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FLUORIDE

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DIETARY FLUORIDE INTAKE IN THE USA REVISITED

In a previous Fluoride editorial the build-up of dietary fluoride (F^-) in the decades 1950 and 1960 was addressed (1). In the intervening years additional details of F^- distribution into the environment have been revealed. Since water fluoridation is the major dietary contributor, it will be the major thrust of this editorial; fluoridated dental health care products will also be considered. This section of the editorial considers adult and young adult exposure; a later section treats infant and toddler exposure.

Baseline Studies

Table 1 presents a summary of a number of the adult and young adult dietary fluoridation studies in the United States including two examples from Canada. When considering a potential increase in dietary F^- over time it is necessary to establish a baseline which is difficult to do for the following five major reasons:

1. There are few studies focused upon areas having a low fluoride background prior to institution of fluoridation.
2. Quantitative analysis for fluoride is difficult due to its ubiquitous presence in the environment, the low concentration at which it is found, and the difficulties inherent in the analytical methods.
3. The analytical method has changed over time and may have resulted in inadvertent sensitivity and selectivity changes.
4. The terms dietary or intake are confusing because some authors have included food alone, others food and beverages at mealtime and still others food, beverages and water at, and between, meals. These differences are crucial in assessing total dietary F^- intake because of the use of water containing even modest amounts of fluoride in the preparation or reconstitution of foods and beverages, and as drinking water impact total dietary F^- intake substantially.
5. The lack, until recently, of comprehensive and reliable data on total water intake (18).

Data from three studies (2-4) predating 1950 were chosen to estimate the F^- baseline and yield a dietary (food) intake of 0.3-0.4 mg/day for an adult. A hypothetical total intake of 0.45-0.55 mg F^- /day was calculated making the assumption that 1.53 liters of beverage plus water with an average fluoride content of 0.1 mg/L is consumed. This total intake baseline is derived prior to fluoridation projects and significant post World War II manufacturing, processing and other potential sources of F^- input to the environment from

• The data of Ershow and Cantor (18) were used to estimate the water and beverage consumption for an adult (20-64 age group). (See Table 1 and 52 of reference 18.) 2.243 L mean total water consumption/day x 0.683 (fraction contained in beverages [excluding milk] and tapwater) = 1.531 L. Ershow and Cantor reported grams of fluid intake. The conversion factor 1000 g = 1 L was used. Similar calculations will be performed when water and beverage intakes are estimated from the Ershow and Cantor data in other sections of this editorial.

which most food and water are derived. This baseline would hold at that time for the majority of locations in the U.S. except for those few having a high level of fluoride naturally in water. Further, the baseline would be rather insensitive to errors in the estimation of total water intake due to the low F^- water concentration.

The data of Ham and Smith (5), Cholak (6) and Hodge and Smith (7) published from 1954-65 represent dietary F^- values for unfluoridated cities at a time when more national community fluoridation programs were being initiated. Their values of 0.58, 0.34-0.80, and 0.3-0.8 mg/day for food alone suggest an increase in F^- content of the dietary (food) baseline for unfluoridated communities.

Fluoridation Impact

The data of Marier and Rose (9) first drew attention to the potential total dietary F^- intake in a fluoridated community. They convincingly documented the F^- increase in food processed with fluoridated city water, demonstrated the profound effect fluid intake could have on F^- exposure, and estimated food and total F^- exposure to be 1-2 mg/day and 2-5 mg/day respectively. Individual beverage consumption ranged up to 2 or 3 liters/day in the small sample of workers (seven) in their laboratory. Marier and Rose based their dietary (food) intake on the high estimated values of Hodge and Smith (7) (see footnote d Table 1) and assumed too large an intake of foods processed in F^- water (17). More realistic values are: 0.3-0.8 mg/day + 0.2 mg/day processing in F^- water + 1-3 mg/day beverages = 1.5-4.0 mg/day total F^- exposure. The Marier and Rose data are striking in that they demonstrate a wide range in beverage consumption in a very small population. Should this occur when beverages were prepared with water artificially fluoridated at what proponents term the "optimum range" (0.7-1.2 ppm F^-), excessive quantities of F^- would be consumed.

Adult Fluoride Intake from Dietary Analysis

In a series of papers (10, 12-14 and references therein) representing work based on well defined adult hospital diets from the mid-1960's through the first one-half of the 1970's, Spencer's group concluded the total F^- intake in the Chicago area (fluoridated) to be 3.0-4.0 mg/day with approximately 2.0 mg/day derived from the diet (food + beverages) and the remainder (1.0-2.0 mg/day) from fluoridated water (14). Additionally, Spencer *et al.* compared the dietary (food + beverages at meal time) F^- intakes of 12 fluoridated (average F^- 0.9 ppm) cities to that of 4 non-fluoridated (average F^- 0.3 ppm) cities (14). Presumably additional fluoride intake would occur from water and between-meal beverages. Assuming an intake of 1 liter of water and between-meal beverages, the following is obtained:

Fluoridated: 1.7-3.4 mg/day diet (food + beverages) + 0.9 mg/day water/other beverages = 2.6-4.3 mg/day total F^- .

Nonfluoridated: 0.8-1.0 mg/day diet (food + beverages) + 0.3 mg/day water/beverages = 1.1-1.3 mg/day total F^- .

These data make it clear that adults in fluoridated cities consume 2.4 to 3.3 times more fluoride than adults in nonfluoridated cities.

It should be noted that while no trend was found with F^- content of individual food items when compared to the F^- water content in the city of study, the mean dietary and water F^- content ratios correlated and were identical: $F^-/\text{non } F^- = 3$ (14). This ratio confirms that the F^- content of the water used to prepare meals and the beverages accompanying them overwhelms the F^- contributions of other dietary components.

While one of Spencer's studies (13) was in progress the water F^- system (0.9 ppm) was shut down, furnishing a unique opportunity to determine the dietary (food + beverage F^- content in which food and beverage preparation occurred at low F^- conditions (0.27 ppm). (See Table 1). Under these conditions dietary F^- was 0.86 mg/day, less than half of the value when the F^- system was operating.

Spencer's group used specified institutional diets to evaluate F^- intake. The F^- exposure derived from these diets may or may not reflect that of the general public, however. The studies by Spencer et al. clearly indicate that adults in unfluoridated cities receive a total dietary F^- intake level originally intended for fluoridated communities, whereas individuals in fluoridated communities may receive 2.5 to 5.0 times in excess of this amount!

Taves (19) called attention to the comparison of F^- analytical techniques by Guy (20) in whose study values for blood fluoride using different analytical techniques were compared. The method utilized by Spencer's group (10,12-14) was found to give elevated F^- levels with this biological sample. More specifically, Singer et al. (15) have analyzed various food groups using the usual sample pretreatment followed by colorimetric and F^- single-ion-electrode determination of F^- . Fluoride values were consistently high for most food categories when the colorimetric method without ashing, used by Spencer et al. was employed. For this reason, Spencer's values for the food portion of the diet are probably somewhat high.

While the absolute F^- values for foods in Spencer's studies may be high, total F^- intakes should be rather close to the actual F^- exposure since the foods category makes smaller contributions to total dietary F^- intake than beverages and water. The data from Spencer's group are extensive, encompass several years, use well-defined, prescribed diets, and are carefully designed. They should be given careful consideration, and are valid for F^- exposure differences between similar experimental diets carried out by the group.

Taves (16) constructed an adult diet from individual components of a hospital diet in a fluoridated city and estimated total daily F^- intake to be 1.8 mg/day. Drinking water was not taken into account. Inclusion of the data of Ershow and Cantor (18) should add a mean value of 0.7 mg to the intake (water fluoridated at 1 ppm assumed). Total intake would then become 2.5 mg/day.

Adult Fluoride Intake from Market Basket Analyses

Three studies in Table 1 approach dietary fluoride evaluation through analysis of a diet constructed using the components from the Food and Drug Administration's Market Basket Program in which food from 12 categories of dietary components typical of a particular region are purchased on the open market and analyzed (11,15,17). The Market Basket approach to sampling

Table 1
Dietary Fluoride Content Summary

Publication Year	Water F ⁻ (ppm)	Food F ⁻ (mg/day)	Beverage-Water F ⁻ (mg/day)	Total F ⁻ (mg/day)*	Comment	Ref.
1942-50	—	0.3-0.4	0.15 ^a	0.45-0.55	Prefluoridation Baseline	(2-4)
1954	—	0.58	—	—	Toronto, Canada	(5)
1960	0.1	0.34-0.80	0.15 ^b	0.49-0.95	Cincinnati, OH	(6)
1965	—	0.3-0.8 ^c	—	—	—	(7)
1966	low	0.5-1.5 ^d	—	—	Hypothetical Unfluoridated City	(9)
	1	1-2 ^d	1-3	2-5	Toronto, Canada	(9)
1969	0.9	—	—	3.6-5.4	Chicago, IL	(10)
1971	1	0.78-0.90 ^e	1.28-1.46	2.09-2.34	Baltimore, MD	(11)
1974	0.9	1.51 ^f	—	—	Chicago, IL/1966-68	(12)
	0.9	1.8 ^f	—	—	Chicago, IL/1969-72	(12)
1974	0.27	0.86 ^{fg}	—	—	Chicago, IL/Fall, 1968	(13)
	0.90	1.56-1.91 ^f	—	—	Chicago, IL/1967-72	(13)
1974	0.3	0.78-1.03 ^f	—	—	4 Unfluoridated Cities	(14)
	0.9	1.7-3.4 ^f	—	—	12 Fluoridated Cities	(14)
1980	1.0	0.333 ^e	0.882	1.215	San Francisco, CA/1975	(15)
	1.0	—	—	1.636 ^e	San Francisco, CA/1977	(15)
	0.8	0.587 ^e	1.133	1.720	Atlanta, GA/1975	(15)
	0.9	0.378 ^e	0.610	0.988	Buffalo, NY/1975	(15)
	0.4	0.368 ^e	0.544	0.912	Kansas City, MO/1975	(15)
1983	~1	0.4	1.383	1.783	Rochester, NY/1977	(16)
1985	>0.7	—	—	1.85 ^e	From 24 Food Collections	(17)
	<0.3	—	—	0.86 ^e	(1978-1982)	(17)

* The drinking water F⁻ contribution and/or between meal fluid intakes were omitted in most studies.

(a) Calculated assuming consumption of 1.53 L at 0.1 ppm.

(b) Calculated assuming 1.53 L consumption.

(c) Originally estimated at 0.5-1.5 mg/day. These authors later revised these figures downward to the values reported above (8).

(d) These values are probably high for food F⁻ as high estimated values from reference 7 were used (see reference 8). In addition an uncommonly large amount of vegetables (1 kg) processed in fluoridated water was assumed. Correcting for those factors yields an estimated of 0.5-1.0 mg/day food and 1.5-4.0 mg/day total intake.

(e) Diets were constructed from the components of a "market basket program" monitoring pesticides in foods.

(f) The values for reference 13 and 14 include beverages (juice and coffee) but exclude water and between meal beverages and snacks. It is unclear if beverages at meal time are included in reference 12.

(g) The low fluoride value was obtained when fluoridation was temporarily interrupted.

seeks to be sensitive to the dietary composition for each region in which the city is located because it is based upon the mean dietary preference surveys taken in the region. The surveys cited were for young adults of age 15- or 16-19 years and represent an average diet. San Filippo and Battistone (11) obtained a total F^- intake range (four different diets for Baltimore [water 1 ppm F^-]), of 2.09-2.34 mg/day in 1971 whereas Singer *et al.* (15) found a total F^- intake range of 0.988-1.720 mg/day for three fluoridated (average F^- of 0.9 ppm) cities and 0.912 mg/day for an unfluoridated (0.4 ppm) city. In another study Singer *et al.* (17) analyzed 24 market basket collections and found total intake values of 1.85 mg/day for fluoridated (> 0.7 ppm) cities and 0.86 mg/day for non-fluoridated (< 0.3 ppm) cities. Singer (15) explained the higher values found by San Filippo and Battistone (11) as arising from differences in the dietary composition and total quantity from each of the 12 categories included in the diet that had been constructed. San Filippo and Battistone (11) included about 1.2 L in category XII (tea, coffee, soft drinks, water) of the diet while Singer *et al.* (15) included about 0.6-0.7 L in this category and did not include drinking water. In light of the total water intake study (18) showing a mean intake from beverages and water of 1.1 L (for the 11-19 age group)*, the lower value by Singer is unreasonably low.

Adult Intake Reviews

A recent World Health Organization report (21) concluded that the total human F^- intake in persons residing in low-fluoride (< 0.4 mg/L) communities did not exceed 1.0 mg/day intake unless modified by national consumption habits (such as diets including unusual amounts of high F^- components such as tea, seafood, etc.). The report found a range of 1.0 to 5.4 mg/day for fluoridated communities; for subgroups in these communities exposures might be much lower and much higher. In addition to dietary exposure, the report identified occupational exposure which is high in certain industries, affecting a narrow group of workers), air pollution (normally minor for the general public), and fluoridated dental health care products (likely to affect a large segment of the population and to contribute at least 0.25 mg/day to F^- intake). Foodstuffs processed with fluoridated water showed elevated F^- (0.6-1.0 mg/kg v. normal 0.2-0.3 mg/kg) levels.

According to Rao (22) the food component of the adult diet is not a major source of F^- exposure, and the quantity of F^- in food has not changed significantly over the past four decades. Beverages, on the other hand, closely reflect the F^- content of the water from which they are made and thus, when prepared with fluoridated water, contribute significantly to the F^- intake. In a fluoridated community, food F^- may contribute approximately one-fourth of the F^- dietary intake. It should be noted that Table 3 in this reference has some values out-of-place. Reference #147 is to young adults, not toddlers and references #93 and #107 are to adults, not toddlers and young adults respectively.

Comparison of the U.S. studies cited in this editorial and the WHO Report (21) reveals that low fluoride or unfluoridated communities have experienced an initial rise in mean total dietary F^- (0.88 ± 0.03 mg F^- /day)(mean value

* This study does not give sufficient data to allow calculations for the 15-, 16-19 age group. Since liquid intake increases with age, the 1.1 L value should be considered a lower limit; 1.3 L would probably be a more realistic value.

for low F^- cities in references 13-15, 17 in Table 1); and 0.75 ± 0.17 mg F^- /day (mean of values from 1954-1980 in Table 3 for low F^- cities in reference 21)) above the prefluoridation range (0.45-0.55 mg F^- /day). This rise came about due to contamination of food and beverage supplies in these communities through the importation of commercial products produced and/or prepared in neighboring communities when they adopted fluoridation programs. The intake in most of the low or unfluoridated communities is now within the range proposed by proponents of fluoridation programs (0.7-1.2 mg F^- /L). After the initial rise to the new higher level, the F^- dietary exposure appears to have remained relatively constant.

In fluoridated communities the mean total dietary F^- exposure is much higher and more variable (2.0 ± 0.9 mg F^- /day, the mean of the fluoridated city values from Table 1 from references 9-17) than that proposed when water fluoridation programs are implemented, primarily due to the F^- contribution from the fluid intake portion of the diet. Originally predicated upon consumption of 1 L of tapwater fluoridated at 1 ppm, the dose of F^- actually delivered in the fluoridated areas is much greater, primarily due to an underestimation of the total quantity of fluoridated water consumed and its effect upon the F^- content of commercially prepared foods and those prepared or reconstituted in the home.

