effective treatment for the victims of skeletal fluorosis. Preventive measures, such as shifting of populations from endemic to nonendemic areas and laying down water pipes for the distribution of pure water from alternate water sources, are impractical and expensive.

The treatment of crippling skeletal fluorosis with disodium EDTA and corticosteroids has been ineffective and is usually associated with the side effects of these drugs. In 1969, we (1) reported that our patients with crippling skeletal fluorosis improved on treatment with a low calcium diet. On the low calcium regime, the calcium balance became progressively negative with the increase in urinary excretion of calcium and fluoride. We then suggested that the low calcium diet had stimulated osteoclastic activity and thus caused mobilization of calcium with secondary release of fluoride from the bone mineral reserves and other sites where they are deposited in excess. The calcium depleting regime in our patients may also have stimulated the secretion of parathormone which further accelerated the removal of calcium and fluoride from the fluoroapatite crystals. Since the low dietary intake of calcium favors fluoride absorption from the bowels, such a regime should be combined as far as is possible, with a fluoride-free diet and low fluoride drinking water.

We believe that any therapeutic regime which would remove fluoride from the drinking water, inhibit absorption of fluoride from the bowels and cause its mobilization from the bone and other calcified tissues would not only produce clinical improvement in patients with skeletal fluorosis but would also be an effective measure in the control of fluorosis. The Geological Survey of India has recently discovered that Serpentine, a naturally occurring metasilicate of magnesium, is capable of taking up large amounts of fluoride over a wide range of pH in aqueous solutions (2).

The purpose of the current communication, therefore, is to report our preliminary results on serpentine and other compounds for their capacity to remove fluoride from the drinking water which had a high natural content of fluoride. In addition, the effects of Serpentine have been studied on fluoride balance in the normal individual and the patient with endemic skeletal fluorosis in order to ascertain the possibility of employing this mineral as a defluoridating agent in the treatment of endemic skeletal fluorosis.

Materials and Methods

In brief, samples of 40 ml of drinking water with a high natural fluoride content (24.4 ppm) were taken in polypropylene containers and to each sample was added one of the compounds to be studied, shaken and kept for 24 hours. The supernatant was removed and analyzed for its fluoride content. The fluoride content in the drinking water, urine and fecal samples were determined by using the specific fluoride ion electrode (Orion) and the diffusion technique (3).

Fluoride balance experiments were performed on two male adults, one normal and one with endemic skeletal fluorosis. The effects of oral administration of metasilicate of magnesium (Serpentine) on intestinal absorption and urinary excretion of fluoride were studied. Each individual was admitted to the Metabolic Ward and received a diet of fixed composition from the metabolic kitchen by the trained dietitian throughout the entire period of study.

The study on the normal adult was continued for 24 days. The first six days served as the stabilizing period for the diet before the collection of metabolic specimens was commenced. The subsequent three periods of six days each included Period I - Diet alone; Period II - Diet + 25 mg Serpentine twice a day administered with meals, and Period III - Diet alone. In order to keep the dietary intake of fluoride as accurate as possible, the patient was put on a fluoride-free diet and was given sodium fluoride supplements; one capsule containing 10 mg of fluoride was administered three times a day with meals.

The patient with endemic skeletal fluorosis was studied for a period of 66 days. He received the diet made up of exactly the same ingredients and so far as possible of the same composition and ratio as he had been eating at his home in the endemic area. The food stuffs and the drinking water were collected from his home in the endemic area and were served from the metabolic kitchen, in order to keep the intake of fluoride and other dietary constituents similar to his intake at home. The fluoride balance was performed over 7 periods of six days each as follows: Period I and II - Diet alone; Period III and IV - Diet + 50 mg Serpentine twice a day with meals; Period V - Diet alone; Period VI - Fluoride-free diet and Period VII - Fluoride-free diet + 50 mg Serpentine twice a day. Following Period IV, the patient was stabilized for 12 days on diet before the metabolic specimens for Period V were collected. Similarly, a stabilization period of 12 days was allowed on the fluoride-free diet following Period V before the specimens were collected for Period VI. The fluoride intake of the patient, solely through drinking water, was 28 mg per day. It was kept the same throughout the period of the study except during the last two periods when he received a fluoride-free diet and deionized water.

The metabolic periods were demarcated by the Carmine markers given at the beginning and at the end of each period. The 24-hour fecal samples were collected in a cellophane membrane in plastic boxes and the 24-hour urinary samples in polythene bottles. All specimens were preserved in the deep freeze until analyzed. The diagnosis of skeletal fluorosis was established by accepted criteria (4).

Results

The results of our water purification experiments showed that aluminum hydroxide, magnesium hydroxide, bone meal, calcium chloride and Serpentine were most effective compounds in taking up fluoride from the drinking water. The other two compounds which removed fluoride were the egg-shell powder and dowex (Table I).

Discussion

Bone meal is a whole bone (hydroxyapatite) preparation gained from the raw bones of young animals and therefore contains all the components of growing bone. One gram of dry extract (mainly water insoluble fractions) of bone was derived from 6.5 g of fresh bone. Each dragee (0.2 g) of bone meal contains 33 mg calcium, 15 mg phosphorus and 56 mg collagen. In vitro studies proved that bone meal, which possesses remarkable properties for binding fluoride from the drinking water, was the most promising compound. Deposition of fluoride in the skeleton of patients with endemic skeletal fluorosis occurs because of the great affinity of the fluoride ions to the hydroxyapatite structure of the bone. Similar mechanisms could be involved in removal of fluoride from the drinking water by bone meal (hydroxyapatite).

