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DEAN BURK, 1904-1988

Both science and the humanities have lost a major intellect with the recent death of Dean Burk, October 6, 1988, at the age of 84. Burk was born March 21, 1904, in Oakland, California, the son of Frederic Burk, who was President of what is now San Francisco State University. He entered the University of California Farm School at Davis at the age of 15 and studied agriculture. A year later, he transferred to the University of California at Berkeley, where he received his B.S. in Entomology in 1923. Four years later, at the age of 23, he earned a Ph.D. in Plant Nutrition and Chemistry (1).

He continued his advanced studies as a fellow, National Research Council/ International Education Board (1927-1929), successively at University College, University of London (with A.V. Hill, a Nobel laureate), the Kaiser Wilhelm Institute for Biology in Berlin, and Harvard University. In Germany he had investigated nitrogen fixation with Otto Meyerhof and also befriended Otto Warburg, both Nobel Prize winners, and their students.

He maintained a forty-year friendship with Warburg, "my greatest mentor," and perhaps the world's greatest biochemist, who worked with him in Bethesda, Maryland, on photosynthesis in 1949. From 1950 up until 1969, the year before Warburg's death, Burk spent most summers in Berlin and translated many of Warburg's "most important contributions on cancer and photosynthesis . . . ." (2). In 1953, he became a Foreign Member of Warburg's Institute.

In 1935 Burk also had the opportunity to study in the U.S.S.R. at the Academy of Sciences (Biochemistry Institute) as a Guest Research Worker, and he returned to study in the summer of 1937 under the famous biochemists A.N. Bach and V.I. Engelhardt. During the period of his government service, he spent parts of many years in England, Germany and the U.S.S.R. He also traveled throughout the world ranging from Europe to Africa and Australia, where he toured extensively in 1977. He attended countless international meetings. Linguistically, he was proficient in German and French and knew some Russian.

Burk's professional career began in 1929 as an Associate Physical Chemist, at the Fixed Nitrogen Research Laboratory, Bureau of Chemistry and Soils, Department of Agriculture in Washington, D.C. (3). In 1939 he left that job to join the National Cancer Institute (NCI) of the National Institutes of Health, with which he was associated until 1974, when he reached the mandatory retirement age of 70. His government career, therefore, spanned 45 years. With the NCI, he was Senior Chemist (1939-1948), Principal Chemist (1948-1951), Head Chemist (1951-1958), and Chief Chemist (1958-1974). He was also simultaneously a faculty member, Associate Professor of Biochemistry, at the Cornell University Medical College during 1939-1941. From 1947 until his death he held the honorary post of Research Master, Graduate Faculty, George Washington University, and during 1974-1976, he was Guest Scientist at the U.S. Naval Research Institute.

His impressive list of memberships and honors is too numerous to cite completely here (4). A few examples of some of these are: Fellow, American

Association for the Advancement of Science (and organizer of cancer research conferences, 1942-1945); American Association of Cancer Research; Foreign Scientific Member, Max Planck Institute of Biochemistry, Munich, Germany and also the Institute for Cell Physiology, Berlin; Society of Experimental Biology and Medicine; Honorary President, German Society of Medical Tumorotherapy; and the Royal Society of Medicine, London. He was a member of the prestigious Cosmos Club in Washington, D.C. and the Commonwealth Club of California.

Aside from his major contribution to scientific literature of more than 250 articles, Dr. Burk was also recognized as a leading American authority on photosynthesis by receiving the American Chemical Society Hillebrand Prize in 1953 "For the experimental discovery of a photosynthetic energy cycle of high quantum efficiency, with demonstration of the applicability of the Einstein law of photochemical equivalence and studies of related biochemical energy transformations in cancer metabolism" (5). According to the American Chemical Society (which he joined in 1931), the stimulus for "Burk, and the principal reason for his receiving the award," was "his discovery with Otto Warburg of one quantum reaction in photosynthesis at 90% utilization efficiency of incident light" (6). Dr. Burk achieved singular honor for his distinguished cancer research in 1965 with the Gerhard Domagk Prize "for the development of procedures for distinguishing the differences between a normal cell and one damaged by cancer" (7) normally reserved for native-born German, Swiss or Austrian citizens (8).

Burk will probably be best remembered to biochemists as the co-author, with Hans Lineweaver, of the most frequently cited paper in biochemistry, "The Determination of Enzyme Dissociation Constants," in the **Journal of the American Chemical Society**, published in 1934 (9). With 20-20 vision of hindsight, we now view the lack of enthusiasm by the six referees of this classic article with amusement -- they recommended rejection of the article, but editor Arthur B. Lamb overruled them! — because we see that "the double reciprocal plot usually provides, automatically and conveniently, a considerably improved weighting for linear graphics of most enzyme velocity data as a function of concentration" (10). The consequences, as one recent review of biochemistry stated: "One can hardly find any enzymological publication in which the kinetic data are presented in a way other than the double reciprocal plot according to Lineweaver and Burk. The results here confirm the opinion of Dowd and Riggs (1965) [J.E. Dowd and D.E. Riggs, J. Biol. Chem., 240:863, 1965] that the popularity of the Lineweaver-Burk method is based upon the ability to provide what seems to be a good fit even when the experimental data are poor . . . ." (11). But, as Burk himself commented, "It is much more importantly used to test the general qualitative correctness of an assigned mechanism formulation before ascertaining the numerical values of the parameter constants involved" (12).

Burk himself has summarized his other major laboratory projects in an interview: "thermodynamics of nitrogen fixation, biochemistry, and cancer; photosynthesis with and without green plants; biology and biochemistry of nitrogen fixation by bacteria; . . . trace mineral elements; B vitamins (co-discoverer of biotin); optical activities of various biochemical racemates; cancer metabolism in all kinds of cancers and leukemia in animals and humans; poloragraphic analysis of cancer and normal blood; antibiotics; cobalt models of hemoglobin-oxygen systems; cell-tissue cultures; mitochondrial control of

metabolism; iron-binding compounds in blood; chromatographic separations; manometric techniques for measurement of gases; efficiency of photosynthesis in green plants; origin of cancer cells; conventional anticancer agents' mode of action; cytotoxic actions of human sera; insulin; correlation between cancer growth rate and magnitude of metabolism (Domagk Prize, 1965); purification of tobacco smoke; effects of amygdalin (Vitamin B-17, Laetrile) on cancer cells" (13). The period from 1975-1988 was sharply focused on fluoridation and cancer.

On November 18, 1974, after his retirement, Burk founded the Dean Burk Foundation "devoted to research on health, nutrition, and chronic and degenerative diseases including cancer." Two major reports (called "Briefs") were published. One focused on vitamin B-17 and also alluded briefly to vitamin B-15 and vitamin B-13 (14). Burk carefully analyzed the federal food, Drug and Cosmetic Act (as amended August 1972), Title 21 USC, Chapter 11 (Definitions), Sec. 201 (321) (f), and demonstrated conclusively that amygdalin (vitamin B-17/Laetrile) is by definition, and long-standing scientific knowledge — to which he had personally contributed — "scientifically to be regarded as a food, a vitamin . . . ." (15). Unfortunately, he caustically added, irresponsible human nature appeared certain to reject such an axiom in the same way the flat earth advocates reject the view of a round earth. What was true about vitamin B-17 was equally true of vitamin B-15 and vitamin B-13. (16).

The other major focus during his retirement years was on the link between fluoridation and cancer. As a result of Dr. Burk's expert views on conventional as well as nonconventional cancer therapies, he became a sought-after speaker before nonmainstream health groups during the late 1960s and an embarrassment to his superiors at the National Cancer Institute (NCI), who advocated only the traditional medical cancer therapies of surgery, chemotherapy, and radiation treatments. The NCI therefore attempted to muzzle Burk when he was invited to speak to many groups on nonorthodox treatments he was acquainted with that had shown promise. Clinton R. Miller, legislative advocate in Washington, D.C., for the National Health Federation (NHF), knew that Burk was being harassed by the NCI — the notoriety of the maverick Head of Cytochemistry was very well known — and lobbied the NCI through Congress to release Burk to speak to health groups. The NCI refused to approve Burk's talks, but they did not positively disapprove his appearances, a distinction bureaucrats might be expected to make. On Sunday, July 16, 1969, during his weekend, for example, Burk delivered a talk on "Healthier Cigarettes and Cancer Prevention," to the International Association of Cancer Victims and Friends, Inc. in Los Angeles. The NCI frowned with disapproval; the IACVF groups clamored for more (17)!

In January 1975, shortly after retiring from the NCI, Burk spoke to the National Health Federation about Laetrile. At that time he first met John Yiamouyiannis, a Ph.D. in biochemistry who had joined the NHF as Science Director and who was a formidable combatant in the fight against fluoridation. Burk couldn't understand why Yiamouyiannis was "wasting his time" pursuing such a dead-end subject (18).

Burk's familiarity with fluoride reached back to the very beginning of his career. As he observed in 1976, "I did my first experiments with fluoride in 1929, and was present in Meyerhof's laboratory in Berlin when Fritz Lipmann

was doing his fluoride experiments in 1928" (19). Burk's friendly, but sharp, advice to Yiamouylannis therefore had deep roots, although Yiamouyiannis doggedly continued to pursue the topic.

In about May 1975, Yiamouyiannis finished an expanded version of his fluoridation efforts comparing cancer mortality rates in some major fluoridated cities with cities without fluoridation. The paper was sent to Clinton R. Miller, who took it to the National Cancer Institute; the reception was negative, and Miller then asked Dean Burk, whom he knew from Burk's Laetrile talks, to read the paper. Burk, again, was typically caustic, and repeated his carefully considered opinion that Yiamouylannis was wasting his time on a "worthless" enterprize — if there were a connection it would have been found already. Nevertheless, he agreed as a favor to Miller to read the paper and criticize it. After spending "all night" and several more days minutely analyzing the data, Burk conceded the arguments were sound, convincing, in fact, despite his strong preconceptions, probably correct (20). He then began to view the fluoridation/cancer link in an entirely new light. Miller took Yiamouyiannis to Burk's house, and an important, lengthy collaboration began.