Total Water-Intake-Based F^- Exposures: Identifying At-Risk Groups

The studies cited in this editorial underestimate the dietary F^- intake for two major reasons.

1. The investigators either omit total adult water intake or make assumptions that usually lead to an underestimation of the quantity consumed, a serious omission because F^- intake is very sensitive to fluid F^- concentration and volume.
2. Assessing the intake of standardized or prescribed institutional adult diets may not identify the actual F^- exposure for the general public. Additionally, the highly variable adult diets actually consumed by the general public fail to identify F^- intakes of those at greatest risk of excessive F^- intake, who consume a diet with an F^- content several standard deviations above the mean.

The appearance of the Ershow and Cantor study of total water and tapwater uptake in the United States (18) furnishes a unique opportunity, not previously available, to produce estimates of total dietary F^- intake. This survey, based upon a stratified random sample, represents persons living in households in the United States. Weekly food purchase and dietary questionnaires, including a follow-up interview within 24 hours of recording household data, provided the database for the study. Inclusion of only those individuals having data for 3 complete days and elimination of those with incomplete questionnaires provided data from 26,446 subjects.

From the data for total water and tapwater intake in the U.S. (18), means of total dietary F^- intake were estimated for adults (20-64 years), young adults (11-19 years), and minimum intakes for a number of groups considered to have intakes considerably above the mean. Because the use of dentifrice containing F^- is so prevalent in this country, intake from that source is also

Table 2
Estimated Daily Fluoride Exposure From Total Water and Tapwater Intake

Exposure Group	Foods ^a (mg)	Fluids ^b (mg)	Tea ^b (mg)	Dentifrice ^c (mg)	Total (mg)
Young Adults (11-19 years)					
Mean	0.4	1.0	0.2	0.3	1.9
Upper 25%	0.4	>1.2	>0.2	0.3	>2.1
Upper 5%	0.4	>1.6	>0.9	0.3	>3.2
Upper 1%	0.4	>1.9	>1.7	0.3	>4.3
Adults (20-64 years)					
mean	0.4	1.4	0.3	0.3	2.4
Upper 25%	0.4	>1.5	>0.5	0.3	>2.7
Upper 5%	0.4	>2.1	>1.3	0.3	>4.1
Upper 1%	0.4	>2.6	>2.3	0.3	>5.6
Male Adults (20-64 years)					
Mean	0.5	— ^d	—	0.3	2.7
Upper 25%	0.5	—	—	0.3	>2.9
Upper 5%	0.5	—	—	0.3	>4.5
Upper 1%	0.5	—	—	0.3	>6.1

(a) The food intake is discussed in assumption 1. All values are rounded to the nearest tenth of a milligram.

(b) The fluids and tea contributions to intake are discussed in assumptions 2 and 3. The values were computed using the total water and tapwater intake values for the indicated populations from the data of Ershow and Cantor (18). All values were rounded to the nearest tenth of a milligram.

(c) The dentifrice contribution is discussed in assumption 4.

(d) The — indicates insufficient data were given to calculate these values. Values for fluids and tea were estimated using the percentage excess total water consumed by adult males compared to that of male and female adults combined and in the same percentile of intake.

included with the total dietary intake. The values utilized for each category appear in Table 2, and the assumptions used in calculating the values are given below.

1. Food F^- contributions included dairy products, meats, grain products, eggs, fruit, vegetables, fats and sweets. For purposes of this estimation the F^- content of these items was assigned 0.4 mg/day except for adult males where their caloric intake was found to be 23.3% greater than the adult mean and the F^- mean for this group was increased accordingly. The food F^- exposure for all higher percentile fluid consumption groups should be viewed as conservative because those having a higher liquid intake most likely also have a higher food consumption; however the difference, if any, could not be extracted from the available data.

2. The fluids category for all mean values includes drinking water, fruit juices and drinks, coffee, carbonated soft drinks, and alcoholic beverages. A contribution of 1 mg/L for fluids was assumed. Tap-water intake values (which include drinking water and water used in preparation of foods and beverages) were substituted for fluid intake for all non-mean groups because detail available from the data was insufficient to extract fluid intake as defined in the first sentence of this section. For this reason, water present in some beverages and food at the time of purchase will be underrepresented. Had this assumption been made for the adult mean values, the under-reporting would have been 11%. The values for these non-mean groups should then be viewed as conservative or minimum values.
3. Tea, a high fluoride beverage, was assumed to have been made with fluoridated water (1 ppm) and the infusion was assumed to increase the F^- concentration to 2 ppm (21,22). A further assumption was that those in the higher consumption percentiles also consumed higher quantities of tea. Such an assumption may tend to exaggerate the total F^- intake because those in the higher water consumption percentiles may, in fact, have consumed more fluid from other beverage categories.
4. From 80-95% of the dentifrice used by the U.S. public contains F^- (22,23), which contributes 0.3 mg F^- /day. A higher intake would be expected if the sample population engaged in multiple brushings/day and used F^- rinses.

The mean young adult (11-19 years) and adult (20-64 years) daily F^- intakes in a fluoridated community were calculated to approximate 1.9 and 2.4 mg/day, respectively, twice the predicted intake of 1 L at 0.7-1.2 ppm F^- put forward by proponents. The mean adult male intake is approximately at 2.7 mg F^- /day, almost triple the intended intake.

The groups most at risk of excessive F^- intake are those whose total water intakes are above the means:

Young Adults (11-19 years) in the upper 25th percentile have an exposure greater than 2.1 mg F^- /day; the upper 5th percentile >3.2 mg F^- /day; and the upper 1st percentile >4.3 mg F^- /day.

Adults (20-64 years) in the upper 25th percentile >2.7 mg F^- /day; the upper 5th percentile >4.1 mg F^- /day; and the upper 1st percentile >5.6 mg F^- /day.

Male Adults (20-64) in the upper 25th percentile >2.9 mg F^- /day; the upper 5th percentile >4.5 mg F^- /day; and the upper 1st percentile >6.1 mg F^- /day.

In his 1945 balance studies McClure (24) noted that fluoride balance did not become significantly positive until intake was above 4-5 mg/day. He stated that this level should not be exceeded. It is obvious from the above estimates of total F^- intake that a significant portion of the U.S. adult population is probably at or above this level. Since adult intakes occur after tooth formation, one would not expect an obvious excess F^- intake marker such as dental

fluorosis, to signal this high level of intake. These estimates of excessive F^- intake in the nontarget population, along with recent studies (25 and references therein) suggesting a world-wide decline in dental caries in both fluoridated and non- and low-fluoride areas, argue strongly for the discontinuation of fluoridation programs.

References

1. Kintner, R. Roy: Dietary Intake in the USA. **Fluoride**, 4:43-46, 1971.
2. Armstrong, W.D. and Knowlton, M.: Fluorine Derived from Food. **J. Dental Research**, 21:326, 1942.
3. McClure, F.J.: Fluorine in Foods, Survey of Recent Data. **Public Health Reports**, 64:1061, 1949.
4. Ham, M.P. and Smith, M.D.: Fluoride Studies Related to the Human Diet. **Can. J. Chem.**, 28F:227, 1950.
5. Ham, M.P. and Smith, M.D.: Fluorine Balance Studies in Three Women. **J. Nutrition**, 53:225, 1954.
6. Cholak, J.: Current Information on the Quantities of Fluoride Found in Air, Food and Water. **Archives of Industrial Health**, 21:312-315, 1960.
7. Hodge, H.C. and Smith, F.A.: **Fluorine Chemistry**, Vol. 4. Academic Press, N.W., 1965, p. 155 and 171.
8. Hodge, H.C. and Smith, F.A.: Minerals: Fluorine and Dental Caries. In: **Advances in Chemistry Series**, No. 94:93-115, 1970.
9. Marier, J.R. and Rose, D.: The Fluoride Content of Some Foods and Beverages - A Brief Survey Using a Modified Zr-SPANDS Method. **J. Food Science**, 31:941, 1966.
10. Spencer, H., Lewin, L., Fowler, J. and Samachson, J.: Effect of Sodium Fluoride on Calcium Absorption and Balances in Man. **Am. J. Clinical Nutrition**, 22:381, 1969.
11. San Filippo, F.A. and Battistone, G.C.: The Fluoride Content of a Representative Diet of the Young Adult Male. **Clin. Chim. Acta**, 31:453-457, 1971.
12. Osis, D., Wiatrowski, E., Samachson, J. and Spencer, H.: Fluoride Analysis of the Human Diet and of Biological Samples. **Clinica Chimica Acta**, 51:211-216, 1974.
13. Osis, D., Kramer, L., Wiatrowski, E. and Spencer, H.: Dietary Fluoride Intake in Man. **J. Nutr.**, 104:1313-1318, 1974.
14. Kramer, L., Osis, D., Wiatrowski, E. and Spencer, H.: Dietary Fluoride in Different Areas in the United States. **Am. J. Clin. Nutr.**, 27:590-594, 1974.
15. Singer, L., Ophaug, R.H. and Harland, B.F.: Fluoride Intake of Young Male Adults in the United States. **Am. J. Clin. Nutr.**, 33:328-332, 1980.
16. Taves, D.R.: Dietary Intake of Fluoride Ashed (Total Fluoride) v. Unashed (Inorganic Fluoride) Analysis of Individual Foods. **Br. J. Nutr.**, 49:295-301, 1983.
17. Singer, L., Ophaug, R.H. and Harland, B.F.: Dietary Fluoride of 15-19-Year-Old Male Adults Residing in the United States. **J. Dent. Res.**, 64:1302-1305, 1985.
18. Ershow, A.G. and Cantor, K.P.: Total Water and Tapwater Intake in the United States: Population-Based Estimates of Quantities and Sources. National Cancer Institute Order #263-MD-810264, Life Sciences Research Office, Federation of American Societies for Experimental Biology, 1989.
19. Taves, D.R.: In: Johansen, E., Taves, D.R. and Olsen, T.O.: **Continuing Evaluation of Fluorides**, AAAS Selected Symposium 11, Westview Press, Boulder, CO, 1979, Chap. 6, pp. 149-157.

20. Guy, *Ibid*, Chapter 5, pp. 125-147.
21. World Health Organization: **Environmental Health Criteria 36, Fluorine and Fluorides**. World Health Organization, Geneva, 1984.
22. Rao, G.S.: Dietary Intake and Bioavailability of Fluoride. **Ann. Rev. Nutr.**, 4:115-136, 1984.
23. Kumar, J.V., Green, E.L., Wallace, W. and Carnahan, T.: Trends in Dental Fluorosis and Dental Caries Prevalence in Newburgh and Kingston, N.Y. **Am. J. Pub. Health**, 79:565-569, 1989.
24. McClure, F.J., Mitchell, H.H., Hamilton, T.S. and Kinser, C.A.: Balance of Fluorine Ingestion from Various Sources in Food and Water by Five Young Men. Excretion of Fluorine Through the Skin. **J. Ind. Hyg. Toxicol.**, 27:159-170, 1945.
25. Yiamouyiannis, J.A.: Water Fluoridation and Tooth Decay. Results from the 1986-1987 National Survey of U.S. School Children. **Fluoride**, 23:55-67, 1990.

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FLUORIDE AND PHOTOSYNTHETIC CAPACITY OF AZALEA (RHODODENDRON) CULTIVARS

by

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SUMMARY: The ability of fluoride to reduce photosynthetic capacity of leaf discs of various azalea (Rhododendron) cultivars was investigated. Leaf discs were floated on buffered 10 mM KCl and 10 mM KF solutions. Fluoride was more effective in reducing photosynthetic capacity of the cultivar "Fashion", a slow photosynthesizer, than of "Hinocrimson", a faster photosynthesizer. Fluoride was more effective in reducing photosynthetic capacity than in reducing chlorophyll concentrations. Fluoride was highly effective in reducing the photosynthetic capacity of "Treasure", a fast growing cultivar with a very high rate of photosynthesis.

KEY WORDS: Azalea; KF; O₂ evolution; Photosynthetic capacity; Rhododendron

Introduction

Azaleas and other members of the genus Rhododendron are potentially useful plants for investigating the phytotoxic effects of fluoride and other air pollutants. These plants have intermediate sensitivity to fluoride (1), but they also retain at least some leaves all year; their leaves are sufficiently large for a number of biochemical and physiological investigations (as compared to conifer needles), and they pass through a period of dormancy which has been thoroughly investigated (2). In addition, there is a considerable variation in photosynthetic rates between various azalea cultivars (3-5). Recently, techniques for determining photosynthetic capacity of leaf discs have been described which make use of the Clark O₂ electrode (6), and the influence of fluoride on reducing photosynthetic capacity of spinach leaf discs has been reported (7).

The purpose of these studies is to describe the effect of fluoride on the photosynthetic capacity of leaf tissue of azalea cultivars with low, medium, and high rates of photosynthesis.

Materials and Methods

Leaves were removed from the middle portions of ten-week-old (from time of pruning) shoots of various azalea cultivars. The plants were growing in a peat moss:sand mixture (1:1 v/v) in a 16°C night glasshouse. They were fertilized once per month with 2.6 g/L ammonium sulfate (21% N) plus 0.75 g/L 20-20-20 (20% N-, 8.8% P-, 16.6% K). Leaf discs were floated on water,

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10 mM KCl, and 10 mM KF, all buffered with 10 mM MES (2-[N-morpholino] ethanesulfonic acid) at pH 5.5. This was necessary because KF can cause a considerable increase in pH, and pH 5.5-5.7 has resulted in a maximum reduction in chloroplast activity by KF (10).

For determinations of photosynthetic capacity the method of Steffen and Palta (6) was adopted. Sixteen leaf discs, 4 mm in diameter, were isolated from 4 leaves with a cork borer, cut in half, and placed in 4.8 mL of 50 mM HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]) and 0.5 mM CaSO_4 at pH 7.2. The leaf segments were vacuum infiltrated twice (to avoid excessive bubbling) for 3 minutes with a water aspirator. Leaf segments were given a 10-minute pre-illumination of $1400 \mu\text{mol}/\text{m}^2/\text{sec}$. Following pre-illumination, 0.1 mL NaHCO_3 (final concentration 12.5 mM) was added, and O_2 uptake and evolution were determined with a Yellow Springs Instruments Model 53 Clark oxygen electrode at 30°C . Respiration (O_2 uptake) was determined before and after illumination. Evolution of O_2 was determined with $2000 \mu\text{mol}/\text{m}^2/\text{sec}$ (presumed to be saturating). The average value of O_2 uptake was added to the value for O_2 evolution to determine gross (true) photosynthesis. As this determination has been made at saturating light and CO_2 levels (high CO_2 should completely suppress photorespiration), it should then be a measure of photosynthetic capacity. Chlorophyll concentrations of leaf segments were made using 96% Ethanol as an extractant, as described by Wintermans and De Mots (8).

All experiments were randomized block experiments with 4 replications. The level of significance was 5% and data were analyzed by Duncan's Multiple Range Test (9).