Serpentine is also capable of taking up enormous quantities of fluoride from the water. The data on fluoride balance (Table 2) in the

TABLE I

Fluoride Content of the Drinking Water Before* and After Treatment with the Chemical Compounds**

Ser. No.	Compound	Dose per 40 ml of drinking water (g)	Fluoride retained in treated water		Fluoride taken up by compound	
			µ	ppm	µ	ppm
1	Bone meal (Ossopan, Robapharm Ltd. Basle)	0.4	290	5.8	930	18.6
2	Bone meal	0.8	11.6	0.23	1208.4	24.17
3	Egg Shell Powder	5.0	665	13.3	555	11.1
4	Dowex-Cl form 100-200 mesh	1.0	500	10.0	720	14.4
5	Calcium Chloride	1.0	253	5.06	967	19.34
6	Serpentine 200 mesh (Metasilicate of magnesium)	1.0	295	5.9	925	18.5
7	Aluminium hydroxide	1.0	35	0.7	1185	23.7
8	Magnesium hydroxide	1.0	250	5.0	970	19.4
9	Aluminium hydroxide + Magnesium hydroxide	1.0	112.5	2.25	1107.5	22.15

*Untreated water samples contained 1220 µM (24.4 ppm) fluoride.
**Other compounds tested and found ineffective in removing natural fluoride from water included Disodium EDTA, Groundnut shell powder, Cellulose Phosphate, D-Penicillamine, Aluminium Sulphate, Sodium Citrate and Calcium Bromidolactobionate.

TABLE 2

Effects of Serpentine on Fluoride Balance in Normal Control and in the Patient with Endemic Skeletal Fluorosis

	Metabolic Periods	Intake mg/day	Urine mg/day	Feces mg/day	Balance mg/day	Experimental Therapy
Normal Control	I	20	11.5	1.9	+6.6	Diet
	II	20	4.0	11.0	+5.0	Diet + Serpentine 25 mg twice daily.
	III	20	13.2	2.2	+4.6	Diet
Endemic Skeletal Fluorosis	I	28	13.5	2.6	+11.9	Diet
	II	28	14.0	2.4	+11.6	Diet
	III	28	9.0	14.6	+4.4	Diet + 50 mg Serpentine twice daily.
	IV	28	10.5	13.0	+4.5	Diet + 50 mg Serpentine twice daily.
						Stabilizing period of 12 days on diet.
	V	28	14.5	3.2	+10.3	Diet
						Stabilizing period of 12 days on fluoride-free diet
	VI	0.2	3.5	0	- 3.3	Fluoride-free diet
	VII	0.2	3.8	0	- 3.6	Fluoride-free diet

normal adult showed that the oral administration of Serpentine in the 25 mg dosage as fine powder passing 200 mesh twice daily with meals caused an increase in fecal fluoride and a decrease in urinary excretion. Similarly, the administration of Serpentine in 50 mg dosage twice daily with meals to a patient with endemic skeletal fluorosis markedly inhibited the intestinal absorption of fluoride and correspondingly reduced its urinary excretion in Period III and IV (Table 2). On discontinuing Serpentine, the fecal and urinary fluoride levels returned to the pretreatment values (Period V). The continuing excretion of fluoride in the urine of this patient on a fluoride-free diet (Period VI) suggested that the fluoride was drawn from the bone and from other calcified tissues where it had been deposited in excess. Further increase in the urinary excretion of fluoride following the administration of Serpentine (Period VII) suggested to us that Serpentine may exert direct action on the bone in mobilizing fluoride. Thus, the results of our short term balance experiments have shown that Serpentine is capable of inhibiting fluoride absorption from the bowels and is also effective in extracting it from the bone of the patient with endemic skeletal fluorosis.

The green variety of Serpentine which is being used in this trial contained 0.17 ppm of fluoride and is sensitive over a wide spectrum of pH (2). It is known that fluoride is deposited on the surface of the hydroxyapatite crystals and displaces carbonate, bicarbonate, citrate and phosphate ions on the surface and also substitutes isomorphically for hydroxyl ions within the hydroxyapatite lattice (1). The exact mechanism of action of Serpentine is not known. It may be assumed that it inhibits the fluoride absorption from the bowels by forming an insoluble complex and that the mobilization of fluoride from the calcified tissues may involve exchange reactions within, or on the surface of, the fluoroapatite crystals in the bone. During this short term study no side effects were observed with the use of serpentine. Whether similar or different mechanisms are involved in the binding of fluoride by bone meal is being investigated.

While evaluating the defluoridating properties of a compound, it is suggested that the local, social, cultural, religious and economic factors of the community involved should also be considered. The defluoridating agent should be inexpensive, easily available, convenient to use and free from adverse effects on prolonged use. The hardness and alkalinity of drinking water, the nature of occupation and the state of nutrition of the population affected also need careful study for the successful prevention of fluorosis. We also suggest that, before a large scale program for the control of fluorosis is launched, the acceptability of the program to the community affected with fluorosis should be first assessed. Unless all the above factors are considered, attempts at controlling the ill-effects of fluorosis in the community may not meet with success.

On the basis of the results of our study it is suggested that a small, simple, portable and inexpensive unit which removes fluoride from water should be developed using a cartridge made of bone meal and serpentine. Such a device would remove from 80 to 90% of fluoride from drinking water. The cartridge so made should be replaceable over three or four months to keep the unit at its maximum efficiency. The filtration material should be safe and should remove only the fluoride leaving the concentration of the beneficial minerals such as calcium, magnesium, phosphorus unaltered.