Burk's preliminary statement on the subject was a publication of his Foundation ("Brief" No. 2) discussing the probable link between fluoridation and cancer and the Delany Amendment to federal law, which prohibits the addition of any carcinogen to food or water (21). If the fluoridation/cancer link is correct, then by law fluoridation must be discontinued immediately. The NCI, however, repeatedly disclaimed any connection.

Burk's preliminary statement was followed by a detailed collaborative effort between himself and Yiamouyiannis — Burk's "second most important paper" (22). The authors showed that crude cancer death rates in the 10 largest fluoridated cities in the U.S.A. "were higher and had risen faster than those in the 10 largest nonfluoridated U.S. cities that had essentially the same crude cancer rates during the decade before fluoridation." They found that this increase occurred in persons 45-65 and 65 and over and that corrections for age, race, and sex did not eliminate the difference as suggested by the NCI (23). The bottom line was a 5-15% higher death rate (unweighted) in the fluoridated over the unfluoridated communities compared in the study (24).

Criticism from fluoridation proponents was swift and hostile: the authors had failed to correct for age, sex, race, and cancer site distribution, it was claimed. When proper "adjustments" (i.e. manipulations) were made — a constant proponent theme — all was well with fluoridation (25). Another author emphasized that population gains (demography) explained the apparent problems away (26). The battle still continues with great vehemence a decade later.

Burk's final statement on the subject, and indeed his last scientific paper, reiterates his strong scientific conviction that demographic changes of the two groups of central cities do not explain away the apparent causal relationship between fluoride and cancer death rate. "It is concluded that artificial fluoridation appears to cause or induce about 20-30 excess cancer deaths for every 100,000 persons exposed per year after about 15-20 years." Burk and his co-authors therefore pleaded: "In light of this conclusion, we urge the governments of civilized countries of the world to bring about a prompt end to artificial fluoridation of public water supplies" (27). Tens of thousands

of unnecessary deaths — at one time he estimated about 40,000 in the U.S.A. alone — would thereby be averted (28). Saving lives was a primary concern of Burk, and he thought his work on fluoridation was the most important he had done during his life (29). There is no doubt that one of his most personally fulfulling moments was experienced when he received the news that he was largely responsible for the termination of fluoridation in the Netherlands (30), despite erroneous claims to the contrary (31), and his impact in Australia was widely reported in the press there (32).

Dr. Dean Burk musician, artist, scientist, sage — lived a rich and valuable life. He published more than 250 scientific articles. He probed abstruse mysteries; he proposed profound answers. He devoted his life to science and mankind. He made an indelible mark where he has passed. The world is infinitely richer having known such a gentle, brave man of genius, industry, and altruism.

References

1. The most detailed biography of Dean Burk is an interview done in 1976. [Don C. Matchen], "Dean Burk: Brilliant, Ethical Scientist, and Humanitarian," **National Health Federation Bulletin** (November 1976) 18-23. Obituaries appear in the **N.Y. Times** (Oct. 10, 1988), B-8, and **Chemical and Engineering News** (C&EN) (January 2, 1989) 43.
2. See his elegant biography of Warburg in **Dictionary of Scientific Biography**, Vol. 14 (1976) 172-177.
3. In biographies such as **Marquis Who's Who in America**, Vol. 38, 1974-1975, and **American Men and Women in Science**, 1986, cited as the Fixed Nitrogen Research Laboratory; however, the classic Hans Lineweaver and Dean Burk, "The Determination of Enzyme Dissociation Constants," **J. Amer. Chem. Soc.**, 56 (Mar. 1934) 658-666, ref. 658, states the work came from "The Fertilizer Investigations Unit, Bureau of Chemistry and Soils, United States Department of Agriculture."
4. See **American Men and Women of Science**, 1986.
5. "Hillebrand Prize to Burk for Photosynthesis Studies," C&EN (March 9, 1953) 998.
7. "Burk," **C&EN**, January 2, 1989, 43.
8. Paul Wasserman and Kryptyna Wasserman, Eds., **Awards, Honors and Prizes, Vol. 2 International and Foreign**, 3rd ed. (1975) 66-67.
9. Lineweaver and Burk, "The Determination of Enzyme Dissociation Constants," **J. Amer. Chem. Soc.**, 56 (Mar. 1934) 658-666.
10. Dean Burk, "Enzyme Kinetic Constants: The Double Reciprocal Plot," **Trends in Biochemical Sciences**, 9, No. 4 (April 1984) 202-204, ref. 203.
11. M. Zydowo, K. Kaletha, and A. Dudek, **Acta Biochimica Polonica**, 18 (1971) 368-371. Cited in **Ibid.**, 203.
12. Burk, **op. cit.** (fn. 10) 203.
13. **National Health Federation Bulletin** (fn. 1) 18-19.
14. Dean Burk, "**Vitamin B-17**, Amygdalin-Nitroloside-Laetrile, **Vitamin B-15**, Panganic Acid-Pangamates, **Vitamin B-13**, Orotates," The McNaughton Foundation, P.O. Box A, Sausalito, California 94965, n.d. [1975], pp. 1-23.
15. **Ibid.**, p. 16.
16. **Ibid.**, p. 17.
17. Clinton R. Miller, "NCI Attempts to Censor and Gag Dr. Dean Burk," **National Health Federation Bulletin** (May 1971) 7-12.
18. Author's interview with John Yiamouyiannis, February 20, 1989.

19. Letter, Dean Burk to George L. Waldbott, M.D., January 6, 1976. Copy in author's possession.
20. Author's interviews with John Yiamouyiannis, February 20, 1989, and Clinton R. Miller, February 8, 1989.
21. Dean Burk, "Fluoridation-Linked Cancer and the Delany Amendment," The McNaughton Foundation, Sausalito, California, n.d. [1975]. See also "Delaney Amendment Could be Death-Knell to Fluoridation," **National Health Federation Bulletin** (Sept. 1975) 4-15.
22. Interview (fn. 18).
23. Dean Burk and John Yiamouyiannis, "Fluoridation and Cancer. Age Dependence of Cancer Mortality Related to Artificial Fluoridation," **Fluoride**, 10 (1977) 102-123.
24. G.L. Walbott, A.W. Burgstahler, H.L. McKinney, **Fluoridation: The Great Dilemma.** Coronado Press, 1978, ch. 13.
25. For example, see R.N. Hoover, F.W. McKay, and J.F. Fraumini, Jr., "Fluoridated Drinking Water and the Occurrence of Cancer," **J. Natl. Cancer Inst.** 57 (1976) 757-768 and R. Doll and L. Kinlen, "Fluoridation of Water and Cancer Mortality in the U.S.A.," **Lancet**, 1 (1977) 1300-1302.
26. D.R. Taves, **Fluoride in Drinking Water and Health**, National Research Council — National Academy of Sciences, Washington, D.C., 1977. Cf. Waldbott, Burgstahler, and McKinney, **Fluoridation: The Great Dilemma.** Ch. 13, pp. 225-232.
27. J.R. Graham, D. Burk, and P. Morin, "A Current Restatement and Continuing Reappraisal Concerning Demographic Variables in American Time-Trend Studies on Water Fluoridation and Human Cancer," **Proc. Penn. Acad. Science**, 61 (1987) 138-146, refs. 138, 145.
28. Burk often estimated "that there now are some 40,000 cancer deaths a year in the United States that are linked with and induced by the fluoridated drinking water which 40% of the American population is forced to drink whether it wants to or not." See "Dean Burk Testifies Before Congressional Committee," **National Fluoridation News**, 26, No. 1 (January-March 1980) 1. In his last article, however, fn. 27 above, pp. 138, 145, he apparently estimated a reduced figure, not specified.
29. Explicitly stated to the author (at the Dean Burk Foundation, Washington, D.C.) and Albert W. Burgstahler on separate occasions. Mrs. Dean Burk (Mildred Burk) has confirmed that Burk thought his fluoridation work was one of his most important contributions during his scientific career. Interview with the author, February 2, 1989.
30. Hans C. Mollenburgh, **Fluoride: The Freedom Fight** (Main Stream Publishing Co., 1987), pp. 183ff. On October 3, 1977, Mollenburgh, a medical doctor from Haarlem who led the Dutch fight against fluoridation, sent Burk a telegram stating he had "PERSONALLY PRESENTED FINDINGS BURK AND YIAMOUYIANNIS JANUARY 1976 TO REPRESENTATIVES HEALTH COMMITTEE FIVE GREAT POLITICAL PARTIES AND SENT WRITTEN EVIDENCE TO ALL OTHER MP'S OF HEALTH COMMITTEE/ FINDINGS MADE DEEP IMPRESSION/ BURK APPEARED ON DUTCH TV FEBRUARY 10 AND ALL OVER THE COUNTRY COMMENTARIES IN PAPERS/ . . . / FINDINGS BURK MENTIONED REGULARLY IN DISCUSSIONS/ . . . / FINDINGS BURK AND YIAMOUYIANNIS TIPPED SCALES AGAINST FLUORIDATION IN NETHERLANDS." Copy in the author's possession.
31. Letter, Dean Burk to Congressman L.H. Fountain, October 12, 1977. Copy in the author's possession. That letter referred to a letter of Dr. J.C.N. Jonkman of the Royal Netherlands Embassy, Washington, D.C., to G.S. Goldhammer, September 7, 1977, in which Jonkman, following orders

from his government, erroneously denied that the fluoride-cancer link had any bearing on the Dutch rejection of fluoridation.

32. For example, **The Courier**, Ballarat [Australia], September 7, 1977, p. 9; **Moorabbin Standard News** [Australia], July 27, 1977, p. 18; and **Geelong Advertiser** [Australia], June 29, 1977, p. 4.