Results

When azalea leaf segments were floated on 10 mM KF, photosynthetic capacity was reduced (Table 1). While photosynthetic capacity of "Hinocrimson" (a rapid photosynthesizer) and "Fashion" (a slow photosynthesizer) were both reduced, in the case of "Fashion" there was a slight light-induced oxygen uptake, indicating the presence of the "Mehler reaction" as discussed by Badger

Table 1

Influence of 10 mM KCl and 10 mM KF on Photosynthetic Capacity of Azalea (*Rhododendron*) Leaf Discs.¹

Cultivar	Photosynthetic Capacity \pm S.D. ($\mu\text{mol. O}_2/\text{mg chlorophyll}/\text{hr}$)			
	Day 0	Day 1		
		H_2O	10 mM KCl	10 mM KF
"Hinocrimson"	$37.2 \pm 16.1^{\text{cde}}$	$46.1 \pm 12.5^{\text{e}}$	$43.9 \pm 10.5^{\text{e}}$	$32.8 \pm 15.7^{\text{bcd}}$
"Fashion"	$22.6 \pm 8.7^{\text{b}}$	$26.7 \pm 8.5^{\text{bc}}$	$24.8 \pm 10.3^{\text{b}}$	$-4.8 \pm 1.5^{\text{2a}}$

¹ Means are of 4 replications and when followed by the same letter or letters are not significantly different at the 5% level.

² This value indicates light-induced oxygen consumption rather than oxygen evolution.

(10). Chlorophyll concentrations in "Fashion" leaf tissue were adversely affected by KF, but KF did not significantly reduce the chlorophyll concentrations of "Hinocrimson" leaf tissue (Table 2). In both cultivars the effect of KF was on reducing light-induced O_2 evolution; that is, on the photosynthetic process, rather than on chlorophyll concentration (Table 3). The photosynthetic apparatus of "Fashion" was more adversely affected by KF than was that of "Hinocrimson".

The azalea cultivar "Treasure" seemed to be unique because its photosynthetic capacity, chlorophyll concentration and shoot growth were all considerably greater than those of "Hinocrimson" (Table 4). Both photosynthetic capacity and chlorophyll concentration were considerably reduced by 10 mM KF (Table 5). Again, the reduction in photosynthetic capacity induced by KF was greater than the reduction in chlorophyll concentration.

Table 2

Influence of 10 mM KCl and 10 mM KF on Chlorophyll Concentration of Azalea (*Rhododendron*) Leaf Discs.¹

Cultivar	Chlorophyll Concentration \pm S.D. (μ g chlorophyll/16-4 mm diam. Leaf Discs)			
	Day 0	Day 1		
		H ₂ O	10 mM KCl	10 mM KF
"Hinocrimson"	76.5 \pm 7.9 ^{ab}	68.7 \pm 8.8 ^a	70.3 \pm 9.4 ^a	63.2 \pm 5.3 ^a
"Fashion"	117.1 \pm 17.8 ^d	90.6 \pm 8.1 ^{bc}	100.0 \pm 18.4 ^c	70.3 \pm 10.7 ^a

¹ means are 4 replications and when followed by the same letter or letters are not significantly different at 5% level.

Table 3

Influence of 10 mM KCl and 10 mM KF on Percent Change in Light-Induced O_2 Evolution and Chlorophyll Concentration of Azaleas (*Rhododendron*) Leaf Discs over 24 Hours.¹

Parameter	Cultivar	Percent from Day 0 \pm S.D.		
		H ₂ O	10 mM KCl	10 mM KF
O_2 Evolution	"Hinocrimson"	+19.5 \pm 29.5 ^c	+16.6 \pm 22.4 ^c	-23.3 \pm 24.2 ^b
	"Fashion"	-1.3 \pm 26.3 ^b	-7.0 \pm 13.6 ^b	-114.5 \pm 6.8 ^a
Chlorophyll ³ Concentration	"Hinocrimson"	-10.0 \pm 1.6 ^b	-12.2 \pm 4.3 ^b	-15.5 \pm 4.0 ^b
	"Fashion"	-21.7 \pm 4.1 ^b	-14.0 \pm 13.9 ^b	-38.0 \pm 8.4 ^b

¹ Means are of 4 replications and when followed by the same letter or letters are not significantly different at the 5% level.

² Calculated initially as μ moles O_2 /hr./16-4 mm leaf discs.

³ Calculated initially as μ g chlorophyll/16-4 mm leaf discs.

Table 4

Photosynthetic Capacity and Chlorophyll Concentration of Leaf Discs and of Shoot Growth of Azalea (*Rhododendron*) Cultivars "Hinocrimson" and "Treasure".

Parameter	Cultivar	
	"Hinocrimson"	"Treasure"
Photosynthetic Capacity ($\mu\text{mol. O}_2/\text{mg Cl/hr.}$)	41.9 \pm 5.8	69.3 \pm 6.2*
Chlorophyll Concentration ($\mu\text{g}/16\text{-}4\text{ mm leaf discs}$)	60.9 \pm 3.2	7.8 \pm 8.1*
Shoot Length (cm/shoot)	7.2 \pm 0.4	11.7 \pm 2.6*
Shoot Dry Weight (mg/shoot)	0.17 \pm 0.10	0.49 \pm 0.18*
Shoot Dry Weight (mg/cm)	0.023 \pm 0.004	0.041 \pm 0.006*

Means are of 4 replications.

- * indicates that mean for "Treasure" is significantly different to respective mean for "Hinocrimson" at the 5% level.

Table 5

Influence of 10 mM KCl and 10 mM KF on Photosynthetic Parameters of Leaf Discs of Azalea (*Rhododendron*) Cultivar "Treasure".¹

Parameter	Day 0	Day 1		
		H ₂ O	10 mM KCl	10 mM KF
Photosynthetic Capacity ($\mu\text{mol. O}_2/\text{mg Cl/hr.}$)	64.3 \pm 7.8 ^b	70.2 \pm 9.2 ^b	57.2 \pm 6.7 ^b	14.3 \pm 13.2 ^a
Chlorophyll Concentration ($\mu\text{g chlorophyll}/16\text{-}4\text{ mm leaf discs}$)	75.0 \pm 0 ^b	68.7 \pm 5.1 ^b	70.3 \pm 7.8 ^b	53.1 \pm 6.2 ^a
Percent change from day 0: Light-induced O ₂ evolution		1.0 \pm 17.3 ^c	-14.7 \pm 21.7 ^{bc}	-84.7 \pm 12.1 ^a
Chlorophyll Concentration ³		8.2 \pm 6.9 ^b	-6.2 \pm 10.4 ^b	-29.0 \pm 8.0 ^b

¹ Means are of 4 replications and when followed by the same letter or letters are not significantly different at the 5% level.

² Calculated initially as $\mu\text{mol O}_2/\text{hr.}/16\text{-}4\text{ mm leaf discs}$.

³ Calculated initially as $\mu\text{g chlorophyll}/16\text{-}4\text{ mm leaf discs}$.

Discussion

These data indicate a considerable cultivar difference in azaleas in fluoride sensitivity, especially in regard to the photosynthesis apparatus (Tables 1-3). The light-induced oxygen consumption that occurred in "Fashion" leaf tissue

following fluoride treatment could be due to a suppression of photosystem II. Photorespiration would be unlikely to occur due to the high concentration of NaHCO_3 present and the only alternative explanation is that the "Mehler reaction" was either stimulated or became detectable following fluoride treatment. This reaction produced superoxide and H_2O_2 - both of which are extremely phytotoxic (11). If photosystem II was inhibited by fluoride in azalea leaf tissue as it was in pea chloroplasts (12), and photosystem I was not affected, the reduced ferredoxin produced by photosystem I would be available to participate in the "Mehler reaction." Other photosynthetic processes that have been found to be sensitive to fluoride include photophosphorylation (13) and such photosynthetic enzymes as chloroplast ATPase (13) and ribulose-1,5-bisphosphate carboxylase-oxygenase (14). Any or all of these factors could be involved in the fluoride inhibition of photosynthetic capacity in azalea leaf tissue.

The effect of fluoride on "Treasure" leaf tissue is interesting because this is a cultivar with a very high rate of photosynthesis and shoot growth (Table 4). The photosynthesis apparatus of this cultivar was extremely sensitive to fluoride, indeed almost as sensitive as leaf tissue of the more slowly photosynthesizing "Fashion". Thus, there does not appear to be a relationship between rate of photosynthesis and fluoride sensitivity in azaleas.

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References

1. Weinstein, L.H.: Fluoride and Plant Life. *J. Occup. Med.*, 19:49-78, 1977.
2. Ballantyne, D.J.: Dormancy and Greenhouse Culture. *Rhodo. Notes and Records*, 2:145-153, 1988.
3. Ceulemans, R., Impens, I. and Gabriels, R.: Comparative Study of Photosynthesis, Transpiration, Diffusion Resistances and Water-Use Efficiency of Two Azalea Cultivars. *Scientia Hort.*, 13:283-288, 1980.
4. Ceulemans, R., Heursel, J., Ibrahim, N. and Impens, I.: Variations Among Physiological, Morphological and Biochemical Characteristics of Evergreen Azalea (*Rhododendron simsii* Planch.) Cultivars. *Scientia Hort.*, 22:147-155, 1984.
5. Ballantyne, D.J.: Influence of Photosynthetic Capacity Irradiance and SO_2 Fumigation on Shoot Growth of Azalea (*Rhododendron*) Cultivars. In: Baltscheffsky, M., Ed.: *Current Research in Photosynthesis*, Vol. IV. Kluwer Academic Publishers, Dordrecht, 1990, pp. 603-606.
6. Steffen, K.L. and Palta, J.P.: Effect of Light on Photosynthetic Capacity During Cold Acclimation in a Cold-Sensitive and Cold-Tolerant Potato Species. *Physiol. Plant*, 66:353-359, 1986.
7. Quick, P., Neuhaus, E., Feil, R. and Stitt, M.: Fluoride Leads to an Increase of Inorganic Pyrophosphate and an Inhibition of Photosynthetic Sucrose Synthesis in Spinach leaves. *Biochim. Biophys. Acta*, 973:263-271, 1989.
8. Wintermans, J.F.G.M. and De Mots, A.: Spectrophotometric Characteristics of Chlorophylls a and b and Their Pheophytins in Ethanol. *Biochim. Biophys. Acta*, 109:448-453, 1965.

9. Li, J.C.R.: **Statistical Inference I**. Edwards Brothers, Inc. Ann Arbor, Michigan, 1964.
10. Ballantyne, D.J.: Fluoride Inhibition of the Hill Reaction in Bean Chloroplasts. **Atmos. Environ.**, 6:267-273, 1972.
11. Badger, M.R.: Photosynthetic Oxygen Exchange. **Ann. Rev. Plant Physiol.**, 36:27-52, 1985.
12. Ballantyne, D.J. and Glover, B.L.: Chloroplast Electron Transport, Protein and RNA in Fluoride-Treated Pea Shoots. **Environ. Exp. Bot.**, 21:83-88, 1981.
13. Pushnik, J.C. and Miller, G.W.: The Influence of Elevated Environmental Fluoride on the Physiology and Metabolism of Higher Plants. **Fluoride**, 23:5-19, 1990.
14. Parry, M.A.J., Schmidt, C.N.G. and Gutteridge, S.: Inhibition of Ribulose P₂ Carboxylase/Oxygenase by Fluoride. **J. Exp. Bot.**, 21:1177-1181, 1984.

THE INHIBITED ENCHONDRAL OSSIFICATION IN EXPERIMENTAL OSTEOPOROSIS IN RATS

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SUMMARY: There is agreement in the literature, that in osteofluorosis the whole bone mass, the osteoid surface and the osteoid volume become enlarged, and the mineralization of osteoid is delayed and irregular. It is not proven whether the enlargement of the whole bone mass is due to increased bone formation and/or decreased bone resorption, increased activity, number, and life span of osteoclasts and/or decreased number, activity and life span of osteoclasts.

The enhanced enchondral ossification of rat femur and vertebrae caused by daily intraperitoneal administration of 0.5 mg and 5 mg sodium fluoride for 3 months was investigated. The enlargement of residual chondroid tissue is a part of complex disturbances of the fluorotic bone explained by the inhibition of bone resorption as a toxic effect of fluoride.

KEY WORDS: Bone fluorosis; Enchondral ossification; Fluorosis in rats; Fluorotic bone; Ossification; Osteofluorosis, experimental; Rats, osteoclasts; Sodium fluoride.

Introduction

It is generally accepted, that the whole bone mass becomes increased under the effect of sodium fluoride (1,2). This takes place in intact, healthy bone in the case of industrial or endemic fluorosis, or occurs in osteoporotic bone when therapeutic fluorosis is created.

The increase of the whole bone mass may be due either to augmented bone formation (3-6), or to decreased bone resorption (7-18). The increased number, or activity, or longer life span of osteoblasts (OBs) may lead to augmented bone formation. Decreased bone resorption may be caused by decrease in number, or activity, or shorter life span of osteoclasts (OCs). The increased number, activity, long life span of OBs, or decreased number, activity, shorter life span of OCs may be absolute or relative. In other words, relative, simultaneous changes of the activity, number, or duration of life of both the OBs and OCs may result in changes — increase or decrease — of the whole bone mass.

Both quantitative and qualitative changes of the whole bone mass take place in osteofluorosis. The osteoid volume is increased (14,19), and mineralization of osteoid is delayed (5,19-21), increased and irregular (3,5,6,19,22). Qualitative alterations in the preexisting bone tissue itself occur (23).

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The present work aimed to find indirect histological evidence of the inhibited resorption activity in osteofluorotic bone tissue.

Materials and Methods

75 female rats weighing 200 g each were divided into 3 groups. 25 animals were given 0.5 mg and 25 animals were given 5 mg sodium fluoride daily, intraperitoneally, for 3 months. The control group – 25 animals – were given physiologic saline solution.

Histological investigation was performed on adjoining bone (tibia and femur) of both knee, and lumbar vertebrae III-V of the animals. The material fixed in 10% formalin solution was decalcified (decalcinating medium was 24 mL 85% formic acid, 50 mL 35% hydrochloric acid, 126 mL distilled water), embedded in paraffin, serially sectioned and stained with Haematoxylin-Eosin (H-E).

The increased bone volume and bone mass, and the volume of residual cartilage islets (as an indirect histological sign of inhibited enchondral ossification) were estimated in the zone of resorption of the epiphyseal cartilage of the vertebrae and tibiae with the help of a 10x10 mm square bar placed into the ocular of the microscope.

Results

The volume of tibia, femur and vertebrae is enlarged in the treated animals in proportion to the administered dose of NaF, compared to control animals (Table 1). Bone mass increase in proportion to the dose of NaF was observed in the treated animals (Table 1). The corticalis became widened and more spongiotic. Thickening of trabeculae of spongiosa could be observed. The medullary space became narrowed (Figure 1a and 1b). Marginal osteophytes were observed with a frequency in proportion to the NaF dose (Figure 2a). Bone having woven structure was more or less circumscribed, mostly subperiosteally (Figure 2b).