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POSSIBLE EFFECTS OF FLUORIDES ON THE THYROID

by

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SUMMARY: A review of the voluminous literature on the relationship of fluorides and the thyroid gland reveals that the fluoride concentration within the thyroid gland on a wet basis is frequently at levels approximating, or above, those of blood and also, frequently, above those of other tissues including the kidneys. According to some investigators the fluoride concentration in the thyroid decreases at a slower rate than in other organs once animals are removed from fluoride intake. Fluoride appears to be actively concentrated by some of the thyroid cells and metabolized into such a form that it is not lost as readily as it is from other tissues when blood levels decrease. Fluorides at sufficient concentration produce profound morphological changes in the thyroid. The lower level of fluoride intake at which this may occur is open to discussion. Functional changes, however, may be seen frequently at daily intakes of 5 mg. Changes in activity of adenyl-cyclase concentrations are frequently encountered in thyroid glands at current levels of fluoride pollution. To date, analytical methods have not permitted these changes to be shown in vivo.

Several investigators presented data for publication by the World Health Organization (1) correlating a high incidence of goiter with endemic fluorosis in various areas of the world. Some concluded that iodine deficiency is the primary cause of goiter but when somewhat deficient, the goiterogenic effects of fluoride can manifest itself. It is stated (2) "No more definite data have been reported since the original suggestions made by Wilson that excessive intake of fluoride may be a causative factor." Others have published similar data. However, frequently investigators have shown a specific fluoride thyroid interaction, but in different directions. Demole (3) stated in a subsequent review by the World Health Organization "Advances in biochemistry, tracer studies in particular, have led to a complete revision in the theory of iodine-fluoride antagonism. We now know that fluoride does not accumulate in the thyroid gland that its presence does not decrease the uptake of iodine by the thyroid and that it has no effect on the synthesis of thyroxine." He called the problem of the toxic effects of fluoride in relation to the thyroid "settled;" and stated "a specific toxicity of fluorine for the thyroid gland does not exist."

The main facts in support of this statement follow: 1. Fluoride does not accumulate in the thyroid. 2. Fluoride does not affect the uptake of iodine by the thyroid tissue. 3. Pathological changes in the thyroid show no increased frequency in regions where the water is fluoridated, either naturally or artificially. 4. The administration of fluoride does not interfere with the prophylactic action of iodine on endemic goiter. 5. The beneficial effect of iodine in threshold dosage to experimental animals is not inhibited by administration of fluoride, even in an excessive dose (4).

Reasons may be offered for the inconsistency of published data. The thyroid is not "an island unto itself" but heeds the input received from the pituitary and central nervous system and exerts its effect peripherally. When the physiology is altered, it may be difficult to pinpoint the exact level within the complex thyroid cycle. But, interpretations of data are further compounded by the introduction of experimental parameters (Table 1). Models of numerous animals ranging from the small mouse to man have been used for in vivo or epidemiological studies, or in vitro studies of homogenates, tissue slices, intact gland, and ultracentrifuge fractions. Such a wide variety of fluoride salts and compounds have been used that one may question whether the fluoride or the cation is actually being studied. The terms describing the concentrations of fluorides used in various experiments may be difficult to decipher. Acute and chronic doses have been scheduled in ranges differing by quantum levels. In consequence of the above variables, the numbers of comparable results are reduced.

TABLE 1

VARIOUS PARAMETERS USED IN FLUORIDE-THYROID STUDIES

Animal	Man, Guinea Pig, Dog, Rat, Chick, Monkey, Cow, Rabbit, Sheep, Mouse
Compounds	NaF, HF, CaF_2, Fluorosilicates, Organic Salts, UF_4, BeF_2, Bone meal
Dose	Chronic Fluoride Poisoning, Endemic Fluorosis, Fluoridated Water, ppm, mg/kg, Mols, m Mols, mgm/M^3, $-Log(F)=X$
Routes of Administration	Oral, intraperitoneal, percutaneous, subcutaneous, gastric tube, intravenous, inhalation
Assay	Epidemiology, histology, BMR, RAI uptake, enzymatic, chromatography, serum iodine levels, tissue homogenates, slices, or ultracentrifuge fractions

To facilitate analysis of the voluminous literature, tables showing the basic data of various investigators have been prepared for possible correlation of fluorides under various conditions with altered morphology and function as well as levels of fluoride in the thyroid gland. Only the most fundamental information will be given. Those with a deeper interest are encouraged to read the original articles given in the bibliography.

Fluoride Content of the Thyroid Gland

Originally, data was compiled to answer the question, "Is fluoride concentrated or trapped in the thyroid?" I have tabulated the levels of fluoride reported by various workers in the thyroid gland for numerous experimental models (Table 2). It should be noted that investigators may have used different units to define fluoride concentration; therefore, values may not be comparable vertically, but are horizontally. Before scanning these values one must consider the fact that plasma makes up approximately 60% of and contains approximately 75% of fluorides in whole blood. The blood component of thyroid or its sections varies markedly according to whether or not the assay is done on the intact gland in vivo or in vitro with some loss of blood and fluid prior to assay. On page 103 of reference 3 it is shown that there is a decrease in the fluoride concentration gradient from plasma to extracellular fluid to intracellular fluid. Accordingly if, on a weight basis, the ratio of thyroid/blood fluoride level approximates or is greater than 1, one must consider the possibility that fluoride has crossed thyroidal cellular membranes by either a diffusion process or as a metabolic thermodynamically controlled process. Also concentration gradients will exist among various tissues and cells so that some areas will have an appreciably higher concentration, and other, appreciably lower than the average values reported in the tables.