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TRACE ANALYSIS OF FLUORINE IN PLANTS AND FOODS

by

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SUMMARY: This paper describes a method for determination of traces of fluoride in plants and foods. The samples are decomposed in an oxygen flask, followed by the multi-standard addition of Gran's method with an fluoride ion-selective electrode. A minicomputer then processes the data using Gran's method. Compared with hydrochloric acid and perchloric acid extraction, this method is simple, rapid, and sensitive. A computer program is presented.

KEY WORDS: Gran's method of ISE; Minicomputer; Oxygen flask method; Trace analysis of fluorine.

Introduction

Plants as well as other foods can adsorb and accumulate micro amounts of fluoride from the air, soil and water. To determine amounts of fluoride in polluted tissues, various analytical methods have been published (1,2,3). Villa (1) developed a method for determination of fluoride with perchloric acid extraction combining fluoride ISE. However, the method is not as sensitive as is desirable when determining minute levels of fluoride. It has been shown that Gran's plot can enhance the sensitivity by 1-2 orders of magnitude. However, the necessary calculations are long and tedious.

This paper recommends Oxygen Flask decomposition in combination with the fluoride electrode using the multi-standard addition of Gran's method and a minicomputer to process the data. Results obtained were in good agreement with those obtained by use of perchloric acid and hydrochloric acid extractions (1,2). This method which is simple, rapid and determines the lower levels of fluoride requires only 25 minutes to complete the entire analytical procedure.

Theory and Computer Program

According to the Nernst equation

$$E = Eo - S \log f \left(\frac{CoVo + CsVs}{Vo + Vs} \right) + Ej \qquad (1)$$

Where Eo and Ej are the normal potential and the liquid junction potential respectively, Co, Vo, and Cs, Vs are the concentration and volume of the sample solution, and of the standard solution. f is the activity coefficient of the F^-. S is the electrode slope.

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From Equation (1) is obtained

$$(Vo + Vs)10^{-E/S} = 10^{-(Eo + Ej)/S} fCsVs + 10^{-(Eo + Ej)/S} fCoVo \qquad (2)$$

If the sample solutions are kept high and almost constant ionic strength, f and Ej do not change appreciably. Therefore only E and Vs are variable, and E is the function of Vs.

For this reason, we can asume

$$Y = (Vo + Vs)\ 10^{-E/S} \qquad X = Vs$$

$$a = 10^{-(Eo + Ej)/S} fCoVo \qquad b = 10^{-(Eo + Ej)/S} fCs$$

from Equation (2), the straight line equation is obtained

$$Y = a + bX$$

After a series of the values of Vs and correlations to potentials E are determined. We get a series of values for X and Y. By means of straight line regression of these values, a and b are obtained.

Assume $Y = 0$

Then $$X = -\frac{a}{b} = -\frac{10^{-(Eo + Ej)/S} fCoVo}{10^{-(Eo + Ej)/S} fCs} = -\frac{CoVo}{Cs}$$

Because $X = Vs$

Therefore $$Co = -\frac{CsVs}{Vo}$$

According to the theory, we worked out a computer program in BASIC to process the data of Gran's plot.

```
10   I=o:J=o:R=o:L=o:M=o
20   INP"N=",N,"Vo=",P,"S=",S,"Co=,C
30   FOR B=1 TO N
40   PRT"E";B;:INP E:PRT"V";B;:INP V
45   Y=(P+V)*(10↑(-E/S)
50   PRT"Y";B;"=";Y
55   I=I+Y
60   J=J+V;R=R+Y*Y
65   L=L+Y*V:M=M+V*V
70   NEXT B
75   I=I/N:J=J/N
80   R=R-N*I*I
85   L=L-N*I*J
90   M=M-N*J*J
95   H=SQR (R*M)
100  W=L/H
```

```
110  PRT "F="; L/(N-1)
120  PRT "G="; W
130  K=L/R:T=J-K*I
140  PRT "A="; K:A=K
150  PRT "B=";T
160  PRT "Y=0"
170  V=T:PRT "v=";T
180  C=-C*V/P
190  PRT "C=";C
200  END.
```

Where, S-58mv/pF, C-concentration of sample solution, G-correlation coefficient.

Experimental Apparatus

Fluoride electrode, made by Chang Sha Semiconductor Factory. Reference electrode, a saturated calomel electrode, sleeve-type, filled with agar (4). Microprocessor Ionalyzer (Orion 901), 0.1 mv., Magnetic Stirrer, with plastic coated stirring bar. Minicomputer, CASIO, Fx-702P, with Mini Electro Printer, CASIO, Fp-10. Oxygen flask, 500 mL. Filter paper with adhesive paper (4).

Reagents

TISAB solution (5). Standard sodium fluoride solution, 1.00×10^{-4}, 5.00×10^{-4}, 1.00×10^{-5}, 5.00×10^{-5} M. Perchloric acid, 0.1N (1).

Procedure

Burn about 40 mg dried and powdered (80-100 mesh) sample of the plants or foods wrapped in filter paper with adhesive paper (4) according to Schöniger's method. Absorb the combustion products in 10.0 mL of TISAB solution in an oxygen flask. Transfer the contents into a 50 mL beaker, wash the flask with 15.0 mL distilled water (5 mL each time) into the beaker. The total volume is 25 mL.

Immerse the electrodes in the solution. Using a magnetic stirring bar, slowly stir the solution at a constant rate. When the readings on the Ionalyzer equilibrate, record the potential E_1(mv). Then add 4 mL standard fluoride solution (1 mL each time) into the sample solution, record the potential values of E_2, E_3, E_4, and E_5. Input E_1, E_2, . . . E_5 and corresponding volumes of standard fluoride solution $V_1 = 0$, $V_2 = 1$, . . . and $V_5 = 4$ into CASIO PROGRAM LIBRARY Fx-702P, Print the fluoride concentration of sample with CASIO Mini-Electro Printer Fp-10.

A blank test should also be determined with each series of samples.

Results and Discussion

From the concentration of sample solution printed, the amounts of fluoride in the sample may be calculated from the formula:

> Sample fluoride (μg/g) = (sample molar concentration - blank molar concentration) x Cp x 25 mL/sample weight (g)

where Cp is the equivalent ppm (μg/mL) of the sample molar concentration.

The results of the determination of samples are given in Table 1.

Table 1

The Results of the Determination of F in Plants and Foods (μg/g)

Method	Sample Number 1	2	3	4	5	6	7	8	9	10
Gran's Method Using Computer to Process	0.65	3.36	9.20	0.78	3.49	4.69	26.7	25.2	57.5	155
	0.65	3.29	9.25	0.85	3.52	4.80	26.4	25.5	58.0	156
	0.64	3.35	9.31	0.82	3.45	4.75	26.8	26.0	57.8	155
Perchloric Acid extraction	0.62	3.28	8.90	0.72	3.35	4.65	25.6	24.1	57.1	151
Hydrochloric Acid extraction	0.61	3.20	9.40	0.75	3.42	4.70		25.7	56.9	156

During experiment, because of various fluoride content of samples, the selections of Gran's standard addition solutions are very important. The different selections are given in Table 2.

Table 2

The Selections of Gran's Standard Addition Solutions

Contents of Fluoride (μg/g)	< 5.0	5.0–20.0	20.0–100.0	100.0–500.0
Gran's Addition Solutions (M)	1.0×10^{-5}	5.0×10^{-5}	1.0×10^{-4}	5.0×10^{-4}

For general tests, it is best to read five potential values, and put them into the minicomputer. After inputting, check the correlation coefficient G. According to mathematical methods of statistics, if the G > 0.999, the possibility of error is below 1%; if the G < 0.99, the set of data should not be entered.

Acknowledgement

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References

1. Villa, A.E.: Rapid Method for Determining Fluoride in Vegetation Using an Ion-Selective Electrode. **Analyst**, 104:545, 1979.
2. Ma, T.S., Wang, C.Y. and Gutterson, M.: Organic Elemental Analysis. **Anal. Chem.**, 54:87R, 1982.
3. Ma, T.S. and Wang, C.Y.: Organic Elemental Analysis. **Anal. Chem.**, 56:88R, 1984.
   Idem. Ibid., 58:144R, 1986.
4. Wang, C.Y.: Fluorine Ion Selective Electrode for Micro-Determination of Fluorine in Organic Compounds. **Microchem. J.**, 27:455, 1982.
5. Frant, M.S. and Ross, Jr., J.W.: Use of a Total Ionic Strength Adjustment Buffer for Electrode Determination of Fluoride in Water Supplies. **Anal. Chem.**, 40:1169, 1968.

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AN EXPERIMENTAL STUDY OF BLOOD BIOCHEMICAL DIAGNOSTIC INDICES FOR CHRONIC FLUOROSIS

by

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SUMMARY: Of a total of 40 Wistar rats 27 were randomly divided into test groups: 13 received 10 ppm and 14 received 30 ppm fluoride, respectively, in their drinking water for eight months; the control group of 13 received low-fluoride water (less than 0.6 ppm). The rats that drank water which contained 30 ppm developed severe chronic fluorosis characterized by skeletal damage; in those receiving 10 ppm fluoride in water only mild chronic fluorosis was detected. Blood biochemical examination showed decrease in glutathione peroxidase activity and reduced glutathione content; the amount of lipid peroxidation and the load of cyclic adenosine monophosphate increased in rats with mild chronic fluorosis. In rats with severe chronic fluorosis the glutathione peroxidase activity and contents of reduced glutathione, albumin and calcium were lowered; but the glutamic-pyruvic transaminase activity and the lipid peroxidation, inorganic phosphate, potassium and cyclic adenosine monophosphate content all increased. Among these biochemical indices, the glutathione peroxidase, potassium, calcium, inorganic phosphate, albumin and cyclic adenosine monophosphate were chosen to diagnose mild and severe chronic fluorosis.

KEY WORDS: Blood biochemistry; Fluorosis; Rat.