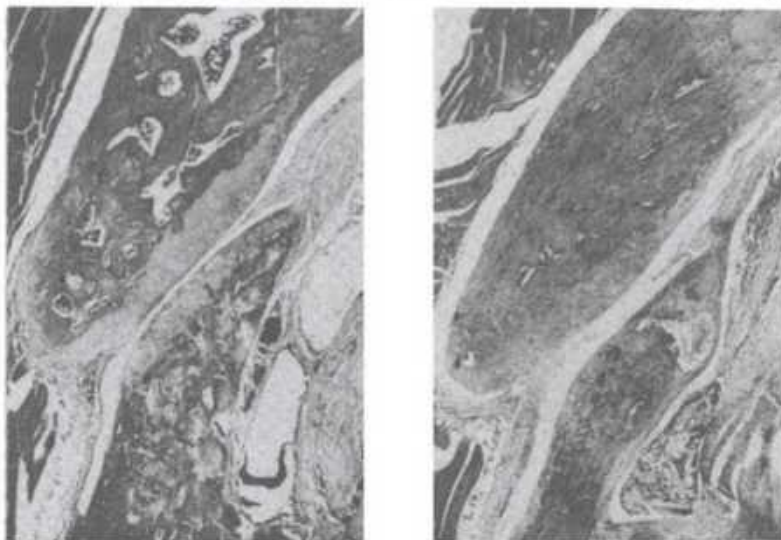
The number of active and inactive osteoblasts and osteoclasts in different files was so varied and scattered (Figure 3a and 3b) that statistical evaluation was not performed. The epiphyseal cartilage became widened, its regular

Table 1
The Percentage Values of Bone Mass and
Residual Cartilage Compared to Whole Bone Volume

	Tibia		Vertebra	
	Bone Mass	Residual Cartilage	Bone Mass	Residual Cartilage
Control	12:20 (60%)	1:20 (5%)	13:21 (62%)	2:21 (9.5%)
0.5 mg NaF	14:23 (61%)	2:23 (8.7%)	16:23 (69.5%)	3:23 (13%)
5 mg NaF	20:25 (80%)	6:25 (24%)	21:25 (84%)	6:25 (24%)

Figures 1a and 1b

Vertebral process: (a) Control. (b) Significantly increased bone mass, narrowed marrow space due to 3 months of daily administration of 5 mg NaF.
H-E 16x (magnification refers to 24x36 mm negative picture)



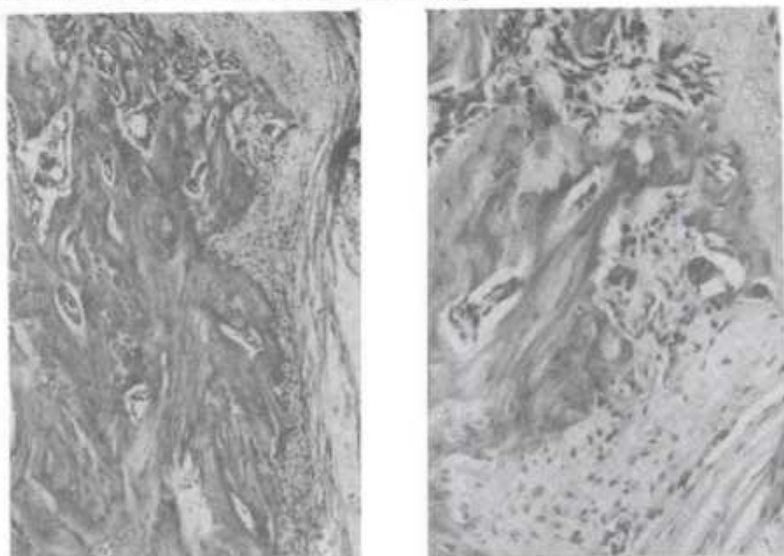
Figures 2a and 2b

Circumscribed, augmented bone formation: (a) Marginal osteophyte. (b) Subperiosteal, newly formed woven bone tissue.
H-E 16x

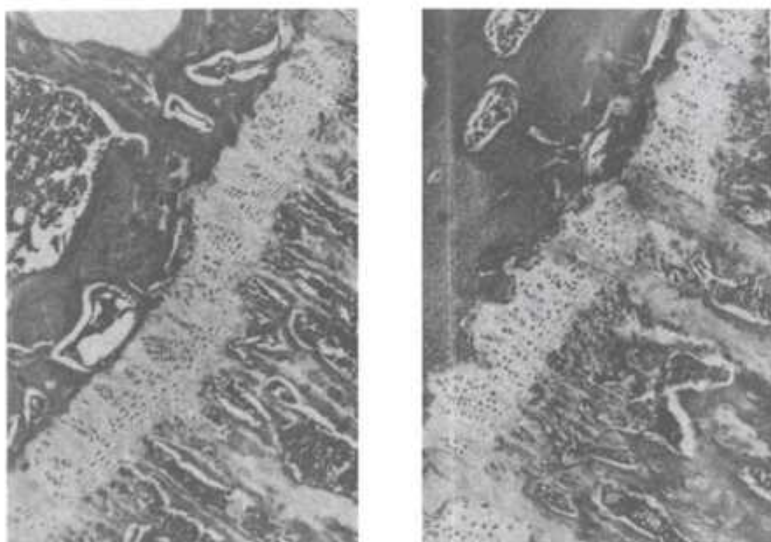


Figures 3a and 3b

Epiphysis of tibia following daily treatment with 0.5 mg NaF. (a) H-E 16x. (b) 100x [same section, higher magnification. Active osteoblasts (+) and inactive osteoblasts (I) in a circumscribed field.]

Figures 4a and 4b

Epiphyseal cartilage of tibia. (a) Control. (b) Irregular calcification of the epiphyseal cartilage due to daily administration of 5 mg NaF. The bone tissue above the epiphyseal cartilage is enlarged, marrow space is narrowed. H-E 16x.



zonality indistinct; the regular, linear arrangement of cells became disorganized (Figures 4a and 4b). Cells frequently became arranged in small groups in the proliferative zone. The degenerative zone became irregularly widened compared to control. The cartilaginous axis of primary spongiosa remained enlarged in the zone of resorption in proportion with the NaF dose. Occasionally islets of cartilage remained within the secondary spongiosa or corticalis.

Daily administration of 0.5 and 5 mg NaF for 3 months resulted in a dose dependent, significant chondral tissue. The proportional numbers in the table give the number of foci of bone or residual chondral tissue seen at the cross points of the square bar placed into the ocular, compared with the total width of femur and vertebra.

Discussion

Increase of bone volume and bone mass was observed in experimental fluorosis in rats. The corticallis became widened, and the number and thickness of spongiotic trabeculae increased. The increased osteoid volume and increased proportion of newly formed woven bone tissue in osteofluorosis (1,2,14,19) may be due not only to the increased number, activity and active life span of OBs (as believed by many authors), but also to the decreased number, activity and shorter life span of the OCs. To determine histologically the exact number of OBs and OCs is possible. To evaluate their activity without using enzyme histochemical methods is questionable. It is impossible to estimate the active life span of OBs and OCs on embedded, fixed material. Therefore, increased bone and osteoid volume alone, as evidence of the activating effect of NaF, is not established.

We aimed to find histological indirect evidence of the inhibited resorptive processes (chondroclasia). Based on the increased number of residual chondral islets, inhibited enchondral ossification during NaF treatment seems to be evident. Enlargement of bone and osteoid volume proposed to be due to a toxic effect (inhibited enzyme activity) as stated by several authors (5,14,24) is more acceptable for us than a supposed activating effect.

References

1. Conrozier, T. and Meunier, P.J.: Traitement de l'ostéoporose cortisonique par le fluorure de sodium. Effects cliniques et données histomorphométriques. *Annales d'Endocrinologie*, 46:369-370, 1985.
2. Qiu Ming-cai, Li Song-ling, Zhang Nai-xim, Bai Cheng-jiang: Is the Severity of Osteosclerosis of Fluorosis Proportional to the Dose of Fluoride Intake? *Chinese Med. J.*, 100:330-332, 1987.
3. Baylink, D.J. and Bernstein, D.S.: The Effects of Fluoride Therapy on Metabolic Bone Disease. *Clin. Orthop. and Related Research*, 55:51-85, 1967.
4. Dominok, G.W.: Die Industrielle Knochenfluorose und Probleme einer Fluortherapie der Osteoporose. *Z. Alternforsch.*, 29:277-283, 1975.
5. Malcolm, A.S. and Storey, E.: Osteofluorosis in the Rabbit: Microradiographic Studies. *Pathology*, 3:39-51, 1971.
6. Vigorita, V.J. and Suda, M.K.: The Microscopic Morphology of Fluoride-Induced Bone. *Clinic. Orthop.*, 177:274-282, 1983.
7. Beickert, A.: Die Behandlung der Glukokortikoidosteoporose mit Natriumfluorid. *Dtsch. Gesundh.-Wes.*, 26:2398-2402, 1971.

8. Bély, M.: Experimental Fluorosis in Rats. Natrium-Fluoratum-Induced Changes in Bone and Bone Marrow. **Fluoride**, 16:106-111, 1983.
9. Bernstein, D.S., Sadowsky, N., Hegsted, D.M., Guri, C.D. and Stare, F.J.: Prevalence of Osteoporosis in High- and Low-Fluoride Areas in North Dakota. **J. Am. Med. Assn.**, 198:499-504, 1966.
10. Charles, P., Mosekilde, L., Taagehoj-Jensen, F.: The Effects of Sodium Fluoride, Calcium Phosphate, and Vitamin D₂ for One to Two Years on Calcium and Phosphorus Metabolism in Postmenopausal Women with Spinal Crush Fracture Osteoporosis. **Bone**, 6:201-206, 1985.
11. Faccini, J.M.: Fluoride and Bone. **Calcif. Tiss. Res.**, 3:1-16, 1969.
12. Goldhaber, P.: The Inhibition of Bone Resorption in Tissue Culture by Nontoxic Concentrations of Sodium Fluoride. **Israel J. of Med. Sci.**, 3:617-626, 1967.
13. Jowsey, J. and Kelly, P.J.: Effect of Fluoride Treatment in a Patient with Osteoporosis. **May Clin. Proc.**, 43:435-443, 1968.
14. Krook, L. and Maylin, G.A.: Industrial Fluoride Pollution. Chronic Fluoride Poisoning in Cornwall Island Cattle. **The Cornell Veterinarian**, 69:7-70, 1979.
15. Messer, H.H., Armstrong, W.D. and Singer, L.: Fluoride, Parathyroid Hormone and Calcitonin: Interrelationship in Bone Calcium Metabolism. **Calc. Tiss. Res.**, 13:227-233, 1973.
17. Milicic, M. and Jowsey, J.: Effect of Fluoride on Disease Osteoporosis in the Cat. **J. Bone Joint Surg.**, 50-A:701-708, 1968.
18. Ream, L.J., Pendergrass, P.B. and Scott, J.N.: Fluoride Ingestion during Pregnancy and Lactation: Morphological Effects on Maternal Rat Bone. **Fluoride**, 16:24-33, 1983.
19. Oláh, A.J., Reutter, F.W. and Schenk, R.K.: Histological Bone Changes after Long Term Treatment with Sodium Fluoride. In: Kuhlencordt, F. and Kruse, H.P.: **Calcium Metabolism, Bone and Metabolic Bone Disease**, Springer, Berlin-Heidelberg-New York, 1975, pp. 146-150.
20. Qiu Ming-cai, Zhu Xian-yi, Li Song-ling, Sun Gui-lian, Ni An-min, Song Wen-zhong and Zang Jia-lan: Bone Dynamic Changes in Experimental Fluorosis in Rats. **Chinese Med. J.**, 100:879-885, 1987.
21. Reutter, F.W.: Aktuelle Aspekte der Klinik und Therapie der Osteoporose. **Therapiewoche**, 26:6212-6227, 1976.
22. Larsen, M.J. and Thorsen, A.: A Comparison of Some Effects of Fluoride on Apatite Formation in Vitro and in Vivo. **Calcif. Tiss. Int.**, 36:690-696, 1984.
23. Bély, M. Pintér, T., Sádorfi, N. and Ratkó, L.: Changes in the Collagen Structure of Bone Tissue in Experimental Fluorosis. **Fluoride**, 21:28-31, 1988.
24. Bernick, S. and Zipkin, I.: Histochemical Study of Bone in Hydrocortisone- and Fluoride-Treated Rats. **J. of Dent. Res.**, 46:1404-1411, 1967.

EFFECT OF CHRONIC FLUORIDE TOXICITY ON GLUCOCORTICOID LEVELS IN PLASMA AND URINE

by
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SUMMARY: Circulating levels of cortisol were estimated in 14 fluorosis patients and 9 healthy control subjects. The plasma cortisol level was markedly decreased in the patient group. To confirm this, the protocol was repeated in fluoride-treated experimental animals with sex and age matched controls. The fluoride-treated group also demonstrated marked hypocortisolemia, and the decrease was consistent at different time periods irrespective of diurnal variation. The 17-hydroxy-corticosteroid levels in urine was also decreased ($p < .001$), clearly suggesting adrenal hypofunction in chronic fluoride toxicity.

KEY WORDS: Calcium osteopenia; Cortisol; Corticosteroid; Fluorosis; Glucocorticoids.

Introduction

Glucocorticoids are one of the least studied calcium regulating hormones in chronic fluoride toxicity and fluorosis. At supraphysiologic level, they are known to inhibit bone formation and stimulate bone resorption, resulting in severe bone loss or osteopenia (1-3). Glucocorticoids are also known to inhibit intestinal calcium absorption (4), possibly by inhibiting the synthesis of a calcium binding protein in intestine (5). Enhanced urinary calcium excretion is also very common in subjects having higher circulating levels of endogenously produced corticosteroid (6), or in patients under prolonged glucocorticoid therapy (7).

In order to understand the role of glucocorticoids, if any, in the pathogenesis of bone lesions in chronic fluoride toxicity in man and animals, investigations on glucocorticoid metabolism in experimental animals and cortisol levels in fluorosis patients were planned and conducted.

Materials and Methods

Osteofluorosis Patients and Control Group: Patients ($n = 14$) were selected from the outpatient clinics and in-patients of the All India Institute of Medical Sciences Hospital, New Delhi. In each case, a detailed clinical and radiological examination was conducted and the diagnosis was reconfirmed. The control group consisted of nine healthy individuals of comparable age, sex and weight.

Experimental Animals: Sixteen male rabbits were randomly distributed in two groups of 8 each and kept under identical laboratory conditions with 16:8 hr dark and light cycles. One group of animals ($n = 8$) was orally administered

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NaF solution at the dose of 10 mg NaF/kg body weight daily for a period of 24 months. The other group (n = 8) served as controls.

Sampling of Body Fluids: In view of the fact that there is a diurnal variation in the plasma concentration of corticosteroids, blood samples were collected from the patients and their matched controls at identical time intervals. The same protocol was maintained for the experimental animals. After the blood was collected in a heparinized tube the plasma was separated by centrifugation, using a refrigerated centrifuge, and stored at -20°C until use.

Twenty-four hour urine was collected for 3 consecutive days; hormone assay was carried out on samples collected on each day; the mean of the 3 day assay value was reported as the final result.

Plasma Cortisol Level in Human Subjects: total cortisol levels in plasma were estimated by Radioimmunoassays according to WHO Assay Method Manual (8).

Corticosteroid Levels in Plasma and Urine in Experimental Animals: Because of the controversy as to whether cortisol and corticosterone is the major corticosteroid in rabbit, both cortisol and corticosterone in the plasma of the rabbit were investigated. Competitive protein binding radioassay procedure was adopted (9). Urinary corticosteroid was also assayed by the same method.

17-Hydroxycorticosteroid (17 OHCS) Levels in Urine: Urinary 17 OHCS in experimental animals were established according to the method of Silber and Porter (10).

Statistical Method: The significance of the differences between the mean values was established by the Student's t-test.

Results

Cortisol Levels in Patient and Control Groups: The 14 Osteofluorosis patients were subdivided into three groups according to their blood collection time and each group had a matched control group with same blood sampling time. All three groups of fluorosis patients showed hypocortisolemia, and the decrease was statically significant compared to control values (Table 1).