Morphological Changes

Morphological changes within the thyroid are shown in Table 3. Since Maumené (19) reported in 1866 "goiter-like swelling" of the thyroid of a dog in which he gave high doses of NaF, there have been doubts by some whether or not his dog demonstrated true goiter and whether or not fluorides produce structural changes within the gland. It is obvious that fluorides produce profound structural changes in the thyroid. Whereas the more obvious changes are produced by high concentrations of fluoride,epidemiological reports indicate that goiterogenic effects are seen at concentrations approaching fluoride pollution levels of many areas. Most of these reports agree essentially with the authors of the WHO (1) publication indicating effects of fluoride only when iodine intake is suboptimal, but several investigators have shown changes in the gland with adequate iodine intake. This and the fact that extra iodine will overcome the physiological action of the perchlorates and thiocyanates in inhibiting the trapping of iodine by the thyroid lends support to the theory that there may be competition between fluorides and iodides at this early stage of the biosynthesis of thyroxin. In this case,those goiters produced by an excess of fluoride would be expected to be of the colloid type at lesser concentrations. At higher concentrations of fluoride, goiters are

TABLE 3

Age, Fluoride Regression Analysis

Population	Y[a]	r	SEE	SEB	N	t
BIGHORN SHEEP						
Wildhorse Island	20.747x + 204.898	0.495	131.478	6.152	37	3.373**
1953-1962	39.525x + 149.525	0.775	101.969	13.164	8	3.002*
(age, ppm)[b]	71.226x + 72.613	0.960	53.492	10.326	6	6.897**
($\bar{x}$ age, $\bar{x}$ ppm)[c]						
($\bar{x}$ age, $\bar{x}$ ppm) oldest deleted						
1971-1972						
(age, ppm)	78.488x + 251.213	0.814	111.377	9.320	38	8.422**
($\bar{x}$ age, $\bar{x}$ ppm)	65.428x + 278.539	0.898	90.706	12.097	9	5.409**
($\bar{x}$ age, $\bar{x}$ ppm) oldest deleted	90.393x + 184.482	0.989	31.631	5.978	7	15.122**
Rock Creek						
(age, ppm)	17.286x + 112.758	0.484	66.208	6.381	26	2.709*
($\bar{x}$ age, $\bar{x}$ ppm)	10.319x + 132.625	0.664	39.610	8.226	4	1.254
($\bar{x}$ age, $\bar{x}$ ppm) oldest deleted	23.433x + 79.908	0.998	4.943	1.550	3	15.115**
National Bison Range						
(age, ppm)	9.853x + 76.008	0.605	29.566	3.592	15	2.743*
($\bar{x}$ age, $\bar{x}$ ppm)	14.184x + 44.600	0.944	17.328	3.496	4	4.057*
PRONGHORN ANTELOPE						
National Bison Range						
(age, ppm)	27.456x + 58.189	0.899	44.625	2.628	28	10.456**
($\bar{x}$ age, $\bar{x}$ ppm)	30.286x + 38.198	0.970	27.991	2.520	10	11.292**
DALL SHEEP						
Brooks Range, Alaska						
1950-1954 (age, ppm)	68.098x + 492.414	0.707	285.379	10.132	48	6.787**
(x age, x ppm)	86.556x + 444.089	0.945	111.843	14.952	6	5.787**
1960-1965 (age, ppm)	53.724x + 412.552	0.532	196.461	14.436	37	3.722**
(x age, x ppm)	56.700x + 405.680	0.997	15.081	2.352	5	24.084**
MULE DEER						
National Bison Range (age, ppm)	41.225x + 85.482	0.826	70.282	9.937	10	4.149**
Sapphire Mountains (age, ppm)	23.142x + 4.137	0.791	33.537	2.638	48	8.774**
(x age, x ppm)	22.900x + 2.950	0.993	5.122	1.620	5	14.139**
WHITETAIL DEER						
National Bison Range (age, ppm)	58.947x + 31.342	0.639	69.165	25.089	10	2.349*
Sapphire Mountains (age, ppm)	15.365x + 18.889	0.699	25.031	4.205	16	3.654**
(x age, x ppm)	17.307x + 15.145	0.974	9.582	2.864	4	6.041**
ELK						
Sapphire Mountains (age, ppm)	33.595x - 2.182	0.825	41.842	3.836	38	8.758**
(x age, x ppm)	37.114x - 13.029	0.969	23.003	4.762	6	7.794**

[a] Y = regression estimate, r = correlation coefficient, SEE = standard error of the estimate, SEB = standard error of regression coefficient, N = sample size, t = sample t, * = significant at t 05 level, ** = significant at t 01 level.

[b] Age in years. [c] $\bar{x}$ = mean.

TABLE 4

Age Specific ppm Fluoride Levels in Populations of Rocky Mountain Bighorn and Dall Sheep