Introduction

Chronic fluorosis is an endemic disease which induces systemic damage to the human body. It is generally believed that biochemical changes appear earlier in the blood than in other organs or tissues (1). Since the 1970s, studies to determine whether biochemical changes in the blood can be utilized to diagnose chronic fluorosis have given contradictory results. To study this problem, mild and severe chronic fluorosis were induced in rats; eighteen biochemical parameters in the blood were analyzed, from which indices that closely relate to chronic fluorosis were chosen.

Materials and Methods

Forty Wistar rats, 22 females and 18 males, weighing 100-120 g, were randomly divided into three groups. Group I constituted the control: 13 rats were fed a standard diet containing less than 4 ppm fluoride and tap water containing less than 0.6 ppm. Group II: 13 rats were fed the same diet as Group I, but their water contained 10 ppm fluoride. Group III: 14 rats were

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fed the same diet as Group I; their water contained 30 ppm fluoride. All rats received their respective diet for eight months.

General Examinations: Fluoride concentrations in the urine were measured with CSB-F-I fluoride ion electrode (Changsa Analysis Instrumentation Co. of China) during the fourth month of the experiment (2). All animals were weighed and examined for dental fluorosis at the end of the experiment, after which they were sacrificed; pieces of femora and ulnae were decalcified and embedded in paraffin and sections were stained with Hemotoxylin-Eosin (H.E.) for light microscopic examination.

Biochemical Analysis of Blood: Eighteen blood indices were analyzed. The serum lactic dehydrogenase (LDH), alkaline phosphatase (ALP), glutamic-pyruvic transaminase (GPT), creatinine, urea nitrogen (BUN), glucose, albumin, globulin, calcium (Ca^{2+}), inorganic phosphate (Pi), potassium (K^{+}), and magnesium (Mg^{2+}) were measured with ILM III fluorescence/light scatter automatic biochemical analyzer (Instrumentation Laboratory, Inc. of U.S.A.). The activities of the serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) were assayed respectively by the methods of Beauchamp et al., and Hafeman (3,4), The contents of the serum lipid peroxidation (Lpo) and erythrocyte reduced glutathione (e-GSH) were measured respectively by the methods of Buege et al. and Ellman (5,6), The hemoglobin (Hb) content was measured. The plasma cyclic adenosine monophosphate (cAMP) level was assayed by the method of Tovey et al. (7), and the radioactivity of the plasma sample containing ^{3}H-cAMP was counted with LSC-700 Liquid Scintillation System (Aloka Co. of Japan).

Statistical Analysis: Groups II and III were compared with controls (Group I). Results of the experiment were evaluated statistically by the Student's t-test. Meanwhile, the function discriminant of diagnosing mild and severe chronic fluorosis was set up with Bayes's method on the computer (8,20), the formula:

$$Yg = Cog + \sum_{i=1}^{m} CigXi.$$

Results

The average increasing rate of rats' body weight in Groups II and III had declined (Figure 1). Urinary fluoride concentration had increased in all Group II and III rats (Figure 2). Dental fluorosis was observed in most Group III rats, but in only two rats in Group II (Table 1).

According to light microscopic examination of bones, cortex and trabeculae were regular in shape and homogeneously stained (Figure 3) in Group I. No significant differences were found in Group II except occasional deposition of chondroitin sulfate in some areas of the cortex. In Group III, focal thickening of the periosteum, widening and irregularity of bony trabeculae, and deposition of chondroitin sulfate around the cortex and bony trabeculae were observed; osteoid formation was observed in some areas (Figures 4, 5). Morphometric studies showed increase in the thickness of the cortex and size of the bony trabeculae but no change in density of osteocytes (Table 2).

Figure 1

Body Weight of Rats

14% and 19% lower in Group II and Group III respectively compared with Group I. BW = body weight.

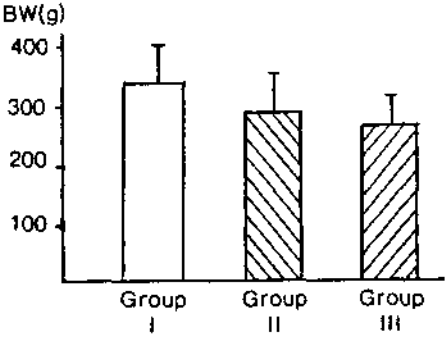


Figure 2

Urinary Fluoride Concentration

2.47 and 3.14 times increase in Group II and Group III respectively compared with Group I. UF^- = urinary fluoride.

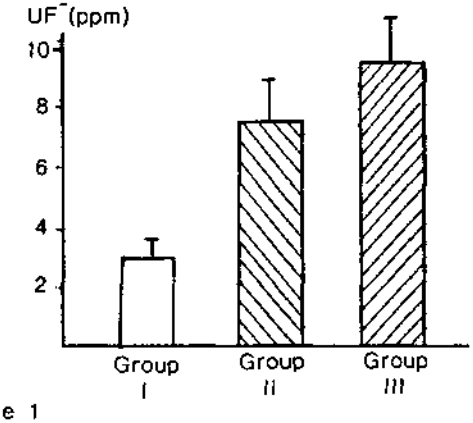


Table 1

Rates of Dental Fluorosis in Rats

Group	n	Numbers of Rats with Dental Fluorosis	%
I	13	0	0
II	13	2	15.39
III	14	11	78.57

Note: n = number of animals

Table 2

Morphometric Indices of Femora in Rats

Group	n	Thicknss of Osseous Cortex (mm) $\bar{X}$ ±S.D.	Size of Bony Trabeculae (μ) $\bar{X}$ ±S.D.	Density of Osteocytes (n/36 check) $\bar{X}$ ±S.D.
I	7	0.468 ±0.047	61.65 ±4.65	7.86 ±0.88
II	7	0.446 ±0.067	62.40 ±7.40	8.29 ±0.73
III	7	0.535 ±0.027**	71.15 ±5.25**	8.33 ±0.51

n = number; **$p < 0.01$, compared with Group I.

Figure 3

Bony trabeculae regular in shape in Group I. HE x 66.

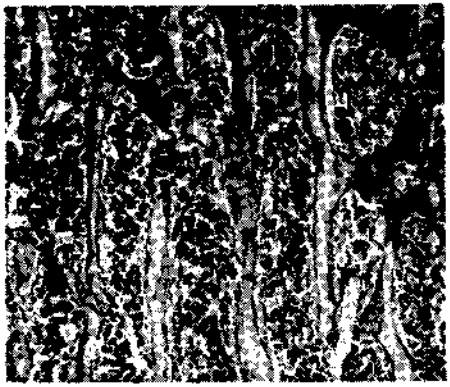
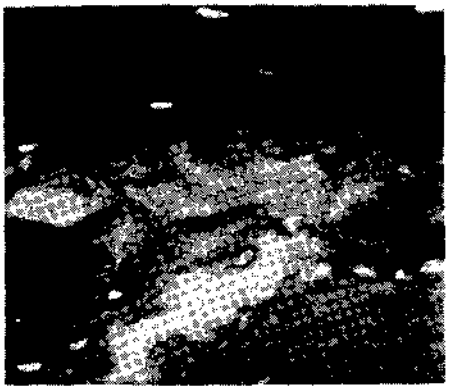


Figure 4

Widening and irregularity of bony trabeculae in Group III. HE x 66.

Figure 5

Deposition of chondroitin sulfate around the cortex in Group III. HE x 132.



GSH-px activity and e-GSH content had decreased, but Lpo and plasma cAMP content had increased in Group II. GSH-px activity and content of e-GSH, albumin, and Ca^{2+} were lower, but content of GPT, Lpo, Pi, K^{+}, and cAMP had all increased in Group III. Other blood biochemical indices, such as LDH, ALP, SOD, BUN, creatinine, glucose, globulin, Hb and Mg^{2+}, had not changed in Groups II and III compared with Group I (Table 3). Thus, the GSH-px, K^{+}, Ca^{2+}, Pi, albumin and cAMP were chosen to set up the function discriminant by computer. The diagnosis rate in accordance with the actual conditions was more than ninety percent in our study.

The function discriminant:

$$Y_1 = -112.53 + 2.07X_2 + 6.85X_9 + 5.30X_{10} + 8.80X_{11} + 8.97X_{16} - 1.36X_{18}$$

$$Y_2 = -96.95 + 1.78X_2 + 7.21X_9 + 5.07X_{10} + 8.30X_{11} + 6.92X_{16} - 0.40X_{18}$$

$$Y_3 = -105.18 + 1.70X_2 + 10.21X_9 + 3.81X_{10} + 10.23X_{11} + 5.05X_{16} + 0.69X_{18}$$

Y_1 = normal rat, Y_2 = mild chronic fluorosis, Y_3 = severe chronic fluorosis; X_2 = GSH-px, X_9 = K^{+}, X_{10} = Ca^{2+}, X_{11} = Pi, X_{16} = albumin and X_{18} = cAMP.

Table 3

Blood Biochemical Indices in Rats

Index	Unit of Measure	Group I ($\underline{n}$ = 13) $\bar{X}$ ±S.D.	Group II ($\underline{n}$ = 13) $\bar{X}$ ±S.D.	Group III ($\underline{n}$ = 14) $\bar{X}$ ±S.D.
Lpo	OD_{535}/0.5 mL	0.142 ±0.012	0.159 ±0.018**	0.164 ±0.023**
GSH-px	U/10 μL	33.84 ±4.67	28.83 ±5.32*	27.26 ±4.60**
SOD	OD_{560}/100 μL	0.218 ±0.026	0.221 ±0.022	0.215 ±0.019
e-GSH	nM/mg Hb	18.24 ±4.03	13.32 ±2.25**	11.93 ±3.54**
LDH	I.U./L	160.46 ±48.87	180.39 ±41.39	170.29 ±45.87
ALP	I.U./L	9.88 ±2.13	10.91 ±4.05	9.80 ±2.08
GPT	I.U./L	19.98 ±4.99	18.87 ±7.39	31.74 ±15.57*
Mg^{2+}	mg%	1.69 ±0.31	1.83 ±0.40	1.82 ±0.44
K^{+}	mEq/L	4.66 ±0.71	4.62 ±0.56	6.03 ±0.94
Ca^{2+}	mg%	11.61 ±1.61	11.39 ±1.58	9.06 ±1.28*
Pi	mEq/L	3.86 ±0.63	3.84 ±0.48	4.95 ±1.12*
BUN	mg%	20.14 ±8.23	20.95 ±10.25	21.05 ±9.23
creatinine	mg%	0.84 ±0.53	0.83 ±0.54	0.89 ±0.61
glucose	mg%	108.25 ±38.18	117.74 ±26.84	117.49 ±34.59
Hb	g %	14.64 ±1.18	14.36 ±0.99	14.18 ±1.17
albumin	g %	3.47 ±0.71	3.02 ±0.58	2.83 ±0.39**
globulin	g %	3.18 ±1.08	2.96 ±0.42	3.19 ±0.82
cAMP	pM/50 μL	2.67 ±0.96	3.64 ±1.22	4.67 ±1.61

n = number; *$p < 0.05$, **$p < 0.01$, compared with control (Group I).