Table 1
Plasma Cortisol (Total) Levels in Fluorosis Patients

	Age (in years) (Mean \pm S.D.)	Total Cortisol ($\mu\text{g}/100\text{mL}$). (Mean \pm S.D.)		
		10:30#	11:30#	12:30#
Control Group (n = 9)	37.22 \pm 9.03	14.01 \pm 0.84 (n = 3)	11.66 \pm 0.77 (n = 3)	9.68 \pm 0.63 (n = 3)
Patient Group (n = 14)	43.28 \pm 11.20	8.68 \pm 1.62* (n = 5)	7.45 \pm 1.12** (n = 5)	5.73 \pm 0.98* (n = 4)

Blood Sample Collecting Times; * $p < 0.001$; ** $p < 0.005$

Corticosteroid Levels in Plasma and Urine in Experimental Animals: Both cortisol and corticosterone levels in plasma were estimated to find out the main corticosteroid in the experimental rabbits. The 8 A.M. blood sample clearly demonstrated cortisol as the main corticosteroid in the experimental animals (Table 2). Plasma cortisol, as well as corticosterone, showed a decrease in the fluoride-treated group compared to the values of the control animals (Table 2).

Plasma cortisol levels at three different time periods namely, 7:00 am, 12:30 pm and 5:00 pm were also investigated to find out whether the hypocortisolemia observed in fluoride-treated animals is maintained at different time periods of diurnal periodicity.

As in Osteofluorosis patents, the fluoride-treated animals also demonstrated hypocortisolemia at all three different time periods of circadian periodicity and the difference was statically significant (Table 3).

The urinary free cortisol, however, did not show any deviation from control values (Table 4).

Table 2
Plasma Corticosteroid Levels in Experimental Animals (8:00 am)

Animal Group	Body Weight (in grams) Mean \pm S.D.	Duration of Fluoride Treatment# (in months)	Cortisol	Corticosterone
			(μ g/100 mL) Mean \pm S.D.	(μ g/100 mL) Mean \pm S.D.
Control (n = 8)	1607.14 \pm 128.50	Nil	7.21 \pm 1.56	2.29 \pm 0.38
Treated (n = 8)	1557.14 \pm 123.92	24	4.35 \pm 0.63*	1.39 \pm 0.41*

#Dose: 10 mg NaF/kg body wt./day; * p < 0.001

Table 3
Plasma Cortisol (Total) Levels in Experimental Animals

Animal Group	Plasma Cortisol Level (μ g/100 mL) (Mean \pm S.D.)		
	7:00#	12:30 pm#	5:00 pm#
Control (n = 8)	7.96 \pm 1.18	4.10 \pm 0.65	2.18 \pm 0.36
Treated (n = 8)	5.01 \pm 0.46 ^a	2.88 \pm 0.45 ^a	1.73 \pm 0.35 ^b

@ Fluoride-treated for 24 months; # Blood Sampling Time;

^a p < 0.001; ^b p < 0.025

Table 4
Urinary Levels of Cortisol and 17-OHCS in Experimental Animals

Animal Group	Urinary Excretion	
	Cortisol ($\mu\text{g}/24 \text{ hr}$) (Mean \pm S.D.)	17 OHCS ($\mu\text{g}/24 \text{ hr}$) (Mean \pm S.D.)
Control (n = 8)	4.37 \pm 0.83	237.50 \pm 31.05
Treated (n = 8)	4.23 \pm 1.01	168.12 \pm 31.61*

* $p < 0.001$

Urinary 17 OHCS Levels in Experimental Animals: Unlike the free cortisol, the 17 OHCS levels in urine was markedly decreased in the fluoride-treated group ($p < 0.001$). This decrease in 17 OHCS is consistent with the decreased corticosteroid levels in plasma, thus suggesting hypofunction of adrenal gland.

Discussion

In chronic fluoride toxicity and skeletal fluorosis the pathogenesis of bone tissue is not clearly understood. Disturbed metabolism of some of the calcium regulating hormones has been considered as one of the main factors responsible for bone lesions in chronic fluoride toxicity and fluorosis. Hypersecretion of parathyroid hormone and osseous changes consistent with hyperparathyroidism are established facts in chronic fluoride toxicity (11). Calcitonin is also raised in chronic fluoride toxicity (12), although some contradictory results have been reported (13). Srivastava *et al.* (14) reported subnormal vitamin D₃ levels in two of the six fluorosis patients investigated, whereas some reports suggest no alteration in vitamin D₃ levels (15).

Although glucocorticoids are very important calcium regulating hormones, they have received scant attention in fluoride toxicity research. Rao and Susheela (16) reported decreased activity of delta 5-3- β hydroxysteroid dehydrogenase, thereby suggesting impaired steroid production in chronic fluoride toxicity (17). Guansheng Li found reduced levels of serum corticosterone in fluoride-treated rats (18).

The observation on hypocortisolemia in skeletal fluorosis patients reported in this communication is perhaps the only one of its kind. The above finding was further confirmed by the results of the experimental animals. In the animal study cortisol level was decreased in the fluoride-treated group irrespective of circadian variation, thus suggesting adrenal insufficiency in chronic fluoride toxicity. Urinary 17 OHCS was also decreased in the fluoride-treated group. As 17 OHCS levels in urine is a specific assessment of overall cortisol production, the decreased levels of 17 OHCS likewise suggest hypofunction of the adrenal gland. This study, however, is inadequate to explain the normal excretory levels of free cortisol and needs further investigation.

It is a well known fact that bone formation is enhanced in chronic fluoride toxicity (19), whereas glucocorticoids at supraphysiologic levels are

a very potent inhibitor of bone formation (1). Therefore, fluoride by inhibiting cortisol production, indirectly may be stimulating different factors responsible for bone formation. The increased intestinal calcium absorption and hypocalciuria observed in chronic fluoride toxicity (20) could also be due to decreased levels of cortisol, since glucocorticoids at supraphysiologic levels have an antagonistic effect.

Thus, in conclusion, it can be stated that fluoride, by inhibiting cortisol production, is probably disturbing the delicate balance between the calcium regulating hormone and bone metabolism, thereby creating an environment/milieu which may be one of the causative factors for the skeletal derangements in fluorosis.

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References

1. Bressot, C. et al.: Histomorphometric Profile, Pathophysiology and Reversibility of Corticosteroid Induced Osteoporosis. *Metab. Bone Dis. Rel. Res.*, 1:303, 1979.
2. Raiz, L.G., Trummel, C.L., Wener, J.A. and Simmons, H.: Effect of Glucocorticoids on Bone Resorption in Tissue Culture. *Endocrinology*, 90:961-967, 1972.
3. Peck, W., Gennari, C., Raisz, L., Meunier, P., Ritz, E., Krane, S., Nuki, G. and Avioli, L.V.: Corticosteroid and Bone. *Calcif. Tissue Int.*, 36:4-7, 1984.
4. Klein, R.G., Arnaud, S.B., Gallagher, J.C., Deluca, H.F. and Riggs, B.L.: Intestinal Calcium Absorption in Exogenous Hypercortisonism. *J. Clin. Invest.*, 60:253-259, 1977.
5. Feher, J.J. and Wasserman, R.H.: Intestinal Calcium Binding Protein and Calcium Absorption in Cortisol Treated Chicks: Vitamins and 1,25-Dihydroxy Vitamin D₃. *Endocrinology*, 104:547-551, 1979.
6. Jowsey, J. and Riggs, B.L.: Bone Formation in Hypercortisonism. *Acta Endocrin.*, 63:21-28, 1970.
7. Pechet, M.M., Bowers, B. and Bartter, F.C.: Metabolic Studies with a New Series of 1,4-Dienestroids. II. Effects in Normal Subjects of Prehnisone, Prednisolone and 9 α -Fluoroprednisolone. *J. Clin. Invest.*, 38:691-701, 1959.
8. WHO Programme for Provision of Matched Assay Reagents for the Radioimmunoassay of Hormones in Reproductive Physiology. Assay Method Manual (10th Edition), 1986.
9. Murphy, B.E.P.: Some Studies of the Protein-Binding of Steroids and Their Application to the Routine Micro and Ultramicro Measurement of Various Steroids in Body Fluids by Competitive Protein-Binding Radioassay. *J. Clin. Endo.*, 27:973-990, 1967.
10. Silber, R.H. and Porter, C.C.: The Determination of 17,21-Dihydroxy-20-ketosteroids in Urine and Plasma. *J. Biol. Chem.*, 210:923-932, 1954.
11. Teotia, S.P.S. and Teotia, M.: Secondary Hyperparathyroidism in Patients with Endemic Skeletal Fluorosis. *Br. Med. J.*, 1:637-640, 1973.

12. Okayasa, I., Tsuchida, N. and Yanagisawa, F.: Hyperplastic Nodules of Thyroid Parafollicular Cells (C-cells) in Rats Induced by Prolonged Low Dose Ingestion of NaF. **Fluoride**, 18:111-117, 1985.
13. Krishnamachari, K.A.V.R. and Shvakumar, B.: Endemic Genu Valgum: A New Dimension to the Fluorosis Problem in India. **Fluoride**, 9:185-200, 1976.
14. Srivatava, R.N., Gill, D.S., Moudgil, A., Menon, R.K., Thomas, M. and Dandova, P.: Normal Ionized Calcium Parathyroid Hypersecretion, and Elevated Osteocalcin in a Family with Fluorosis. **Metabolism**, 38:120-124, 1989.
15. Teotia, S.P.S., M., Singh, R.K. Teotia, N.P.S., Taves, D.R., Heels, S. and D'Mello, U.P.: Plasma Fluoride, 25-hydroxycholecalciferol, Immuno-reactive Parathyroid Hormone and Calcitonin in Patients with Endemic Skeletal Fluorosis. **Fluoride**, 11:115-119, 1978.
16. Rao, K. and Susheela, A.K.: Effect of Sodium Fluoride on Adrenal Gland of Rabbit. I. Studies on Ascorbic Acid and delta 5-3-beta Hydroxysteroid Dehydrogenase Activity. **Fluoride**, 12:65-71, 1979.
17. Li, G.S., Wang, J.X. and Jing, L.: The Antifluoride Effects of Zhonghua Maifanshi, An Experimental Study. **Fluoride**, 23:37-42, 1990.
18. Baylink, D. and Bernstein, D.: Effect of Fluoride Therapy on Metabolic Bone Disease. **Clin. Orthop.**, 55:51-85, 1968.
19. Das, T.K. and Susheela, A.K.: Effect of Long-Term Administration of Sodium Fluoride on Plasma Calcium Level in Relation to Intestinal Absorption and Urinary Excretion in Rabbits [Communicated].

EFFECTS OF VITAMIN C AND CALCIUM ON THE REVERSIBILITY OF FLUORIDE-INDUCED ALTERATIONS IN SPERMATOZOA OF RABBITS

by

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SUMMARY: The present study was designed to investigate the effects of fluoride on the metabolism and functions of cauda epididymal spermatozoa of rabbits. The studies on reversibility of fluoride-induced effects by fluoride withdrawal, Vitamin C (ascorbic acid, AA) or Calcium (Ca^{+2}) and combined AA + Ca^{+2} ingestion were also investigated.

Adult Male rabbits, Group II and III, were fed 20 and 40 mg/kg body weight sodium fluoride, respectively, for 30 days. Afterwards, cauda epididymal spermatozoa were obtained by micropuncture technique. Alterations in the activities of some specific androgen-dependent enzymes on sperm namely, ATPase, ACP, SDH, and protein, as well as reduction in Na^{+} and K^{+} levels in the spermatozoa, might be due to altered and hostile internal milieu of the epididymis in NaF-treated rabbits. Reduction in sperm motility, count, and changes in their morphology and metabolism led to the significant decline in fertility of the treated animals. After withdrawal of NaF treatment for 30 days (Group IV), no recovery was obtained in all the parameters which were altered, including fertility rates.

During the withdrawal period, AA or Ca^{+2} and combined AA + Ca^{+2} were administered to Groups V, VI, and VII, respectively. With both AA or Ca^{+2} marked recovery occurred from all induced effects. The extent of recovery, however, was somewhat more pronounced by AA treatment than that brought about by Ca^{+2} administration during the withdrawal period. The combined treatment with AA + Ca^{+2} manifested a synergistic effect for recovery of all parameters. The effects of fluoride are therefore transient and reversible, in agreement with earlier data. Moreover, AA and Ca^{+2} have therapeutic importance in fluorotic animals. These findings have a direct bearing on human subjects exposed to high fluoride levels.

KEY WORDS: Ascorbic acid; Calcium; Fluoride; Spermatozoa; Vitamin C.

Introduction

The clinical manifestations of fluorosis due to excessive ingestion of fluoride are fairly well documented. Some studies have reported the effects of fluoride on soft tissue (1), but there is a paucity of data on reproductive organs, a topic which is rather controversial (2,3). Chinoy and Sequeira (4-6)

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have reported that reproductive organs of mice were affected by sodium fluoride (NaF) ingestion (10 and 20 mg/kg body weight) for 30 days. A decrease in sperm density and sperm motility which occurred, led to loss of fertility. The sperm acrosomal integrity, morphology, and metabolism were altered in NaF-treated mice. However, fluoride-induced changes were transient and reversible after 2 months of withdrawal of treatment. A microdose of NaF when directly injected, in retrograde direction, into the distal vas deferens of rats also caused alterations in structure of reproductive organs and their metabolism as well as reduction in fertility (7). The present study was designed to investigate the effects of fluoride on the metabolism of cauda epididymal spermatozoa of rabbits in light of earlier data. The studies on the reversibility of induced effects by NaF withdrawal, vitamin C or calcium ingestion and combined vitamin C + calcium administration during the withdrawal period were also investigated.

Materials and Methods

Adult rabbits (*Oryctolagus cuniculus*) weighing between 1 and 1.8 kg were divided into seven groups (Table 1). The first group was given the control diet. The next three groups of animals were fed sodium fluoride (NaF) orally at doses of 20 and 40 mg/kg body weight/day respectively for 30 days. At the end of the treatment period, epididymal fluid was collected by micro-puncture technique. It was used in studying the microenvironment of epididymis (8). After appropriate dilution of the luminal contents, the sample was centrifuged to separate luminal fluid and sperm suspension; they were utilized for requisite biochemical analysis. The same animals were utilized for withdrawal

Table 1
Experimental Protocol

Group	Treatment	Duration (days)	Day of Necropsy*	No. of Animals
I	Control	30	Along with treated	5
II	NaF 20 mg/kg B.W./day/animal	30	31	5
III	NaF 40 mg/kg B.W./day/animal	30	31	5
IV	NaF 40 mg/kg B.W./day/animal then withdrawal for 1 month	30	31	5
V	NaF then withdrawal 1 month then vitamin C 100 mg/kg B.W./day/animal	30	31	5
VI	NaF then withdrawal 1 month + Ca 125 mg/day/animal	30	31	5
V	NaF then withdrawal 1 month + Vitamin C 100 mg/kg B.W. + Ca 125 mg/day/animal	30	31	5

* From beginning of withdrawal period.

studies. NaF treatment was discontinued for 30 days to Group IV; to Group V rabbits ascorbic acid (AA) at the dose of 100 mg/kg body weight and to another group (Group VI) osteocalcium tablets containing 125 mg calcium were administered. To Group VII, combined AA + Ca^{+2} was administered for 30 days during the withdrawal period. At the end of 30 days the cauda epididymal fluid was collected and subjected to further procedure as in the case of control and NaF-treated animals.