Population	Age Class	N	$\overline{x}$	SD	CV	SE$\overline{x}$	EXE$\overline{x}$
BIGHORN SHEEP							
Wildhorse Island							
1953 - 1962	0.5 years	4	35.0	14.3	40.9	7.2	20.5
	1	3	205.0	102.8	50.1	59.3	28.9
	2	3	309.7	85.1	27.5	49.2	15.9
	3	4	347.2	25.4	7.3	12.7	3.7
	4	-	-	-	-	-	-
	5	3	463.7	57.0	12.3	32.9	7.1
	6	2	499.5	82.7	16.6	58.5	11.7
	7	6	405.2	62.8	15.5	25.6	6.3
	8+	11	353.8	129.3	36.5	38.9	11.0
	0.5-3.5	14	219.5	142.7	65.0	38.1	17.4
	4.5-5.5	4	398.8	137.9	34.6	68.9	17.3
	6.5-7.5	8	428.8	75.5	17.6	26.7	6.2
1971-1972	0.5	4	239.2	49.4	20.7	24.7	10.3
	1	1	352.0	-	-	-	-
	2	3	349.7	38.5	11.0	22.2	6.3
	3	13	501.0	153.8	30.7	42.7	8.5
	4	5	597.0	56.8	9.5	25.4	4.2
	5	4	696.0	81.3	11.6	40.6	5.8
	6	4	770.8	33.8	4.4	16.9	2.2
	7	3	750.3	27.5	3.7	15.9	2.1
	8^+	1	639.0	-	-	-	-
	0.5-3.5	21	422.4	162.7	38.5	35.5	8.4
	4.5-5.5	9	641.0	82.5	12.8	27.5	4.3
	6.5-7.5	7	762.0	30.7	4.0	11.6	1.5
Rock Creek	0.5-3.5	7	136.7	96.6	70.6	36.5	26.7
	4.5-5.5	11	201.1	58.2	28.9	17.6	8.7
	6.5-7.5	6	241.7	34.8	14.4	14.2	5.8
	8+	2	193.5	65.8	33.9	46.5	24.0
National Bison Range	3.5	2	81.5	21.9	26.9	15.5	19.0
	4.5-5.5	7	132.4	29.5	22.3	11.1	8.4
	6.5-7.5	4	139.8	10.2	7.3	5.1	3.7
	8+	2	186.5	27.6	14.8	19.5	10.4
DALL SHEEP							
Brooks Range, Alaska							
1950 - 1954	6 months	5	488.4	302.7	62.0	135.4	27.7
	18	11	523.4	114.4	21.9	34.5	6.6
	30	11	644.8	212.9	33.0	64.2	10.0
	48	4	759.2	514.8	67.8	257.4	33.9
	72	7	1,156.6	373.4	32.3	141.1	12.2
	115	9	1,177.8	373.4	31.7	124.5	10.6
1960 - 1965	6	2	431.5	85.6	19.8	60.5	14.0
	24	10	530.9	144.3	27.2	45.6	8.6
	48	10	632.3	231.2	36.6	73.1	11.6
	72	11	726.4	213.3	29.4	64.3	8.8
	103	4	904.5	246.2	123.1	123.0	13.6

N = sample size, $\overline{x}$ = mean, SD = standard deviation, CV = coefficient of variation in %, SE$\overline{x}$ = standard error of the mean, and EXE$\overline{x}$ = experimental error of the mean in %.

seen which are of the parenchymatous type. According to some authors, time may be an important parameter in the production of morphological changes so that lower concentrations of fluorides may produce changes over a long interval of time, whereas such changes are lacking for a higher dose over a short acute period of time. This temporal factor as well as others such as diet, hardness of water, water contamination, etc., may well be operative variables which explain some of the divergent findings.

Sundstrom (5) gave to three groups of 200 gram rats, 4 controls, 8 drinking distilled water with 40 ppm fluoride ad libitum for two months and to 4 drinking distilled water for two months and then injected intraperitoneally sodium fluoride (20 mg/kg body weight/day in one dose) for four consecutive days. He found no morphological differences among the C-cells in the thyroid gland studied by both light and electron microscopy with special reference to degranulation or signs of intracellular autodigestion of the granules. Often however in the cells of both experimental groups he did find extended endoplasmatic reticulum in the follicular cells. At times, the C-cells of the experimental animals could be differentiated from that of the control animals by their hypertrophy or hyperplasia.

Thyroid Physiology

Reports of altered thyroid physiology are given in Table 4. A survey of these results again indicates that fluorides at various concentrations alter thyroid physiology. Many of these changes occur at higher concentrations than encountered in most environments. Some workers report physiological changes at levels of fluoride intake of approximately 5 mg daily in man which approximates daily ingestion in many of our polluted communities.

Of interest is Galletti's (4) report on a small group of hyperthyroid patients. Although he showed symptomatic improvement with NaF tablets in only 6 out of 15 patients, in these 6 and many others the basal metabolism rate and protein bound iodine values returned to normal. His work is frequently reported to show that the thyroid does not take up fluoride based upon the fact that he injected radioactive fluoride into one normal and one hyperthyroid patient. In both individuals the thyroid picked up less than 1% of the ^{18}F; however, Galletti noted later in the article that due to the amount of carrier 1% would amount to approximately 200 μg of fluoride per hour as compared to approximately 10 μg I per hour. Because of the insensitivity of his test, he suggested it be repeated with carrier-free ^{18}F.

Leone (6) compared 106 individuals drinking water containing 3.48 ppm F with 109 in another community drinking 0.09 ppm F. No mention is made of dietary intake. He gives a spectrum of PBI findings for the two groups, showing 7/106 vs. 4/109 abnormal volume in the conventional range and 10/106 as compared to 16/109 for the range as given by the author. The author's conclusion that there is no significant difference may be true;