Discussion

Rats with severe chronic fluorosis due to drinking water containing 30 ppm fluoride showed a retarded increase in body weight, increased urinary fluoride, and specific dental and skeletal lesions. Osteosclerosis was prominent in bones, characterized by thickening of the cortex and periosteum and irregular widening of the bony trabeculae. In addition, deposition of chondroitin sulfate, evidence of osteoporosis, was also found in some areas. The changes in bone structure were in accordance with Huo's findings (9). In rats administered 10 ppm fluoride in drinking water, fluorosis was mild, body weight increased more slowly, urinary fluoride increased, dental fluorosis was occasional, but no skeletal fluorosis was observed.

In recent years, studies of blood biochemical changes in response to chronic fluorosis have shown that the activities of serum LDH, ALP and GPT were increased, reduced or unchanged (10,11). According to some reports,

in humans or animals with chronic fluorosis, serum Ca^{2+} and Mg^{2+} content had declined; serum K^{+} and BUN had risen; serum Pi had slightly or significantly increased; however, contrary results have also been reported (12-14). That serum albumin content was reduced in response to excessive fluoride intake has been widely recorded (15,16). One study showed that increased fluoride can reduce the hemoglobin content by inhibiting the synthesis of erythrocyte protoporphyrin (1,13). Some reports suggest that the plasma cAMP level increased because excessive fluoride can activate adenylate cyclase (17,18). The diversity of experimental results shows the complexity of pathogenic mechanisms and the wide variety of damage in chronic fluorosis. Different changes may also be related to the degree of injury in the course of this disease.

In our experiments, serum GPT activity and serum Pi, K^{+} and cAMP concentrations increased; serum albumin and Ca^{2+} decreased; whereas in Group III, serum LDH, ALP, BUN, creatinine, glucose, globulin, Mg^{2+} and Hb remained within the normal range. The manifestations indicated that excessive fluoride influenced different organs or metabolic processes by varying degrees. The sensitivity of different organs and their ability to tolerate fluoride should also be taken into account. In Group II, all above-mentioned blood biochemical indices were close to controls except the cAMP level which had increased. Among the remaining biochemical measurements in blood, serum Lpo had increased, and serum GSH-px and e-GSH had declined in Groups II and III. Serum SOD activity was unchanged. It is reasonable to assume that fluoride promotes Lpo production or decreases elimination of Lpo by depressing anti-oxidation of GSH-px and e-GSH. The imbalance between production and elimination of free radicals can induce a wide range of impairments of the body in which the higher Lpo level stemming from free radicals plays an important role (19). Excessive Lpo may be an important mediating factor for the pathogenesis of chronic fluorosis.

GSH-px, K^{+}, Ca^{2+}, Pi, albumin and cAMP from eighteen blood biochemical indices in our study have been chosen to set up a diagnostic function discriminant of mild and severe chronic fluorosis by the computer. It has been shown that the six indices are more closely related with fluorosis than other blood biochemical measurements when all parameters are analyzed simultaneously in this experiment.

Conclusion

This study showed that mild and severe chronic fluorosis can be produced in rats fed water containing different fluoride concentrations. Changes in blood biochemical indices resulted in obvious differences between mild and severe chronic fluorosis in animal models. Some biochemical indices in blood may be chosen to diagnose mild and severe chronic fluorosis.

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References

1. Editorial Review: Nonskeletal Fluorosis. **Fluoride**, 11:111, 1978.
2. Hall, L.L., Smith, F.A. and Hodge, H.C.: Direct Determination of Ionic Fluoride in Biological Fluids. **Clin. Chem.**, 18:1455, 1972.
3. Beauchamp, C., and Fridovich, I.: Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. **Anal. Biochem.**, 44:276, 1971.
4. Hafeman, D.G.: Effect of Dietary Selenium on Erythrocyte and Liver Glutathion Peroxidase in the Rat. **Nutrition**, 104:580, 1974.
5. Buege, J.A. and Aust, S.D.: Microsomal Lipid Peroxidation. In: Fleischer, S. and Packer, L., Eds.: **Methods in Enzymology**. Vol LII, Academic Press, New York, 1978, pp. 302-310.
6. Ellman, G.L.: Tissue Sulfhydryl Groups. **Arch. of Biochem. and Biophys.**, 82:70, 1959.
7. Tovey, K.C., Oldham, K.G. and Whelan, J.A.M.: A Simple Sensitive Direct Assay for Cyclic AMP in Plasma and Other Biological Samples Using an Improved Competitive Protein Binding Technique. **Clinica Chimica Acta**, 56:221, 1974.
8. Huo, D.J. and Zhan, C.W.: Radiologic, Light Microscopic and Scanning Electron Microscopic Observations on Bones of Experimental Animals in Skeletal Fluorosis. **J. Guiyang Medical College**, 10:246, 1985.
10. Kessabi, M., Boudarine, B. and Broun, J.P.: Serum Biochemical Effects of Fluoride in Sheep of the Darmous Areas. **Fluoride**, 16:214, 1983.
11. Ferguson, D.B. and Stephen, K.W.: Plasma Alkaline Phosphatase Levels in Subjects Taking Fluoride Tablets. **Fluoride**, 14:42, 1981.
12. Kessabi, M.: Serum Biochemical Effects of Fluoride on Cattle in the Darmous Area. **Fluoride**, 18:227, 1985.
13. Usiu, B.: Effect of Fluoride on Hemoglobin and Hematocrit. **Fluoride**, 14:38, 1981.
14. Marier, J.R.: Observations and Implications of the (Mg vs F) Interrelations in Biosystems: A Review and Comments on Magnesium Intake and Fluorine Intake in the Modern Day World. **Fluoride**, 14:142, 1981.
15. Kaur, R., Singh, P. and Makhni, S.S.: Long-term Effects of Fluoride Administration — An Experimental Study. ii] Effect on Serum Protein. **Fluoride**, 11:25, 1978.
16. Yu, M.-H., Stoehr, M.P. and Driver, C.J.: Electrophoresis of Serum Proteins in Growing Chicks Fed a Diet Supplemented with NaF. **Fluoride**, 13:20, 1980.
17. Singh, M. and Susheela, A.K.: Adenyl Cyclase Activity and Cyclic AMP Levels Following F^- Ingestion in Rabbits and Human Subjects. **Fluoride**, 15:202, 1982.
18. Strochkova, L.S. and Zhavoronkov, A.A.: Fluoride as an Activator of Enzymatic Systems. **Fluoride**, 16:181, 1983.
19. Harman, D.: Free Radical in Biology. In: Pryor, W.A., Ed.: **The Free Radical Theory of Aging**. Vol. 5, Academic Press, New York, 1982, pp. 255-275.
20. Anderson, T.W.: **Introduction to Multivariate Statistical Analysis**. Wiley, New York, 1958, pp. 20-56.

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SOIL FLUORINE AS AN INDICATOR OF PROFILE DEVELOPMENT IN THE YAMUNA ALLUVIAL PLAIN, INDIA

by

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SUMMARY: Four soil profiles were exposed, representing the various aged soils, and samples were collected by horizon. 299 surface samples from various physiographic units were also collected to learn the spatial distribution of soil fluorine (F).

Fluoride in sand and silt fractions decreased in the direction of older soils, but increased in soil and clay size fraction. F content increased in depth in older soils (P_3 and P_4), evidently in response to adsorption of F weathered from minerals in coarser fractions (sand and silt) of the epipedon. Hence, F distribution in the profile which closely followed soil development trends, is well expressed by clay content ($r = 0.89$). Multiple regression analysis showed that clay alone explained 68 percent variation in soil F.

The cumulative frequency plot of F on probability paper suggested that spatial distribution of F, mainly governed by soil physiography, is independent of development sequence.

KEY WORDS: Alluvial plain; Fluorine; India; Soil; Yamuna.

Introduction

Fluorine is widely dispersed in nature and is a common constituent of most soils and rocks. It is the 13th most abundant element, averaging 650 ppm in the earth crust (1).

The total F of surface soils from different places has been reported by various workers (2-6), and F varied from 43 to 198 ppm in coarse textured soils and from 248 to 15,000 ppm in heavy textured soils. Thomas et al. (7) reported that F content of various clays and clay minerals ranged from 44 ppm (nontronite) to 51,800 ppm (herderite). They also observed that clays formed under hydrothermal conditions are in general relatively high in F content, provided the hydrothermal waters are high in F content.

From a soil genesis point of view, it is uncertain as to how much of the original F is retained in deterital sediments during the course of weathering. Studies (8,9) on F distribution in various aged soils show that it closely follows soil development trends. Hence the wide range of F in soils and its relationship to profile development need further investigation since F may be a good indicator of soil development and the origin of sediments.