The percent sperm motility and sperm count of the epididymis from control and all treated and withdrawal groups of rabbits were determined by means of a Neubauer chamber of the Haemocytometer according to the method of Prasad *et al.* (9) and expressed as percentages and millions/mL, respectively. The acrosomal integrity of the sperm from the cauda epididymis of control, treated, and withdrawal groups was studied using the modified silver nitrate technique (10).

The following parameters were investigated in sperm suspension of all the experimental groups:

- The enzyme adenosine triphosphatase (ATPase) activity was assayed following the method of Quinn and White (11). The enzyme ATPase hydrolyses the substrate ATP into adenosine diphosphate and inorganic phosphate (ip). The ip formed at the end of the incubation was assayed to determine the rate of the reaction.
- The activity of succinate dehydrogenase (SDH) was determined by the method of Beatty *et al.* (12) and expressed as μg formazan/mL/30 minutes.
- The acid phosphatase (ACP) activity was assayed by the method of Bessey *et al.* (13) and was expressed as μ moles of p-nitrophenol liberated/mL/30 minutes.
- The protein content was determined by the method of Lowry *et al.* (14) and expressed as μg of protein/mL of sperm suspension.
- The sodium and potassium contents in the epididymal spermatozoa were estimated by the Systronics Flame Photometer, Digital Unit type 125, according to the method of Dean (15).

Results

Body Weight: Body weight decreased after NaF treatment in comparison to the controls. Recovery occurred throughout the withdrawal period in rabbits of Groups V to VII only. No recovery occurred in Group IV animals from which NaF treatment was withdrawn for one month. However, recovery was significantly greater with vitamin C (AA) supplementation than with calcium. Body weight recovered significantly with the administration of vitamin C and calcium together (Group VII) during the recovery period (Table 2).

Sperm Motility: The percent of cauda epididymal sperm motility decreased significantly ($p < 0.001$) in a dose related pattern, after NaF treatment compared to control (Table 2). Sperms were sluggishly motile. Head to head agglutination and deflagellation was also observed.

Sperm motility was recovered by vitamin C as well as by calcium ingestion. However, recovery was more significant by vitamin C than by calcium. Moreover, the synergistic effect of vitamin C and calcium was manifested to enhance sperm motility during the recovery period (Group VII) (Table 2). No recovery was observed in Group IV, i.e. withdrawal of NaF.

Sperm Count: The cauda epididymal sperm count of treated rabbits decreased with both doses of NaF compared to the controls. The sperm count recovered more by vitamin C in comparison to calcium treatment (Table 2). The sperm count further recovered almost to control level by the combined treatment with vitamin C and calcium (Table 2), but no recovery occurred by withdrawal of treatment alone.

Fertility Rate: The fertility rate was 95% positive in control rabbits. However, after administration of NaF (both doses), it was reduced to 33% and 0% respectively ($p < 0.001$) compared to the controls. Combined treatment with vitamin C and calcium during the withdrawal period resulted in significant recovery of fertility compared with the individual treatments of vitamin C or calcium but not by withdrawal alone (Table 2).

Adenosine Triphosphatase (ATPase): Significantly decreased ($p < 0.001$) spermatzoal ATPase activity was observed due to the fluoride treatment. However, administration of vitamin C and calcium in combination, caused significant recovery. Moreover, vitamin C alone had more effect than that of calcium in recovery of the enzymic activity, which failed to recover by NaF withdrawal alone (Table 3).

Table 2

Showing Body Weight, Sperm Motility, Sperm Count and Fertility Rate of Control, NaF Treated, NaF Withdrawal, and Vitamin C, Calcium, and Vitamin C + Calcium to NaF Withdrawal Groups of Rabbits.

Group	Treatment	Body wt. (kg)	Sperm Motility (%)	Sperm Count (10^6 /mL)	Fertility Rate (%)
I	Control	1.7 \pm 0.1	77 \pm 1.6	49 \pm 0.2	95-100
II	NaF 20 mg/kg B.W.	1.3 \pm 0.3	37 \pm 1.8*	36 \pm 1.2*	33*
III	NaF 40 mg/kg B.W.	1.1 \pm 0.3	27 \pm 2.0*	34 \pm 1.3*	0*
IV	NaF 40 mg/kg B.W. withdrawal 1 mo.	1.2 \pm 0.5	33 \pm 1.5	37 \pm 0.7	0*
V	NaF, Withdrawal, + Vitamin C	1.4 \pm 0.1	66 \pm 0.6	46 \pm 0.9	75*
VI	NaF, Withdrawal, Ca	1.3 \pm 0.1	49 \pm 1.3	40 \pm 0.2	67*
VII	NaF, Withdrawal + Vitamin C + Ca	1.8 \pm 0.1	72 \pm 1.1	48 \pm 0.9*	95-100

Values are Mean \pm S.E.

* $p < 0.001$

Table 3

Showing Adenosine Triphosphate (ATPase), Succinate Dehydrogenase (SDH), Acid Phosphate (ACP) and Protein Concentration in Spermatozoa of Control, NaF Treated, and NaF Withdrawal and of Vitamin C, Calcium and Vitamin C + Calcium to NaF Withdrawal Groups of Rabbits.

Group	Treatment	ATPase (μ mol ip/ mL/30')	SDH (μ g formazan/ mL/30')	ACP (μ mol p- nitrophenol/ mL/30')	Protein (μ g/mL)
I	Control	5.3 \pm 0.2	208 \pm 6.3	0.7 \pm 0.04	484 \pm 13
II	NaF 20 mg/kg B.W.	3.0 \pm 0.1*	112 \pm 3.7*	0.3 \pm 0.03*	303 \pm 16*
III	NaF 40 mg/kg B.W.	2.1 \pm 0.2*	78 \pm 1.3*	0.4 \pm 0.05*	286 \pm 7*
IV	NaF 40 mg, withdrawal 1 mo.	2.7 \pm 0.1	108 \pm 3.1	0.3 \pm 0.01	318 \pm 21
V	NaF, Withdrawal + Vitamin C	4.9 \pm 0.3	187 \pm 7.7	0.6 \pm 0.004	417 \pm 18
VI	NaF, Withdrawal + Calcium	3.1 \pm 0.2	131 \pm 10	0.4 \pm 0.02	386 \pm 17
VI	NaF, Withdrawal + Vitamin C + Ca	5.2 \pm 0.2*	201 \pm 3.1*	0.6 \pm 0.06*	477 \pm 10*

Values are Mean \pm S.E.

* $p < 0.001$

Succinate Dehydrogenase (SDH): Succinate dehydrogenase activity in the spermatozoa decreased significantly in treated rabbits compared to controls ($p < 0.001$). The decrease was dose dependent. During the recovery period, SDH activity recovered more by vitamin C than by calcium. Further, the synergistic therapeutic effect of vitamin C and calcium was greater in combination than the individual treatments or by NaF withdrawal alone (Table 3).

Acid Phosphatase (ACP): NaF treatment with both doses caused a significant decrease in acid phosphatase activity in spermatozoa. Administration of combined treatment of vitamin C and calcium resulted in recovery which was more significant than the individual treatments of vitamin C or calcium (Table 3). NaF withdrawal alone did not cause recovery of enzymic activity.

Protein: The protein concentration of spermatozoa also showed a significant dose-dependent decline ($p < 0.001$) after fluoride treatment. During the recovery period the protein concentration was enhanced by the combined administration of vitamin C and calcium. Moreover, the effect of vitamin C was more pronounced than that of calcium alone. However, NaF withdrawal (1 month) was not conducive to recovery (Table 3).

Na⁺ and K⁺ Levels: Na⁺ levels in the spermatozoa were significantly reduced throughout the treatment compared to the controls ($p < 0.001$) (Table 4). K⁺ levels in spermatozoa were also decreased significantly ($p < 0.001$) after

Table 4

Showing Na⁺ and K⁺ Levels in Spermatozoa of Control, NaF Treated, NaF Withdrawal, Vitamin C, Calcium and Vitamin C + Calcium to NaF Withdrawal Groups of Rabbits

Group	Treatment	Na ⁺ (ppm)	K ⁺ (ppm)
I	Control	144 ±5.3	162 ±4
II	NaF 20 mg/kg B.W.	123 ±2.6*	141 ±2
III	NaF 40 mg/kg B.W.	97 ±4.5*	96 ±5
IV	NaF 40 mg/kg B.W. withdrawal 1 mo.	100 ±3.5	116 ±5
V	NaF, Withdrawal + Vitamin C	121 ±3.3	129 ±3
VI	NaF, Withdrawal + Ca	102 ±3.9	147 ±3
VII	NaF, Withdrawal, + Vitamin C + Ca	139 ±6.3*	152 ±3*

Values are Mean ±S.E.

* p < 0.001

NaF treatment. During the withdrawal period both the treatments with vitamin C as well as with calcium administered individually had a significant effect on the recovery of Na⁺ and K⁺ levels of the spermatozoa. However, calcium produced a better recovery in the K⁺ levels than did vitamin C (Table 4). The combined treatment by vitamin C and calcium manifested a synergistic effect for recovery of both Na⁺ and K⁺ in sperm (Table 4). On the other hand, NaF withdrawal alone did not cause recovery.

Morphology of Spermatozoa - Silver-Nitrate Staining Technique: The silver-nitrate staining of cauda epididymal spermatozoa of rabbit revealed a clear differentiation of the acrosomal, post acrosomal and mid-piece regions (Figure 1). But in rabbits administered NaF, and staining was diffused with no proper demarcations. Deflagellation and agglutination of spermatozoa was also observed due to the effect of NaF (Figures 2 and 3). In withdrawal Group V and VI silver-nitrate staining

Figure 1

Cauda Epididymal Spermatozoa of Normal Rabbit Stained with Silver Nitrate Showing Clear Differentiation of Acrosomal and Post Acrosomal Regions. Note Intact Acrosomal Cap. x1735.

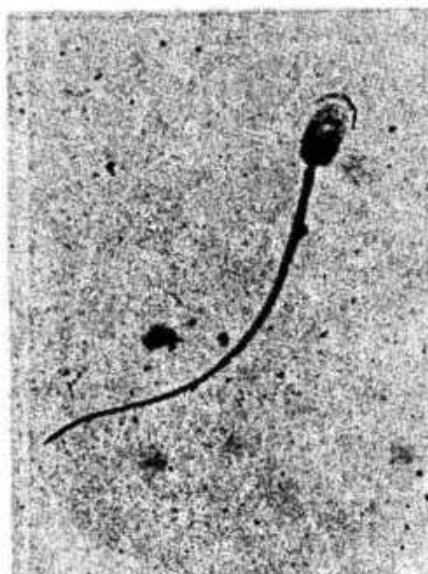


Figure 2

Cauda Epididymal Spermatozoa of Rabbit Treated with NaF 20 mg/kg Body Weight. Note Poor Differentiation of Acrosomal Region, Deflagellated Spermatazoa and Coiling of the Tail. x1450.

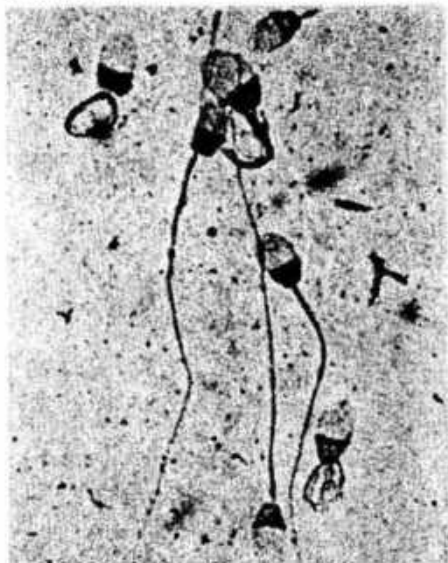


Figure 3

Silver-Nitrate Staining Showing Agglutination of Spermatozoa of Cauda Epididymis of Rabbit Treated with NaF 40 mg/kg Body Weight. x1450.



revealed spermatozoa which appeared quite normal (Figures 4 and 5).

Discussion

The present study was undertaken to investigate the effects of sodium fluoride (NaF) at a low and high dose (20 and 40 mg/kg body weight) on cauda epididymal spermatozoa of rabbits as well as to compare the extent of recovery after NaF withdrawal and the effects of vitamin C (AA) or calcium (Ca^{+2}) ingestion during the withdrawal period. The data revealed that NaF treatment resulted in a decrease in body weight as reported by others (16).

Fluoride ion is an inhibitor that has been extensively used in sperm metabolic studies. Schoff and Lardy (17) have demonstrated the effect of fluoride and caffeine on the metabolism and motility of ejaculated bull sperm. The sperm motility, glycolysis, and respiration could be altered by fluoride. Sperm treated with 30 mM fluoride became immobile within 2 minutes and the flagella assumed a linear, rod-like conformation. In the present study too, the motility of cauda epididymal spermatozoa decreased significantly in NaF-treated rabbits. Cauda epididymal sperm-ATPase activity was significantly affected throughout the NaF treatment. It has been reported that fluoride

Figure 4

Rabbit Cauda Epididymal Spermatozoa Treated with Vitamin C during Withdrawal Period. Clear Differentiation of Acrosomal and Post-Acrosomal Regions Are Observed Similar to Normal. x1650.

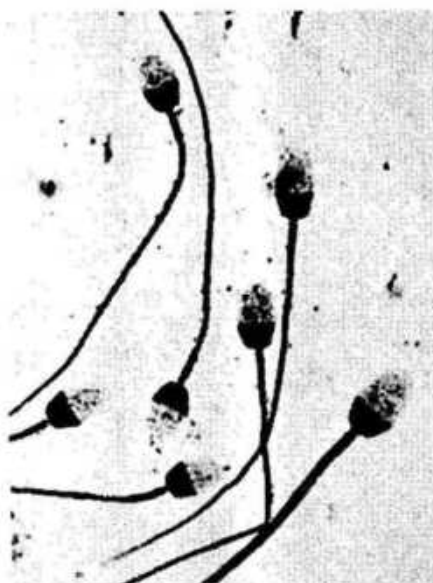


Figure 5

Cauda Epididymal Spermatozoa of Rabbit Treated with Calcium during Withdrawal Period Showing Recovery. x1650.



could directly inhibit the dynein ATPase in cilia (18), or else its decrease might be due to some structural alterations in the mitochondria in the mid-piece region, thereby causing a decrease in sperm motility. Therefore, in the future, ultrastructural studies of the sperm are necessary in NaF-treated animals.

The activity of succinate dehydrogenase (SDH), a mitochondrial oxidative enzyme also decreased in cauda epididymal spermatozoa after NaF treatment. It is likely that the mitochondrial structure and/or metabolism may have been affected, as mentioned above. Similar results were also obtained in cauda epididymal spermatozoa of mice (6) treated with NaF.

Underwood (19) has reviewed enzyme changes in chronic fluorotic animals. Fluoride has been used to block protein phosphates and thereby to "freeze" the phosphorylation states of protein in sperm homogenates. In the present study, activity of acid phosphatase in spermatozoa was reduced significantly throughout the treatment. NaF toxicity involves inhibition of spermatozoa was reduced significantly throughout the treatment. NaF toxicity involves inhibition of enzyme activities, particularly those in which divalent metal cations act as cofactors (20). Hence it is probable that the alterations in ATPase, SDH, and ACP in spermatozoa might be due to the fact that they are either Mg^{+2} , Ca^{+2} , or Zn^{+2} metallo-proteins.