TABLE 4

THYROID FUNCTIONS ALTERED BY FLUORIDES

Reference	Animal Model	Dose of Fluoride	Observations
Smith, (45)	Man	50 ppm in H_2O	Thyroid sensitive with functional & st
Leone (6)	Man	3.48 vs .09 ppm in H_2O; food intake not considered	7-10/106, 4-16/109 abnormal PBI
Galletti (4)	Man (Hyper-thyroid)	2-6 mg q.d. / 2-3 months	6/15 symptomatically improved BMR & remaining 9 cases BMR & PBI values o hibition RAI uptake; F_{18} IV no activity only to 200 μg F^{18} per hr compared to thyroid recurred when I intake was incr
Gedalia (42)	Girls	High F H_2O considered	No change if I intake adequate
Frada (7)	Man (400)	5.2 ppm H_2O 39 ♂	7>50%, 0<26% RAI
		13 ♀	1>50%, 0<30% RAI 2% goiter
Gushchin(46)	Rabbit	NaF, 18 or 36 mg/kg/q.d. 10 da	I content of tissues reduced
Harris, N.O. (47)	Rat	NaF, 1.8 mg, IP	Normal thyroid function
Harris (47)	Rat	NaF 10, 50, 100ppm F in H_2O/43 d	Normal thyroid function
Stormont (48)	Rabbit	NaF, 3 mg/kg IV/2 months	Thyroid I content not decreased
Demole (49)	Rat	NaF, 1-5 mg/kg 8 days	thyroid function normal
(50)	Rat	NaF, 1-4 mg/kg-2 days	no change in RAI
(50)	Rat	NaF_2, 1.85 mg/kg/2 months	no change in RAI
Galletti (51)	Man	NaF; 2-3 mg q.d., 6-14 mo.	RAI, BMR, PBI, serum cholesterol nor
Korrodi (52)		5 mg q.d./2-5 mo.	RAI increased
Jentzer (53)	Rabbit	NaF; 0-18 mg/kg/2.5 mos IV	decreased activity BMR↓2/3
		0.09 mg/kg/2.5 mos IV	decreased glandular activity, BMR↓3/
		4.52 mg/kg/2.5 mos IV	follicles very small
Galletti (51)	Rat	NaF; 0.026 mg/da/42 days (diet)	normal thy. wt., RAI increased 51
		0.90 mg/da/42 days	normal thy. wt., RAI increased
		above repeated, low I intake	RAI as above; wts. higher than those w but = to controls on low I intake.
Wadhwani (54)	Rat	NaF 0.9/kg q.d./20-24 wks	I content ↑ in the thyroid
		4.5 mg/kg q.d./24 wks	above + epithelial atrophy
Shrewsbury (55)	Lamb	F as rock phosphate, 1.5-6 mg F q.d./170 235 days	Thy. wt. decreased, I content and col epithelium decreased.
Levi (56)	Man	Fluorosilicate 4 mg q.d./3-10 wks	No significant increase in RAI
Macchia (57)	Rat	Tissue hemogenates in 0-40mMF	Adenyl-cyclase activity of normal and In tumor, F 2X more stimulating than
Burke (58)	Sheep	0.1-100mM F	TSH & F stimulated; adenyl cyclase ac action different Max.at 10 mM, presen
Willems (59)	Dog	$150 \mu C^{131}$ subcutaneously 150 mg Thyroid powder q.d./ 3 day. 5-10 mMF Thyroid slice	F inhibits TSH induced colloid accumula aerobic glycolysis in follicular cell, st oxidation & I binding to proteins.
Kendall-Taylor (60)	Mouse	Intact thyroid	
		3mM F c̄ TSH	I T_3 T_4 decreased
		10 mM F c̄ & s̄ TSH	Adenyl cyclase inhibited, Iodoprotein↑1 effect.
		s̄ TSH	I_1 T_3 T_4 ↓ 4X
Rantanen (8)	Pig	Basal diet + 2 mg/K/9 mo. 50% thyroidectomy/10 mo.	Thyroidectomy reduced or rereversed seen with F.

however, might a type II error be present? Of interest is the fact that in the fluoridated area one individual with a low PBI had had a thyroid cyst removed and three individuals with high PBIs were on iodine medication for non-thyroidal disease. Either the sample populations were poorly chosen or a deeper search should be made in greater numbers for possible etiology of these rationalizations.

Fradà (7) studied 400 individuals living in an endemic fluorosis area drinking 5.2 ppm F water. Of the 400, he chose 52 for RAI uptake and conversion ratios studies. The method of selection is not stated. Of the 52, 39 were males and 7 of these had 24 RAI uptakes in excess of 50%. One of the 13 females had readings in excess of 50%. None of the males had an uptake of less than 26% and none of the females less than 30%. Although not definitely stated, it appears that the normal range in this community is 15 to 50%. It would appear that an 18% incidence of hyperthyroidism among adult males and even 8% among adult females would be a significant increase. But then possibly this is a low iodine area and the uptake values are physiologically high. The goiter incidence in the 400 individual studies are 2% which may be the anticipated value of that particular area but would be appreciably higher than normal in our patient population.

The more recent enzyme studies are of interest because of the importance of adenyl-cyclase in normal thyroid functions, even though the data are conflicting. The initial step in the action of TSH is a binding of the hormone to a superficial cell site, probably the cell membrane. The next step is activation of the adenyl-cyclase which leads to a conversion of ATP to cyclic AMP, resulting at an intracellular target site to stimulation of glucose and phospholipids metabolism, colloid droplet formation, thyroxin secretion, RNA and protein synthesis, and iodine uptake. Fluoride is among those agents which may alter this sequence of action at some point. Although there is appreciable work showing the effect of fluoride on the adenyl-cyclase -- some have suggested that fluoride be used as a model to study these inter-reactions -- there is confusion as to just what these effects are: some studies show stimulation, others inhibition.