With such considerations in mind, the present study was undertaken to

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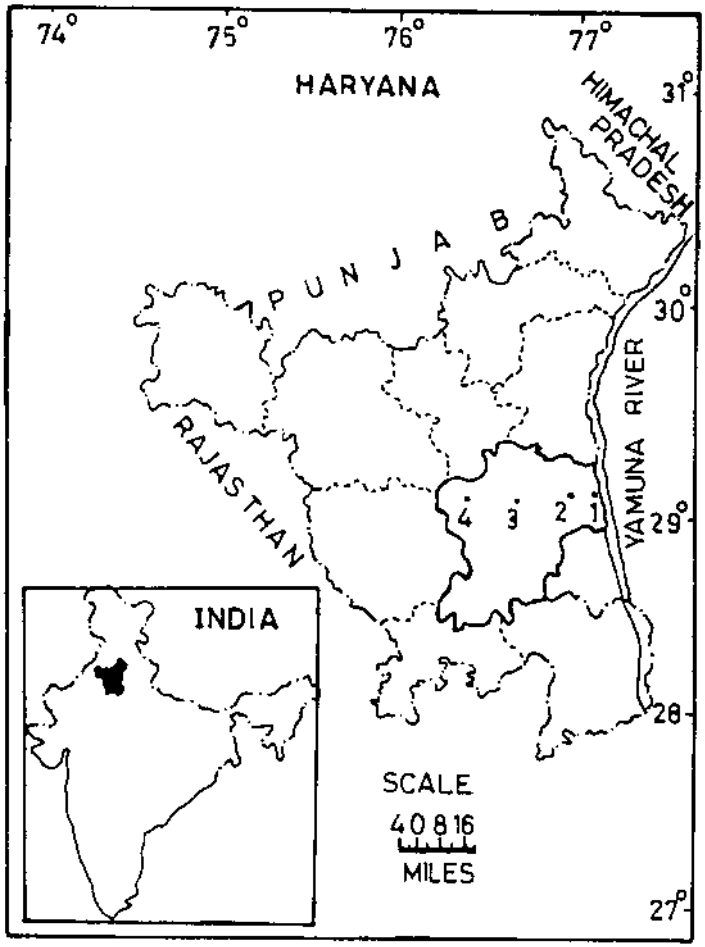
determine the relationship of F with the profile development in a chronosequence (10) in the Yamuna alluvial plain, India.

Materials and Methods

Four soil profiles in order of increasing degree of soil development in Yamuna aluvial plain (10) were selected for this study (Figure 1). Soil profiles were studied according to Soil Survey Manual (11) and samples were collected by horizon. In addition, two hundred and ninety surface samples were collected to learn spatial variability of F in various aged soils, the complete description of which is found elsewhere (10).

Figure 1

Index Map of Haryana and Location Sites of Soil Profiles in Yamuna Alluvial Plain, India



Air-dried ground samples of soil profiles were further fractionated into sand, silt and clay (12). Samples were fused with Na_2CO_3 (13) and F was distilled (14) from the melt after acidification. Distilled F was estimated spectrophotometrically using Zirconium-Erichrome Cyanine R Lake (15).

Moreover soil samples were analyzed for physio-chemical properties, O.C., pH and elemental composition as outlined in Soil Chemical Analysis (16). Amorphous iron and allophanes were extracted using CBD and 0.5 M KOH extractants, respectively (19). Free silica in soil samples was extracted using 5 percent KOH. The mineralogical analysis of oriented clays was done on a Phillips Diffractometer using Cu-Kα radiations at 30 KV and 20 mA.

Results

Fluorine distribution in different soil fractions (Table 1) and their mean, variance, standard deviation, and coefficient of variation (Table 2) indicate that F ranges high in clay size followed by silt and sand. Mean F in soil and clay size fraction increased from P_1 (114 and 460 mg/kg) to P_4 (257 and 707 mg/kg). However, in case of sand fraction, F content decreased from P_1 (29 mg/kg) to P_4 (13 mg/kg) soils. No trend could be observed in silt fraction. Water soluble F (W.S.F.) increased in the direction of older soils (12 to 24 mg/kg).

Sand and silt fractions contributed three quarters or more to the total F in recent soils (P_1 and P_2) and < 40% in older soils. F in soils as well as in different fractions increased in depth in older soils and showed no depth-wise trend in recent soils.

The means and coefficient of F variations in different fractions were compared statistically using the Student's t-test to find their relationship with the profile development. Mean values of F in soil, as well as sand and silt fractions differed significantly between P_1P_3; P_1P_4; P_2P_3 and P_2P_4. Similarly the coefficient of variations of F in soil as well as sand and clay differed significantly among P_1P_3; P_2P_3; P_1P_4 and P_2P_3. However, the means and coefficients of F variations in silt fractions were not significant.

Table 3 presents mean, variance and standard deviation of surface soil F in different soils. Surface soil F ranged from 61 to 637 mg/kg with a mean of 323 mg/kg. Means and coefficient of F variations in different soils were also compared statistically using Student's t-test F in P_1 differed significantly from that in P_2, P_3 and P_4.

To confirm the above test, F data were likewise treated graphically by plotting on a probability paper following the Lapeltier method (18). The ln graph indicates that F is normally distributed but cumulative frequency plot (Figure 2) shows at least four subtle population breaks (See arrows in Figure 2) which give 5 populations of F. The first population (≤ 150 mg/kg) occupies sand dunes and levees in older soils. The second lowest population (150-350 mg/kg) is located in a recent flood plain (P_1). The third population (350-500 mg/kg) is found in undulating landscape of P_2 and P_3. The second highest population (500-600 mg/kg) as well as the highest population (≥ 600 mg/kg) (P_4) is concentrated in the south-western part of Sonepat, where soils are poorly drained, and highly saline sodic (esp. > 15).

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Table 1

F Content of Soil and Soil Fractions and Amorphous Silica in Soil.

Horizon	Depth (cm)	Sand (> 50 μ) mg/kg	Silt (2-50 μ) mg/kg	Clay (< 2 μ) mg/kg	Soil (< 2 mm) mg/kg	Contribution to Total Fluorine Sand (%)	Silt (%)	Clay (%)	Water Soluble Fluorine in Soil (mg/kg)	Amorphous Silica in Soil (mg/g)
					P_1 (Udifluvent)					
Ap	0-24	15	262	535	268	1.5	52.8	41.5	19	7
$11C_1$	24-44	23	273	443	90	20.2	40.9	34.9	12	6
$11C_2$	44-95	23	388	428	129	12.9	61.9	25.9	14	13
$11C_3$	95-116	28	254	491	63	36.8	51.6	9.5	8	5
$111C_4$	116-120	16	185	407	24	63.1	12.0	20.7	7	5
					P_2 (Ustifluvent)					
Ap	0-16	33	254	472	108	25.6	29.9	46.2	17	8
C_1	16-48	29	267	565	134	15.8	28.7	50.6	19	7
C_2	48-60	26	304	489	99	20.5	36.7	45.8	11	5
C_3	60-150	28	295	426	93	23.4	40.5	35.9	6	3
					P_3 (Eutrochrept)					
Ap	0-19	9	197	548	69	10.4	30.3	62.7	27	19
B_2	19-43	17	257	756	125	10.2	30.5	57.1	22	7
B_3Ca	43-106	10	273	820	144	5.1	35.4	61.7	25	5
CCa	106-200	15	301	975	176	6.5	32.3	56.5	15	4

				P_4 (Ochraqualf)						
Ap	0-15	10	236	467	107	5.1	16.5	78.4	15	7
B_{21}	15-51	14	345	589	162	3.9	23.6	73.1	21	6
B_{221}	51-75	19	280	764	292	3.4	27.5	67.0	26	15
B_{31gt}	75-107	18	390	972	399	2.5	31.9	64.7	28	14
B_{32}	107-200	21	337	743	327	3.6	35.3	61.5	30	9

Table 4

Soil Variables Used in Multiple Regression Analysis.

	Clay (%)	Silt + Clay (%)	Organic Carbon (O.C.) (%)	Amorphous Iron (Fe_2O_3) (%)	Allo-phanes (%)	Mica (< 2 μ) (%)	pH	P_2O_5 (mg/kg)	$\frac{K_2O\ SO_2\ (mg/g)}{Al_2O_3}$	$\frac{Al_2O_3}{Fe_2O_3}$	$\frac{Na_2O+K_2O+CaO+MgO}{Al_2O_3+Fe_2O_3}$
Mean	14.5	30.4	0.19	0.68	0.34	65	8.1	508	7.90	5.17	0.88
S.D.	10.1	15.8	0.13	0.39	0.24	10	0.8	138	1.24	0.87	0.23
Low Range	1.0	2.5	0.04	0.25	0.14	40	7.0	292	7.16	4.35	0.87
High Range	35.9	70.2	1.06	1.41	0.85	85	10.5	864	11.97	8.09	1.22

Table 2

Statistical Parameters of F in Various Fractions of Profile.

Property	Mean m (mg/kg)	Variance s^2	Standard Deviation s	Coefficient of Variation 100 (s/m)
SF_1	21.00	29.50	5.43	25.86
SF_2	29.00	8.67	2.94	10.15
SF_3	12.75	14.91	3.86	30.29
SF_4	16.40	19.30	4.39	26.79
S_1F_1	272.40	5362.29	73.23	26.88
S_1F_2	280.00	548.66	23.42	8.37
S_1F_3	257.00	1930.67	43.94	17.10
S_1F_4	317.60	3610.30	60.09	18.92
CF_1	460.80	2676.19	51.73	11.23
CF_2	488.80	3343.33	57.82	11.85
CF_3	774.75	31304.92	176.93	22.84
CF_4	707.00	36573.50	191.24	27.05
TF_1	114.80	8803.70	93.83	81.73
TF_2	108.50	327.00	18.08	16.67
TF_3	128.50	2016.33	44.90	34.94
TF_4	257.40	14453.30	120.22	46.71
WF_1	12.00	23.50	4.85	40.40
WF_2	13.25	34.90	5.90	44.60
WF_3	22.25	27.60	5.25	23.60
WF_4	24.00	36.50	6.04	25.17

SF, F in sand fraction; S_1F, F in silt fraction; CF, F in clay fraction; WF, water solubile F in soil and 1-4, profiles P_1 (Udifluvent), P_2 (Ustifluvent), P_3 (Ustochrept) and P_4 (Ochraqualf), respectively.