Epididymal proteins are important as sperm antigens and for sperm viability (21). Therefore, a change in sperm protein might alter their motility and fertilizability as in the present study. According to McIvor *et al.* (22), fluoride is known to produce a marked K^+ efflux from intact cells. In the present study also, a significant decrease in the Na^+ and K^+ levels of the sperm was observed. This decrease might be due to the active K^+ efflux, disturbing the electrolyte balance and thereby reducing sperm motility. The above-mentioned alterations in sperm motility and metabolism might be the outcome of altered and hostile internal milieu of the epididymis of NaF-treated rabbits, since it is known that normal epididymal structure and its internal microenvironment are important for sperm maturation and for maintaining them in a viable, motile state (21,23). The present findings are in agreement with those of others (4,5). The reduction in sperm motility, sperm count and changes in their metabolism led to the significant decline in fertility of treated rabbits. Similar loss of fertility in NaF-treated male mice and rats has also been reported (5). Another factor leading to reduction of fertility may be due to the large number of deflagellated spermatozoa as well as their acrosomal, mid-piece and tail abnormalities, as observed by the modified silver-nitrate staining technique (10).

The withdrawal-of-treatment groups studied were: Withdrawal of NaF treatment alone for a month (Group IV), Withdrawal of treatment as in Group IV but fed vitamin C (Group V), and in Group VI calcium was fed during the withdrawal period. In Group IV virtually none of the induced effects was recovered with withdrawal of treatment. However, significant recovery was more pronounced with ascorbic acid than with calcium. It is known that ascorbic acid and its free radical monodehydroascorbic acid (MDHA) was potent reducing agents which activate several oxido-reduction reactions in tissues and have beneficial effects in drug-treated animals (24). Yu and Hwang (25) have also reported that high intake of vitamin C mitigated the effects of fluoride in mice.

The therapeutic effects of calcium against fluoride have also been long known. Narasinga Rao *et al.* (26) have studied calcium turnover in endemic fluorosis and reported its mitigating influence after calcium ingestion in fluoride poisoning in rats. Calcium has an important role to play in epididymal and vas deferens smooth muscle contraction (27), permeability of cell membranes and capillaries, sperm motility, interaction with cAMP (28), and activation of enzymes like APTase and succinate dehydrogenase. Hence, in the present study, when calcium was administered to rabbits during the withdrawal period, significant recovery in most of the induced effects was observed.

In Group VII animals, combined treatment of ascorbic acid + Ca^{+2} was found to result in greater recovery of all parameters affected by NaF than by individual administration of AA or Ca^{+2} . The recovery in the activities of ATPase and SDH is important, since these enzymes are involved in energy-releasing reactions and in oxidative metabolic processes of the sperm. Acid phosphatase is a lysosomal enzyme that has been in association with human acrosome. Recovery in this enzyme as well as protein concentration and Na^+ and K^+ levels of the spermatozoa caused normalization in their structure and functions.

It was observed that rats treated with NaF + AA or NaF + Ca^{+2} for 30 days also revealed recovery in several parameters of reproductive and

non-reproductive tissues compared to those treated with NaF alone (29,30). It is therefore suggested that the effects of administration of combination of ascorbic acid and calcium might be highly beneficial in the recovery of fluoride-treated animals. This has a direct bearing on ameliorating the sufferings of fluorosis-afflicted humans also, since they are incapable of synthesizing vitamin C. Such studies are now underway.

Acknowledgement

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References

1. Susheela, A.K.: **Fluoride Toxicity**. Proceedings of the 13th Conference of the International Society for Fluoride Research. November 13-17, 1983, ISFR, New Delhi, 1985.
2. Tao, S. and Suttie, J.W.: Evidence for a Lack of an Effect of Dietary Fluoride Level on Reproduction in Mice. **J. Nutr.**, 106:1115-1122, 1976.
3. Messer, H.H., Armstrong, W.D. and Singer, L.: Influence of Fluoride Intake on Reproduction in Mice. **J. Nutr.**, 103:1319-1326, 1973.
4. Chinoy, N.J. and Sequeira, E.: Effects of Fluoride on the Histoarchitecture on Reproductive Organs of the Male Mouse. **Reprod. Toxicol.**, 3:261-268, 1989.
5. Chinoy, N.J. and Sequeira, E.: Reversible Fluoride Induced Fertility Impairment in Male Mice. **Fluoride**, [in press].
6. Chinoy, N.J. and Sequeira, E.: Fluoride Induced Biochemical Changes in Reproductive Organs of Male Mice. **Fluoride**, 22:78-85, 1989.
7. Chinoy, N.J., Rao, M.V., Narayana, M.V. and Keelakanta, E.: Microdose Vasa Injection of Sodium Fluoride in the Rat. **Reprod. Toxicol.**, [Comm.], 1990.
8. Chinoy, N.J., Rao, M.V. and Verma, R.J.: Micropuncture Techniques in Studying Microenvironment of Epididymis. **Indian J. Med. Res.**, 86:410-411, 1987.
9. Presad, M.R.N., Chinoy, N.J. and Kadam, K.M.: Changes in Succinic Dehydrogenase Levels in Rat Epididymis under Normal and Altered Physiological Conditions. **Fertil. Steril.**, 23:186-190, 1972.
10. Chinoy, N.J., Ranga, G.M., Highland, H.N. D'Souza, K.J. and Sequeira, E.: A Modified Method for the Differential Staining of Spermatozoa Using Alcoholic Acidic Silver Nitrate. **Int. J. Fertil.** [in press], 1990.
11. Quinn, P.J. and White, I.G.: Distribution of Adenosine Phosphatase Activity in Ram and Bull Spermatozoa. **J. Reprod. Fertil.**, 15:449-452, 1968.
12. Beatty, C.H., Basinger, G.M., Dully, C.C. and Bocek, R.M.: Comparison of Red and White Voluntary Skeletal Muscle of Several Species of Primates. **J. Histochem. Cytochem.**, 14:590-600, 1966.
13. Bessey, O.A., Lowry, O.H. and Brock, M.J.: A Method for the Rapid Determination of Acid and Alkaline Phosphatase with 5 Cubic mm of Serum. **J. Biol. Chem.**, 164:321-329, 1946.
14. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.: Protein Measurement with Folin Phenol Reagent. **J. of Biochemistry**, 193:265-275, 1951.
15. Dean, N.A.: **Flame Photometry**. McGraw-Hill Book Co., Inc., 1960, p. 295.

16. Saralukumari, D., Varadacharyulu, N.Ch. and Ramakrishna Rao, P.: Effect of Fluoride Toxicity on Growth and Lipids in Liver, Kidney and Serum in Rats. **Arogya - J. Health Sci.**, 14:24-29, 1988.
17. Schoff, P.K. and Lardy, H.A.: Effects of Fluoride and Caffeine on the Metabolism and Motility of Ejaculated Bovine Spermatozoa. **Biology of Reproduction**, 1037-1046, 1987.
18. Blum, J.J. and Hayes, A.: Specific Anion Effects on ATPase Activity, Calmodulin Sensitivity and Solubilization of Dynein ATPase. **J. Cell Biochem.**, 25:197-212, 1984.
19. Underwood, E.J., (Ed.): **In: Trace Elements in Human and Animal Nutrition**. Academic Press, New York, Sanfrancisco, London, 1977.
20. Hodge, H.C. and Smith, F.A.: **Fluoride in Metallic Contaminants and Human Health**. Lee, D.H.K. (Ed.), Academic Press, New York, 1972, p. 163.
21. Chinoy, N.J.: Structure and Function of Epididymis in Relation to Vulnerable Points of Intervention for Male Fertility Regulation. **Indian Review of Life Sciences**, 4:37-68, 1984.
22. McIvor, M.E., Cummings, C.C., Mower, M.M., Baltazar, R.F., Wenk, R.E., Lustgarten, J.A. and Solomon, J.: The Manipulation of Potassium Efflux during Fluoride Intoxication: Implication for Therapy. **Toxicology**, 37:233-239, 1985.
23. Prasad, M.R.N. and Rajalakshmi, M.: Recent Advances in the Control of Male Reproductive Functions. **In: Greep, R.O. (Ed.): International Reviews of Physiology, Reproductive Physiology II**. University Park Press, Baltimore, MD, USA, 13:153-199, 1977.
24. Chinoy, N.J.: Ascorbic Acid Turnover in Animals and Human Tissue. **J. Anim. Morphol. Physiol.**, Silver Jubilee Vol.: 68-85, 1978.
25. Yu, M.H. and Hwang, H.L.S.: Influence of Protein and Ascorbic Acid on Fluoride-induced Changes in Blood Composition and Skeletal Fluoride Deposition in Mice. **In: Tsunoda, H. and Yu, M.H. (Eds.): Fluoride Research, 1985, Studies in Environmental Science**. Vol. 27., Elsevier/Amsterdam, p. 203-210.
26. Narasinga Rao, B.S., Krishnamachari, K.A.V.R. and Vijayasathy, C.: ^{45}Ca Turnover in Endemic Fluorosis and Endemic Genu Valgum. **Brit. J. Nutr.**, 41:7-14, 1979.
27. Chinoy, N.J. and Chinoy, M.R.: Differential Contractile Response of Normal Vas Deferens in Rodents in Correlation to Their Calcium and Electrolite Levels. **J. Biosci.**, 5:173-180, 1983.
28. Rasmussen, H.: The Cycling of Calcium as an Intracellular Messenger. **Scientific American**, October: 66-73, 1989.
29. Sharma, M.: **Effects of Ascorbic Acid and Calcium on Some Vital Organs of Sodium Fluoride Ingested Male Rats**. M. Phil. Dissertation, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India, 1990.
30. Reddy, V.V.V.S.N.G.L.P.C.: **Effects of Ascorbic Acid and Calcium on Reproductive Functions of Sodium Fluoride Ingested Prepubertal Male Rats**. M. Phil. Dissertation, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India, 1990.

FLUORIDE LEVEL IN CATARACT LENSES IN AN URBAN AREA OF INDIA

by

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SUMMARY: Fluoride levels have been determined in ten samples of lenses with cataracts obtained from patients aged 39-73 yrs. The highest fluoride level was found in the patient 73 yrs old. Patients from 55-60 yrs showed the presence of more than 2% of fluoride in cataracts. Fluoride was undetectable in apparently cataract-free lenses obtained from deceased persons. The level of fluoride in drinking water supplied through wells and river in the area of the study was 1.2-2.4 mg/L, or 2.8 mg/L, respectively

KEY WORDS: Cataracts; Fluoride in cataract lenses; Fluoride in drinking water; Lenses.

Introduction

Cataracts, one of the major causes of blindness throughout the world, are said to be responsible for nearly 40% of blindness in India. As many as 1.2 million people must have surgery for cataracts (1) every year at the world level. The human crystalline lens is completely transparent at birth. Throughout life, it is susceptible to a variety of noxious influences that affect its clarity. The term "cataract" is used to describe any opacity of the crystalline lens. The main symptom is gradual failure of vision; distortion of vision, color changes and double vision may occasionally occur. The main sign is a greyish white opacity in the pupil (2). The most common cataract is the senile variety. It usually begins after age 50 and consists of cortical, nuclear or posterior, subcapsular opacities, three forms which frequently coexist in various combinations (3).

The causative factors which vary in nature are occupational conditions of persons, accidental entry of foreign bodies into the eyes, exposure to radiation such as ultra-violet light, X-rays, gamma rays, and intense heat (4-9). A variety of substances (naphthalene, iodoacetic acid, thallium, dinitrophenol, triparanol, etc.) have produced cataracts when administered clinically (10). Today it is recognized that the prevalence of cataracts in the Punjab and other north-western parts of the Indian sub-continent is very high. The scourge of cataracts is not equally distributed over India; its distribution seems to be related to environmental factors. The day-heat in the plains forces farm workers to drink copious volumes of water which have a high mineral content. Fluorosis has been identified as another disease of the plains of the north-western areas of the sub-continent (11), suggesting that the same environmental factors may contribute to the incidence both of fluorosis and cataracts.

Waldbott (12) described fluoride as a systemic poison, liable to reach any part of the body. The same author also recorded 77.3 ppm fluoride in a

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cataract lens (13). Based on the above evidence, the fluoride level was determined in cataracts obtained from patients of Balaghat town, a district head-quarter in south Madhya Pradesh, India. The fluoride levels in well and river water, determined in a number of samples collected from the same area were 1.2-2.4 mg/L and 2.8 mg/L respectively (14). Linkage between fluoride in drinking water and cataract incidence in that area was investigated.

Materials and Methods

Sample Collection: Ten samples (9 from males and one from female) of cataracts from patients between 39 and 73 years of age were collected through an eye camp clinic of the area. The cataract patients were all farm workers by profession. Two samples were also collected of apparently cataract-free lenses of deceased persons.

Sample Preparation: The cataract lenses were washed with distilled water, dried at room temperature and weighed. A 5 g weighed quantity of fluoride-free calcium oxide and 10 mL of water were added to each lens. After thorough pulverization, the paste was transferred quantitatively to a pressure digestion bomb and heated in an oven at 180°C for 4 hrs. The contents, after cooling, were transferred to a platinum crucible, heated first slowly and then at 500°C for 30 minutes to decompose the tissues.

Table 1
Fluoride Levels in Cataract Lenses

No.	Sex/Age of patients	Weight of lenses (mg)	Weight of Fluoride found (mg)	Concentration of Fluoride in Lenses (%)
1.	M 65	88.8	2.50	2.81
2.	M 40	167.3	1.00	0.59
3.	M 50	157.4	2.50	1.59
4.	F 55	162.3	3.25	2.00
5.	M 60	159.2	3.25	2.04
6.	M 73	115.0	5.75	5.00
7.	M 57	147.0	3.00	2.04
8.	M 39	120.5	2.50	2.07
9.	M 60	130.0	5.25	4.04
10.	M 59	149.0	3.00	2.01
11.	M 40* (w/o cataract)	147.4	Nil	Nil
12.	M 55* (w/o cataract)	150.0	Nil	Nil

* Samples from deceased persons; "Nil" denotes undetectable

Fluoride Determination: The treated samples were transferred to a distillation flask, mixed with concentrated H_2SO_4 (50 mL), distilled water (50 mL), and Ag_2SO_4 (200 mg), and distilled at $180^\circ C$ (15). The distillate was made up to 250 mL with distilled water. An aliquot (5 mL) of distillate was treated with acid-zirconyl-alizarin reagent, and the absorbance was measured at 525 nm (15). A calibration graph was prepared with standard solution of anhydrous sodium fluoride (analytical grade), after being subjected to similar treatment. A blank run was also made. The results are shown in Table 1.

Results and Discussion

The results obtained (Table 1) show that fluoride was present at significant levels from about 0.5% to 5.0% of the weight of the cataract lens, whereas it was undetectable in apparently cataract-free lenses (obtained from deceased persons). The data show that the highest level of fluoride occurred in the oldest patient, aged 73 yrs. Except in one case of 39 yrs, fluoride at a level of 2% or more was found in patients between ages 55 to 65 yrs. It can thus be inferred that, under the environmental conditions prevailing in the area of study, fluoride is one of the causative factors in the incidence of senile cataract.