Rantanen et al.(8) demonstrated a complex relationship between fluoride and the thyroid. Using a basal daily ration deficient in calcium and phosphorus, he measured a number of bony changes produced by F intake of 2 mg/kg body weight for an approximate 9 month period. The pigs were allocated randomly into 8 groups of 5 each and one half of these (4 groups) were thyroidectomized at 10 weeks of age. Bony changes produced by fluoride were reduced or reversed by thyroidectomy. The thyroidectomized animals which had been given iodinated casein to replace thyroid hormone activity did not show the retarding effect of fluoride on cortical bone remodeling. This observation suggested to the authors that the fluoride effect may be mediated through the formation, release, or enhanced action of thyrocalcitonin.

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DIETARY FLUORIDE INTAKE OF INFANTS

by

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(Abstracted from Pediatrics, 55:517-22, 1975)

Several reports have been published documenting dietary fluoride intake of infants in Europe. Since many major municipalities in the USA are fluoridated and many foods are prepared with fluoridated water, an evaluation of dietary fluoride for infants was considered desirable.

The authors carried out about 300 fluoride determinations on a variety of commercially available infant formulas and baby foods such as cereals, fruits, fruit juices, vegetables and meats for infants from 1 week of age to 6 months. They used the diffusion method of Singer and Armstrong.

Liquid concentrate of a homogenized milk formula had the highest fluoride concentration averaging .83 mg/liter diluting the milk with equal parts of fluoridated water did not change the fluoride concentration. A "ready to use" preparation averaged 0.29 mg/liter Evaporated milk contained considerably less fluoride namely 0.05 mg/liter (average).

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Fruit products showed a range of .54 to .80 mg/kg except for apricots which averaged 1.17 mg/kg. Cereals with fruit had a fluoride content of .69 to 1.05 mg/kg; most of the strained vegetable products contained .39 to .59 mg/kg except for creamed spinach which averaged 2.01 mg/kg. Six types of meat were low in fluoride with a range of .2 to .4 mg/kg, turkey contained 0.6 mg/kg. Chicken, however, averaged from 1.5 to 3.14 mg/kg fluoride. The total daily dietary intake of fluoride was lowest in infants 1 to 4 weeks of age. Because of the general increase in food consumption there was a gradual rise in fluoride intake in each successive age group. In infants 4 - 6 months of age, the dietary fluoride intake was four times greater than that of the newborn. In evaluating dietary fluoride consumption per body weight, the newborn consumed an average of .07 mg/kg fluoride and infants 4-6 months of age averaged twice that of the newborn namely, .16 mg/kg.

These data indicate that supplemental fluoride is not necessary in infants below the age of 6 months.

THE ROLE OF CALCIUM AND FLUORIDE IN OSTEOPOROSIS IN RHESUS MONKEYS

by

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(Abstracted from the Invest. Radiol., 10:263-68, 1975)

The authors studied the effect of fluoride on bone metabolism in rhesus monkeys with varying dietary intake of calcium fluoride. Thirty-one female rhesus monkeys, about two years of age, were divided into four subgroups and were fed purified diets containing varying levels of calcium and fluoride for a period of 60 months. Six animals (group I) constituted the normal controls. Groups I and II received about 2 grams of calcium per day which is currently accepted adequate for rhesus monkeys; groups III and IV were given only 0.3 grams of calcium per day.

Radiographs of skulls, vertebral column and limbs were taken every three months. After 48 months on the diet, bone mineral was analyzed by photon absorptiometry with a ^{125}I point source on the cortical bone of the left arm at the midpoint of the shaft of the radius and ulna. This

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bone fluoride, the iliac crests on 20 autopsied cases were examined 19 of whom had been residing in fluoridated communities; the length of their residence was not known. The results were expressed as ppm of fluoride per wet weight of bone.

In plasma, fluoride readings ranged from 0.019 to 0.112 ppm with a mean of 0.047 ppm; the ages of the individuals studied were 17 to 82 with a mean of 52 years. Between the two factors a positive linear correlation of 0.53 was obtained with coefficient of determination amounting to 28%.

In the iliac crest, the fluoride values ranged from 1295 ppm to 5745 ppm with a mean of 2824 ppm for ages 21 to 77 years (mean of 50). A positive linear correlation of 0.67 was obtained between fluoride concentration and age and the coefficient of determination was 45 percent. Thus this study demonstrates a significant correlation between bone fluoride and plasma fluoride levels with age.

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LANGZEITERGEBNISSE NACH KOLLEKTIVEN MUNDSPULENGEN MIT NATRIUM FLUORID LÖSUNG IN DER REPUBLIK KUBA

by

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(Abstracted from Zahn-, Mund-u. Kieferheilk.62: 683-689, 1974)

Since 1969, more than 900,000 children throughout the Republic of Cuba have been subjected to systematic brushing of teeth and mouth washing with a 0.2% sodium fluoride solution. In pre-school children, the teeth were brushed twice monthly with tooth brush and cleaned with sodium fluoride solution. The mouths of pre-school and school children were rinsed with the fluoride solution about 16 times throughout the year for approximately one minute. After 28 months, a sampling of 25 boys performance was repeated every three months over the course of one year. Tetracycline labeling and fluorescent staining were made on iliac crest and on two vertebrae of the tail after 54 months. Calcification fronts were determined by measuring the distance of the two fluorescent labels in undecalcified tissue sections. On each biopsy 30 to 50 measurements were made. The thickness of osteoid seams was determined by measuring the total area occupied by osteoid with a planimeter and by dividing this figure by the total perimeter of osteoid seams. The resultant figure was termed the index of osteoid thickness. The x-rays of the lungs were examined for the trabecular patterns, cortical widths, epiphyseal fusion and radiographic density.