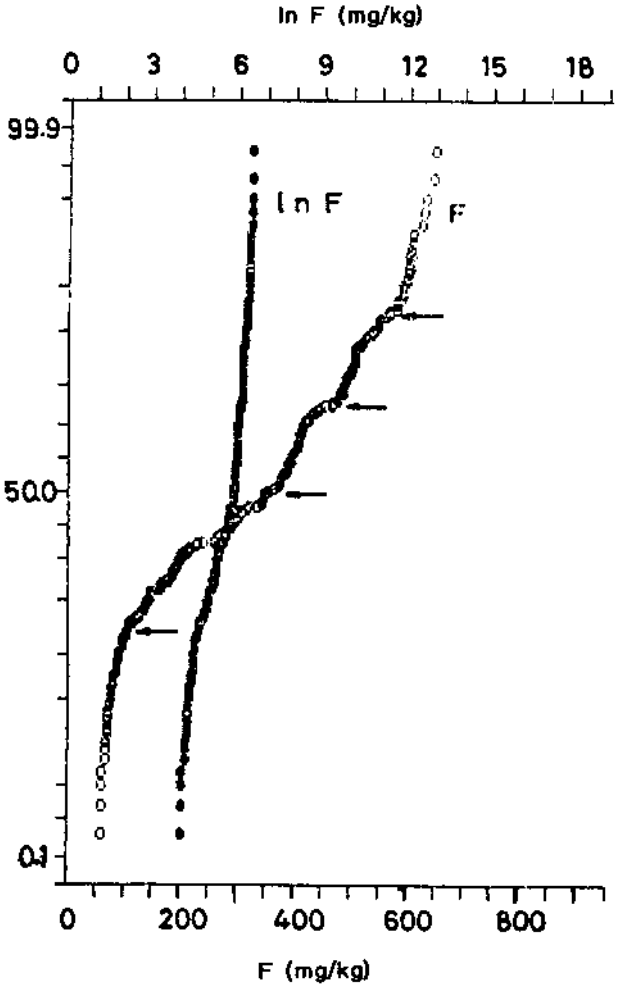
Table 3

Statistical Parameters of F in Surface Soil Samples of Different Terraces

Terraces	Mean m	Variance s^2	Standard Deviation s
P_1	196	865	29.41
P_2	364	13124	114.56
P_3	378	11071	105.22
P_4	352	8743	136.91

Figure 2

Cumulative Frequency Plot for F in Yamuna Alluvial Plain, India.



Soil F was correlated with different soil properties (Table 4). Clay (r = 0.89**), silt + clay (0.75**), amorphous Fe (r = 62**) bear significant correlation, whereas allophanes, P, K, mica and molar ratios of Si, Al, Fe, Ca, Mg, K, and Na are not significantly correlated with soil F. Molar ratios were evaluated to assess the effect of weathering, P and K to find the origin of F.

Multiple regression analysis, performed to determine individual soil property contribution toward variation in soil F, indicated that 94 percent (R^2 = 0.94) variation in F was due to these properties. Clay alone explained 68 percent

variation in soil F, whereas silt + clay, amorphous Fe and pH contributed 19, 5 and 2 percent respectively. Multiple regression equation relating percentage of clay (X_1), silt + clay (X_2), O.C. (X_3), amorphous Fe (X_4), allophanes (X_5) and soil pH (X_6) to soil F (mg/kg) is:

$$F = -61.59 + 9.47 X_1 + 0.89 X_2 - 9.55 X_3 + 67.23 X_4 - 90.27 X_5 + 6.84 X_6$$

Discussion

As stated in the results, F in sand and silt fractions decreased from P_1 to P_4. The dominance of biotite mica (19) may be responsible for high F in sand and silt fractions of recnt soils. The release of F as a result of biotite weathering and its subsequent leaching to sub-surface horizons have resulted in depletion of F in coarse fraction of surface horizon in older soils. The release of F is supported by a high amount of W.S.F. in older soils. The presence of amorphous silica in older soils also supports this view. F in soil and clay size range in soil F and clay size increased in sub-surface horizons with soil age due to the fact that the high amount of released F is adsorbed by clay (20) in sub-surface horizons. The highest adsorption maximum (715 mg/kg) was observed in $B_{31}gt$ horizon of P_4.

The increase in soil F with depth in older soils is explained by leaching of F with soil colloids as indicated by its strong relationship with clay ($r = 0.89^{**}$) where 68 percent variation in soil F is explained by clay alone. The spatial distribution of F failed to coincide with the development sequence of soils as indicated by non-significant difference of mean and coefficient of F variance in various soils, except P_1. Results of the graph plotted on a probabilty paper likewise indicated that 5 populations of soil F were mainly based on soil physiography and independent of development sequence.

Conclusion

Findings of the foregoing are as follows:

1. Mica (illite) may be the possible source of soil F.
2. Sand and silt fractions released a large amount of F in the course of weathering; subsequently soil colloids in sub-surface horizons of older soils were leached and adsorbed.
3. F distribution in soil profile closely follows soil development trends which are especially well expressed by soil colloids.
4. The spatial distribution of F fails to coincide with the development sequence, but is mainly explained by soil physiography.

References

1. Fleischer, M. and Robinson, W.D.: Some Problems of the Geochemistry of Fluorine. R. Soc. Can. Spec. Pap., 6:58-75, 1963.
2. Steinkoeing, L.A.: The Relation of Fluorine in Soils, Plants and Animals. Ind. Eng. Chem., 11:463-465, 1919.
3. Gommeil, G.D.: Fluorine in New Zealand Soils. N.Z. J. Sci. Teh., 27B:1657-1662, 1959.

4. Nommic, H.: Fluorine in Swedish Agricultural Products, Soils and Drinking Water. **Acta Polytect.**, 127:1-121, 1953.
5. Vinogradov, A.P.: **Geochemistry of Rare and Dispersed Chemical Elements in Soils.** 2nd Ed., Consultants Bur. Enterprises, New York, 1957.
6. Omueti, J.A.I. and Jones, R.L.: Regional Distribution of Fluorine in Illinois Soils. **Soil Sci. Soc. Am. J.**, 41:771-774, 1977.
7. Thomas, Josephus, Jr., Glass, H.D., White W.A., and Trandel, R.M.: Fluoride Content of Clay Minerals and Agillaceous Earth Materials. **Clays and Clay Miner.**, 25:278-284, 1977.
8. Korting, S.: Ein Beitrag zur Geochemie des Fluor. **Geochim. Cosmochim. Acta**, 1:89-116, 1951.
9. Omueti, J.A.I. and Robert, L. Jones: Fluorine Distribution with Depth in Relation to Profile Development in Illinois. **Soil Sci. Soc. Am. J.**, 44:247-249, 1980.
10. Shanwal, A.V. and Ghosh, S.K.: Soil in Surfaces Differing in Age, Yamuna Alluvial Plain, India. **Pedologie**, 37:169-186, 1987.
11. Soil Survey Staff: **Soil Survey Manual. Handbook No. 18.** USDA, 45, Govt. Office, Washington, 1951.
12. Anderson, J.U.: An Improved Pretreatment for Mineralogical Analysis Containing Organic Matters. **Clays, Clay Miner.**, 10:380-388, 1963.
13. Babko, A.K. and Phillipenko, A.I.: **Photometeric Analysis Methods of Determination of Non-metals.** Mir Publishers, Moscow, 1976, pp. 1-371.
14. Fox, E.I. and Jackson, W.A.: Steam Distillation of Fluorine from Perchloric Acid Solutions of Aluminoferrous Ores. **Anl. Chem.**, 31:1657-1662, 1959.
15. Megregian, S.: Rapid Spectrophotometeric Determination of Fluoride with Zirconium-Erichrome Cyanine R. Lake. **Anal. Chem.**, 26:1161-1166, 1954.
16. Jackson, M.L.: **Soil Chemical Analysis.** Prentice-Hall, Englewood Cliffs, (New Jersey), 1970.
17. Jackson, M.L.: **Soil Chemical Analysis — Advanced Course,** Dept. Soil Science, University of Wisconsin, Madison, (Wisconsin), 1976.
18. Lapeltier, C.: A Simplified Statistical treatment of Geochemical Data by Graphical Representation. **Econ. Geol.**, 64:538-550, 1969.
19. Shanwal, A.V.: Pedogenic Characterisation of Yamuna Aluvial Plain, Haryana India. Doctoral Thesis, Indian Agricultural Research Institute, New Delhi, India, 1985.
20. Shanwal, A.V.: Fluorine Adsorption by Soils and Soil Clays of Yamuna Alluvial Plain, India. **J. Ind. Soc. Soils Sci.**, [Submitted], 1987.

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BLOOD SERUM ALKALINE PHOSPHATASE IN RATS FOLLOWING DEXAMETHASONE AND FLUORIDE SUPPLEMENTATION

by

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SUMMARY: Alkaline phosphatase activity in the blood serum of rats has been studied following heavy dexamethasone (a glucocorticoid) administration as well as after fluoride supplementation.

A significant increase in blood serum alkaline phosphatase activity has been recorded following fluoride supplementation which is considered indicative of increased osteoblastic stimulation as a result of fluoride intake. Dexamethasone, on the other hand, failed to alter significantly blood serum alkaline phosphatase activity.

KEY WORDS: Alkaline phosphatase; Blood serum; corticoids; Dexamethasone; Fluoride supplementation; Osteoblastic and osteoclastic activity; Osteoporosis.

Introduction

Albright et al. (1), who first reported fluoride (F) supplementation for management for osteoporosis, were subsequently followed by many workers who used heavy F supplementation to arrest developing osteoporosis (2). The efficacy of F in the management of osteoporosis, although accepted, has been interpreted differently and is perhaps two pronged:

(i) F supplementation increases bone apatite crystallinity (3) which renders bones more resistant to chemical dissolution (4,5), reduces both bone resorption (6) and osteoclastic activity (7).