Acknowledgement

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References

1. Randall, J.O.: Occupational Eye Disorder. In: Rom, William N., Ed.: **Environmental and Occupational Medicine**, Little, Brown and Co., Boston, 1983, p. 357-372.
2. Bisley, Geoffrey O.: **Handbook of Ophthalmology for Developing Countries**. The English Language Book Society and Oxford University Press, London, 1980, p. 68-76.
3. Gardner, Thomas W. and Schoch, David E.: **Handbook of Ophthalmology**, Appleton and Lange, Norwalk (Conn.)/Los Altos (Calif.), 1987, p. 139-147.
4. McCaslin, M.F.: Cataracts, **J. Occup. Med.**, 12:345, 1970.
5. Keeney, A.H.: Intralenticular Foreign Bodies. **Arch. Ophthalmol.**, 86:499, 1971.
6. Edbrooke, C.M. and Edwards, G.C.: Industrial Radiation Cataracts: The Hazards and Protective Measures. **Arch. Occup. Hyg.**, 10:293, 1976.
7. National Institute for Occupational Safety and Health: Criteria for a Recommended Standard. **Occupational Exposure to Ultraviolet Radiation**. DHEW Pub. No. (NIOSH) HSM 73-10009, U.S. Government Printing Office, Washington, D.C., 1972.
8. Littleton, J.T. Durizch, M.L. and Perry, N.: Radiation Protection and Users. **Radiology**, 129:795, 1978.
9. Pitts, D.G.: The Ocular Effects of Ultra Violet Radiation. **Am J. Optm. Physiol. Opt.**, 55:19, 1978.
10. Williams, F. Hughes: **Year Book of Ophthalmology**. Year Book Medical Publishers, Chicago, Ill., 1969, p. 172-189.
11. Singh, A., Jolly, S.S., Bansal, B.C. and Mathur, O.G.: Endemic Fluorosis: Epidemiological, Clinical and Biochemical Studies of Chronic Fluoride Intoxication in Punjab (India). **Medicine (Baltimore)**, 42:229, 1963.

12. Waldbott, G.L.: Symposium on the Non-skeletal Phase of Chronic Fluorosis, **Fluoride**, 9:7-10, 1976.
13. Waldbott, G.L.: The Physiologic and Hygienic Aspects of the Absorption of Inorganic Fluorides, **Arch. Environ. Health**, 2:155-167, 1961.
14. Shukla, N.S. and Pandey, G.S.: Study of Fluoride Occurrence in Ejected Human Teeth in a Selected Urban Area in India. [Private Communication].
15. APHA-AWWA-WPCF: **Standard Methods for the Examination of Water and Waste Water**. 14th Edition, 1975.

FDA COMMITTEE SPURNS FLUORIDE

by

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(Medical World News, November 13, 1989, p. 25)

NIH-funded teams found that fluoride does not prevent fractures according to the chairman of an FDA advisory committee that reviewed fluoride's effect on fracture incidence

Dr. Saul Genuth, director of the radioimmunoassay laboratory at Cleveland's Mt. Sinai Hospital, regretfully joined in the endocrinologic and metabolic drugs advisory committee's unanimous opinion that fluoride has yet to prove its worth in the treatment of postmenopausal osteoporosis.

The FDA committee based its opinion on the recently completed NIH-sponsored trial of long-term, immediate-release sodium fluoride. The results of the study, reported earlier this fall at a bone research meeting in Montreal, were presented to the committee by Dr. Michael Kleerekoper, head of the bone and mineral division of Henry Ford Hospital in Detroit, and Dr. L. Joseph Melton III, head of clinical epidemiology at the Mayo Clinic in Rochester, Minnesota.

Although bone mass increased significantly, the teams found no statistically significant differences in vertebral fracture rates. Moreover, more nonvertebral skeletal lesions were observed in the fluoride-treated patients, as well as a higher incidence of painful lower extremity syndrome. The advisory committee, referring to a French team's findings published in *The Lancet* last year, recalled that between months 18 and 24 the percentage of patients sustaining new fractures was the same — 18% in the fluoride-treated group and 17% in the nonfluoride group — suggesting that the benefit diminished over time.

Dr. Baylink, a professor of medicine at Loma Linda (California) University, called the lack of a relation between bone density and fracture frequency "one of the most disturbing aspects" of the NIH data. It had not been available when his group acted.

KEY WORDS: Bone density; Bone fractures; Fluoride therapy; Osteoporosis.

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GENOTOXIC EFFECTS OF FLUORIDE AND IMPLICATIONS FOR ITS USE IN THE TREATMENT OF OSTEOPOROSIS

by

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(Abstracted from *New Zealand Medical Journal*, 101:213, 1988)

Recent publications have suggested that fluoride may be genotoxic at concentrations between 10 $\mu\text{g/mL}$ and 300 $\mu\text{g/mL}$ F. Fluoride can accumulate in bone, particularly during therapy with medium to high daily doses of fluoride. Cells which resorb bone could be exposed to significant concentrations of fluoride in extracellular bone fluid during resorptive and remodelling processes. To assume that potentially genotoxic concentrations of fluoride can arise in extracellular bone fluid especially in patients undergoing fluoride therapy for osteoporosis may be premature. However one report in the literature showed that three elderly osteoporotic patients, who received 16-150 mg NaF daily for 1 to 36 months, developed giant monocytoïd cells, suggestive of reticuloendothelial malignancy in the bone marrow. After discontinuation of therapy these abnormal cell growths disappeared.

KEY WORDS: Bone malignancy; Fluoride therapy; Genotoxicity of F^- ; Osteoporosis therapy.

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GENOTOXIC EVALUATION OF CHRONIC FLUORIDE EXPOSURE: SISTER-CHROMATID EXCHANGE STUDY

by

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(Abstracted from *Mutat. Res.*, 227:159-165, 1989)

This study was conducted to examine the genotoxic effects of chronic exposure to sodium fluoride (NaF) in drinking water on the frequency of sister-chromatic exchange (SCE) in the bone-marrow cells of male Chinese hamsters. Animals at about 3 weeks of age, randomly assigned to 6 groups, each with at least 3 hamsters, were maintained on a low fluoride diet (less than 0.2 ppm F) throughout the experiment. At 4 weeks of age the animals in Groups I-V began to receive drinking water containing fluoride at concentrations of 0, 1, 10, 50, and 75 ppm, respectively. Group VI was treated with cyclophosphalide and served as the positive control. The animals were sacrificed at 24 weeks of age by cervical dislocation; fluoride in humeri and plasma had increased with the increase in fluoride concentration in drinking water.

Slides of chromosomes from bone-marrow cells were prepared and blindly examined for the frequency of SCE. The mean scores of SCE for the hamsters receiving drinking water containing F concentrations up to 75 ppm for 21 weeks, ranged from 4.28 to 6.28 per cell. They were not significantly different from those of the negative controls (4.6-5.44/cell). Thus chronic fluoride exposure had no effect on the frequency of SCE in Chinese hamster bone-marrow cells under the conditions of the present investigation.

KEY WORDS: Chinese hamster; Genotoxic evaluation; Sister chromated exchange.

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IS FLUORIDATION A FRAUD?

by

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(Abstracted from *Sci. Total Environ.*, 76:167-184, 1988)

During the past 15 years tooth decay rates have declined markedly amongst children in many parts of the industrialized world. Over the same period, however, decay rates have increased dramatically in children in developing countries. For many years it has been claimed that water fluoridation is the most important and cost-effective method for controlling dental caries. A series of recent papers in the scientific literature have challenged the "fluoridation hypothesis", since the decline in decay reported in developed countries has occurred in both fluoridated and unfluoridated areas.

In some countries of the developing world, tooth decay is now reaching crisis proportions. Hence it is important to know whether fluoridation really can "reduce tooth decay by about 60 percent", or if the dental profession has been promoting a "flawed" hypothesis for more than 40 years.

KEY WORDS: Dental caries decline; "Flawed" hypothesis; Fluoridation.

REPRINTS: G.E. Smith, 56 Surrey Road, South Yarra, Melbourne 3141, Victoria, Australia.

FLUORIDE-INDUCED CHANGES IN THE TOOTH GLYCOSAMINOGLYCANS:
AN IN VIVO STUDY IN THE RABBIT

by

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(Abstracted from *Arch. Toxicol.*, 62:328-330, 1988)

In rabbits administered 10 mg NaF/kg body weight orally at 24 hr intervals for 9 months, tooth matrix glycosaminoglycans was significantly reduced compared to normal teeth. Furthermore, Sephadex G-75 chromatography and DEAE-cellulose ion exchange chromatography revealed the presence of small molecular weight glycosaminoglycans molecules and an increase in the charge density heterogeneity in the sulphated glycosaminoglycans of the fluoride-treated rabbit tooth compared to controls. These changes may be related to the dedifferentiated tooth matrix and an increase in the dermatan sulphate content in the fluoride-treated tooth matrix reported earlier.

KEY WORDS: Glycosaminoglycans changes; Rabbits; Sodium fluoride; Tooth matrix.

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EFFICACY OF LONG-TERM FLUORIDE AND CALCIUM THERAPY
IN CORRECTING THE DEFICIT OF SPINAL BONE
DENSITY IN OSTEOPOROSIS

by

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(Abstracted from *J. Clin. Epidemiol.*, 42:1067-1074, 1989)

To determine whether long-term fluoride therapy for osteoporosis increases the thickness of vertebral trabeculae as seen on spinal radiographs, quantitative computed tomography was utilized to measure trabecular vertebral body density (TVBD), in the lumbar spine of 18 female osteoporotic patients, all of whom had been treated with sodium fluoride, 77 ± 13 mg/day (mean \pm S.D.), and calcium, 1000 mg/day, for 57 ± 24 month. TVBD in these fluoride-treated osteoporotic patients (132 ± 82 mg/cm³) was found to be significantly greater than mean TVBD for an age-matched group of untreated female osteoporotic patients (51 ± 21 mg/cm³), $n = 89$, $p < 0.001$). The value for TVBD in the long-term fluoride-treated osteoporotics was not only similar to previously published values for TVBD (104 ± 30 mg/cm³) in normal females of similar

age, but was also above the calculated TVBD "fracture threshold" of 100 mg/cm³ for females.

Only one of the 18 fluoride-treated osteoporotics continued to have spinal fractures during therapy, accounting for 4 fractures per 87.2 patient-years of observation, a value which is significantly lower than the published incidence of 76 fractures per 91 patient-years for untreated osteoporotic patients ($p < 0.001$). Together these findings indicate that long-term fluoride and calcium therapy for osteoporosis increases TVBD in the majority of patients within a reasonable time frame and significantly reduces the risk of spinal fractures.

KEY WORDS: Calcium and fluoride therapy; Osteoporosis treatment; Spinal bone fractures.

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RISK OF ENAMEL FLUOROSIS ASSOCIATED WITH
FLUORIDE SUPPLEMENTATION, INFANT FORMULA,
AND FLUORIDE DENTIFRICE USE

by

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(Abstracted from *Am. J. Epidemiol.*, 130:1190-1208, 1989)

A case-control study of the association between enamel fluorosis and exposure to fluoride supplements, infant formula, and/or fluoride dentifrice was carried out on 850 11- to 14-year-old residents of nonfluoridated communities in Massachusetts and Connecticut, born between 1972 and 1975. The effect of median household income, an indicator of socio-economic status, was also examined.

Risk factor exposure was assessed via a mailed questionnaire with a response rate of 80%. Mild-to-moderate enamel fluorosis was strongly associated with fluoride supplementation during the first six years of life (odds ratio = 4.0) and with median household income (odds ratio = 6.6). Subjects in the middle median household income group who had used fluoride supplements through the first six years of life had a 28-fold increase in risk of fluorosis compared with unexposed subjects in the lower median household income group. An odds ratio of 1.7 associated with infant formula use was suggestive of an increased risk of enamel fluorosis as was an odds ratio of 2.9 associated with fluoride dentifrice use.

A total of 680 or 80 percent of the questionnaires were returned completed after three mailing rounds. Response rates were similar regardless

of the case or control status of the subjects. A strong association between a history of exposure to fluoride supplementation and enamel fluorosis was observed. The combination of fluoride supplementation and median household income conferred a much higher risk of subjects' developing enamel fluorosis than would be expected by merely summing the odds ratios associated with exposure to each of these factors.

All cases in this study possessed fluorosis in the mild-to-moderate range; no severe fluorosis was observed. Even in its less severe forms, enamel fluorosis can be an esthetic problem to socially conscious preteens or teenagers and their parents.

The results of this study suggest a pressing need to identify the breadth and magnitude of enamel fluorosis in children of similar and later occurring birth cohorts and to identify appropriate modifications in the supplementation schedule.

KEY WORDS: Dental fluorosis; Enamel fluorosis; Fluoride dentifrice; Fluoride supplementation; Fluorosis risk; Infant formula; mottled enamel.

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DELETERIOUS EFFECT OF SODIUM FLUORIDE ON GASTROINTESTINAL TRACT

by

A. Fujii and T. Tamura
Chiba, Japan

(Abstracted from *Gen. Pharmacol.*, 20:705-710, 1989)

A single oral dose (300 mg/kg) of NaF caused blood flow rate in rat stomach mucosa to be only 30% of the initial rate during 30-60 min. Addition of NaF (final NaF concentration: 50 and 100 ppm) in vitro caused 10 to 28% reduction, respectively, of initial free calcium ion levels in rat blood. These results indicate that oral ingestion of excess amount of NaF caused dilation of blood vessels and greatly decreased blood flow rate to accumulate the circulating blood in the mucosa of gastrointestinal tract and cause redness.

KEY WORDS: Gastrointestinal tract; Rats; sodium fluoride; Stomach mucosa.

REPRINTS: Department of Pharmacology, Nihon University School of Dentistry, Chiba, Japan.

FLUORIDE'S FRACTURE AID DISPUTED

by

Annette Oestreicher

(Medical World News, October 23, 1989, p. 42)

In a major trial, use of fluoride therapy for osteoporosis failed to reduce vertebral fractures. This finding revealed at the first joint meeting for the International Conferences on Calcium Regulating Hormones and the American Society for Bone and Mineral Research, by Dr. B. Lawrence Riggs, professor of medical research at the Mayo Foundation and Mayo Clinic in Rochester, Minnesota, contradicted more optimistic results of an uncontrolled trial reported earlier this year. According to Dr. Riggs, a four-year double-blind trial at the Mayo Clinic and Henry Ford Hospital, Detroit, consisted of post-menopausal women, aged 51-75, all of whom had a history of at least one vertebral fracture. Of 202 women enrolled, 135 completed the study - 66 received sodium fluoride and 69 a placebo. Both groups received 1,500 mg/day of calcium carbonate during the study. The median dosage of fluoride given to the treated group by the end of the study was 71 mg/day.

The fluoride-treated patients demonstrated a linear increase in bone density of about 10% per year, a 35% total increase over the study period, Dr. Riggs said. In fluoride-treated women bone mass increased 10-12% in the neck and Intertrochanter of the proximal femur. In the radius shaft, however, fluoride-treated patients had a significantly greater rate of bone loss - about 4% - than the placebo-treated patients. A 15% decrease in vertebral fracture rates per 1000 patient-years among fluoride-treated women was not considered statistically significant. Fractures increased threefold in treated patients, namely 11 fractures compared with four in the placebo group. According to Dr. Riggs, "Under the conditions of this study, sodium fluoride was not an effective treatment."

KEY WORDS: Bone density; Bone fractures; Fluoride therapy; Vertebral fractures.

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INSTRUCTIONS TO AUTHORS

Fluoride, the official journal of the International Society for Fluoride Research (ISFR) publishes quarterly (Winter, Spring, Summer, Autumn) reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference appear in **Fluoride**. Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

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