Results

In group I the epiphyseal fusion was considered normal. Group II showed a definite increase in overall density but the cortices and epiphyseal fusion were within normal range. Group III manifested a uniform decrease in density with thin cortices and accelerated epiphyseal fusion. Group IV exhibited thin cortices with increased density, irregular trabecular patterns, indicative of osteomalacia with wide flared epiphyseal plates (equivalent to rickets) and also abnormal trabecular patterns.

Scanning revealed a marked decrease in bone mineral and reduced widths of bones in group III. Group IV exhibited normal bone minerals but the width of the bones was slightly less than in group I. However, in group IV the bone appeared sclerotic and the osteoid seams were greatly thickened. In group IV also the distance between the two fluorescent labels was significantly narrower than in groups I, II, and III.

These results indicate that a low calcium diet leads to development of osteoporosis with decreased radiographic density, decreased bone mineral content, and a decrease in width with no increase in osteoid, i.e. reduced bone mass or osteoporosis. Addition of fluoride to a diet containing a normal amount of calcium does not increase the width of bone, nor its bone mineral content, although it increased radiographic density. It appears that the addition of fluoride to a low calcium diet prevents osteoporosis. However, in these animals fluoride interfered with mineralization of osteoids, which resulted in marked thickening of osteoid seams as seen in osteomalacia. Fluoride seems to alter osteoid so that it will calcify only in the presence of adequate dietary calcium but simple calcium deficiency without added fluoride does not result in similarly altered osteoid.

The authors concluded that, under conditions of their study, fluoride reduced the metabolic activity of both osteoblast and osteoclast.

INCREASED CARIES-INCIDENCE BY ORAL INOCULATION OF CARIOGENIC BACTERIA IN RATS AFTER DIETARY FLUORIDE

by

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(Abstracted from Archives of Oral Biology, 18:1445-1450, 1973)

Previous studies on rats have suggested that the cariostatic action of dietary fluoride during tooth development and eruption must be accounted for by a mechanism other than reduction of enamel solubility. One possible mechanism was an alteration in the bacterial flora of rats that would be transmitted from dams to the pups.

The authors had previously observed that dietary NaF administered to rats during the formative and eruptive stages of tooth development does not significantly reduce the enamel solubility in acid buffer solution. They hypothesized that NaF reduces the cariogenicity of the bacterial flora.

In order to test this hypothesis, rats from the same litter were divided into three groups all of which received a cariogenic diet. Group one received no fluoride. Groups two and three were supplemented with 50 ppm NaF, from day 1 to day 21. On day 21, the pups were weaned and all groups placed on the diet without the NaF supplement. In the first study, one of the 21-day-old NaF-supplemented groups was inoculated by smears of fecal material from the control animals that did not receive NaF supplement. The second NaF group was not inoculated. The third group which had received NaF was not inoculated and served as control.

In a second study, cariogenic <u>Strep. mutans</u> 6715 was used as the inoculum in place of the fecal smear.

In both studies, the inoculation of a NaF group increased the caries to about 70 percent of the control group, while the mean scores on the non-inoculated NaF group were about 50 percent of the control group. These results indicate that alteration of the transmissible flora may be an important factor in the cariostatic action of dietary fluoride in experimental animals. This observation supports the suggestion that fluoride may alter the cariogenic flora.

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FLUORIDE TOOTHPASTE: A CAUSE OF ACNE-LIKE ERUPTIONS

by

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(Abstracted from the Arch. of Dermatology, 111:793, 1975)

The author described closed comedonal or papular acne in about 65 adult females, aged 20 to 40, in a slightly fan-like distribution on the corner of the mouth and the chin and the proximal area of the cheeks. All patients had had extensive dermatological treatment including dietary control, tetracycline special washing agents, etc. The localization of the lesions suggested to the author that some kind of chemical carried in the saliva might be draining in the areas and in the follicles of the skin and induce this process. In view of the fact that erythematous eruptions resembling acne have been described following application of fluoridated steroids and after exposure to industrial halogen fumes the author suggested that his patients switch to a nonfluoridated toothpaste. In approximately one half of the patients, the lesions cleared within two to four weeks. When the remaining patients were asked to switch from their dentrifice containing brightening and other unknown chemicals, to baking soda and a commercially available mouthwash, nearly all those treated improved considerably; in most of them the acne-like eruptions cleared up completely.

Several patients were concerned about their dental health and resumed the use of fluoride toothpaste; they promptly developed the same distribution of the acne-like eruption that had previously been present.

RELATIONSHIPS OF HUMAN PLASMA FLUORIDE AND BONE FLUORIDE TO AGE

by

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(Abstracted from the Calcif. Tiss. Res. 16: 335-338, 1974)

There is evidence that fluoride levels in plasma correlate with the fluoride content in bones. The authors determined whether or not fluoride in plasma and bones might correlate with age.

In 41 inpatients at the University Hospital, Iowa City, 36 of whom had been residing in fluoridated communities plasma fluoride was determined in the fasting stage by the fluoride ion selective electrode. For and girls for each group between 6 and 13 years were chosen totaling 400 examinations. The teeth of these children were compared with those of a neighboring city where the natural fluoride content in drinking water was 0.1 ppm. For the second sampling 42 months following the beginning of the program only 10 and 11 year old children - who had been 6 and 7 years old at the beginning of the experiment - were selected. Twenty-five boys and girls in each group were compared with a similar group of children as controls.

After 28 months (approximately 33 rinsings with sodium fluoride solution) the DMF index in the fluoride-treated children was 18.4% less than in the controls. In the second group among 10 and 11 year old children after 42 months with 55 rinsings the difference was 35.0%. The author acknowledged that factors other than applications of sodium fluoride may have contributed to the prevention of caries in the fluoride-treated groups.

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