(ii) Increased skeletal density, following fluoride ingestion, is likewise attributed to new bone formation (8) owing to osteoblastic stimulation (9,10) and because of F substitution (8).

(iii) On the other hand, heavy and prolonged corticoid administration had been used to produce experimental osteoporosis in many laboratory animals (11).

(iv) Blood serum levels of alkaline phosphatase have been directly related to the degree of osteoplastic stimulation and hence provided a fairly good indication of new bone formation (12-14).

Materials and Methods

To determine blood serum alkaline phosphatase following F supplementation, 21 rats weight of which ranged between 140-160 g housed in the animal

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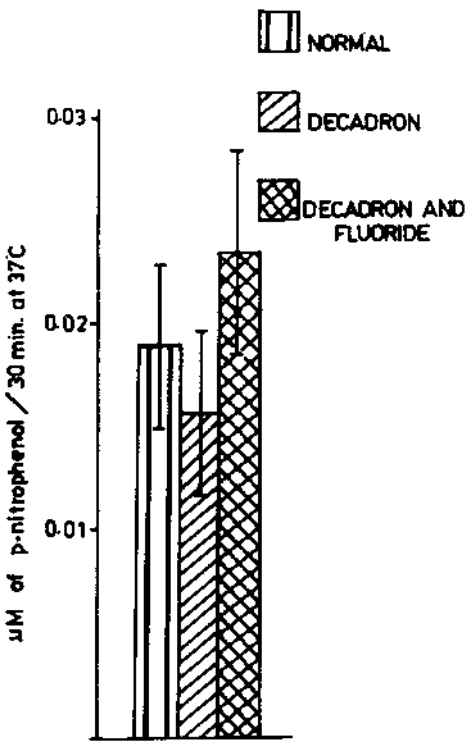
house and maintained at room temperature prior to being sacrificed, were provided laboratory feed (Hindustan Lever, Bombay) and water (F content less than 1.5 ppm) ad libitum unless indicated otherwise. Experimental animals were segregated into 3 groups (A, B and C) of 7 rats each. Group A, the controls (untreated), were administered normal (0.9%) saline (0.1 mL each) subcutaneously, twice a week for 10 weeks. Groups B and C were administered Decadron (Dexamethasone, sodium phosphate, a Merck Sharpe & Dohme product) at a rate of 400 µg/0.1 mL/animal subcutaneously twice weekly for 10 weeks. During Decadron treatment, the rats had free access to tap water as in Group A. After the Decadron treatment was completed, Group C rats were kept on fluoridated water (200 ppm F as NaF in tap water) for another 6 weeks during which Groups A and B continued to receive tap water as before.

Alkaline phosphatase activity in blood serum was estimated according to the Bassey et al. (14) method. The unit of enzyme activity was defined as µM of p-nitrophenol liberated per 30 minutes at 37°C.

Figure 1

Alkaline Phosphatase Activity in Blood Serum.



Results

Blood serum alkaline phosphatase activity dropped marginally although insignificantly following Decadron treatment (Group B). The present observations, showing marked and statistically significant increase in the serum alkaline phosphatase in the fluoridated group compared to the Decadron-treated group confirms the work of Rich et al. (12) who related elevated plasma alkaline phosphatase activity to accelerated bone formation in fluoridated animals. Similar results were recorded by Miller and Shupe (15) in cattle and by Deshmukh et al. (16) in rats.

These data indirectly support many earlier workers who reported enhanced osteoblastic activity for which enhanced plasma alkaline phosphatase is considered a marker parameter in the wake of F supplementation (8-10). Insignificant fall in serum alkaline phosphatase following Decadron treatment compared to control animals can be related to the established failure of corticoids to create osteoporosis-like manifestations in the rat because of highly efficient gut uptake of calcium in these animals (17,18). Urist (19) reported no significant fluctuations in levels of this enzyme in osteoporotic patients.

References

1. Albright, F.: Cushing's Syndrome. **Harvey Lectures.** 38:123-186, 1942-1943.
2. Cohen, P., Nicholas, G.L. and Banks, H.H.: Fluoride Treatment of Bone Rarefraction in Multiple Myeloma and Osteoporosis. **Clin. Orthop.**, 64:221-246, 1969.
3. Armstrong, W.D. and Singer, L.: Distribution in Body Fluids and Soft Tissues. In: **Fluorides and Human Health.** Geneva, 1970, p. 94-104.
4. Adams, P.H. and Jowsey, J.: NaF in the Treatment of Osteoporosis and Other Bone Diseases. **Ann. Intern. Med.**, 63:1151-1155, 1965.
5. Eanes, E.D., Zipkin, I., Harper, R.A. and Posner, A.S.: Small Angle X-ray Diffraction Analysis of the Effect of Fluoride on Human Bone Apatite. **Arch. Oral Biol.**, 10:1961-1973, 1965.
6. Gedalia, I. and Binderman, I.: Effect of Fluoride on Hypervitamin D in Rats. **J. Dental Res.**, 45:825-829, 1966.
7. Faccini, J.M.: Inhibition of Bone Resorption in the Rabbit by Fluoride. **Nature**, 214:1269-1270, 1967.
8. Cass, R.M., Croft, J.D. Jr., Perkins, P., Nye, W., Waterhouse, C. and Terry, R.: New Bone Formation in Osteoporosis Following Treatment with NaF. **Arch. Int. Med.**, 118:111-116, 1966.
9. Jowsey, J., Phil, O., Kelly Patrick, J. and Riggs, B.L.: Quantitative Microradiographic Studies of Normal and Osteoporotic Bone. **J. Bone Joint Surgery**, 47A:785-872, 1965.
10. Parkins, F.M.: Fluoride Therapy for Osteoporotic Lesions. **Ann. Otol. Rhinol. Laryngol.**, 83:626-634, 1974.
11. Sterner, W., Messow, C. and Schultz, A.: Osteoporotic Bone Changes due to Immobilization and Their Treatment. **Med. Welt.**, 26:1146-1153, 1975.
12. Rich, C., Ensink, J. and Ivanovich, P.: The Effects of NaF on Ca Metabolism of Subjects with Metabolic Bone Diseases. **J. Clin. Invest.**, 43:545-556, 1964.
13. Guyton, A.C.: **Text Book of Medical Physiology**, Saunders, Philadelphia, 1981, p. 981.
14. Bessey, O.A., Lowry, O.H. and Brock, M.J.: A Method for Rapid Determination of Alkaline Phospatase with 5 ml of Serum. **J. Biol. Chem.**, 164:321-324, 1946.
15. Miller, G.W. and Shupe, J.L.: Alkaline Phosphatase Activity as Related to Fluoride Ingestion of Dairy Cattle. **Amer. J. Vet. Res.**, 23:24-31, 1962.
16. Deshmukh, D.S., Meranger, J.C. and Shah, B.G.: The Effect of Dietary F on Ca and P Metabolism. **Can. J. Physiol. Pharmacol.**, 48:503-509, 1970.
17. Kanwar, K.C., Dhar, S. and Chhabra, R.: Fluoride Supplementation and Skeltetal Calcium Turnover. **Ind. J. Exptl. Biol.**, 25:238-239, 1987.
18. Yasamura, S. and Ellis, K.J.: Effect of Graded Doses of Cortisol on Total Body Ca in Rats. **Amer. J. Physiol.**, 231:1760-1763, 1976.
19. Urist, M.R.: Observations Bearing on the Problem of Osteoporosis. In: Rodahl, K, Nicholson, J.T. and Brown, E.M., Eds: **Bone as a Tissue.** McGraw-Hill Book Co., Inc. New York, 1960, pp. 18-45.

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FLUORIDES IN BLAST-FURNACE GAS CLEANING SLUDGE

by

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SUMMARY: The genesis of blast-furnace gas cleaning sludge is described. The major components of the sludge matter were determined by conventional methods. The fluoride content was determined spectrophotometrically.

KEY WORDS: Blast-furnace gas cleaning sludge; Insoluble fluoride; Spectrophotometry.

Introduction

The use of blast-furnace gas as a fuel is based on its high calorific value of 900 kcal/m^3. However, along with blast-furnace gas, a substantial quantity of dust is discharged which reduces the heat content of the fuel gas. This leads to accummulation of dust and wear of the equipment utilizing the gas. The dust consists mainly of coke, iron ore, iron oxide fumes and lime. The dust content (about 1000 grains/100 ft^3) is not a problem as large heavy particles separate rapidly. The major difficulty is the removal of fine dust <5 μ in size (1). Blast-furnace gas is used for heating coke ovens and water tube boilers, and for these purposes the dust content must be reduced to 0.5 grains/100 ft^3. The cleaning of the blast-furnace gas is done in three stages: coarse, semi-fine and fine cleaning. The coarse cleaning is done in the dust catchers and cyclones by the dry method. The gas is then taken to the lower end of a gas scrubber, where it comes in contact with water sprayed from the top of the scrubber. The larger dust particles are deposited at the bottom, and the gas with smaller particles slowly moves up. The fine particles are led into an electrostatic precipitator where most of them are separated from the furnace gas. Effluents from the gas scrubber and electrostatic precipitator are allowed to settle in a settling tank resulting in the formation of a sludge at the bottom of the tank (2). A considerable supply of water is necessary for the blast-furnace gas cleaning at a plant, the average requirements is about 46 gal/1000 ft^3 (1). A blast-furnace plant making 1000 tons of iron per day yields 4.2 x 10^6 ft^3/hr of blast furnace gas, and requires a minimum of about 125,000 gal/hr of water in the cleaning operation of the gas (1). The effluent is usually clarified and recirculated. The sludge is often returned to the sintering plant for reuse (3).

The sludge of the blast-furnace gas cleaning plant has been examined here for its fluoride content.

Material and Methods

Sample Collection: Three samples (1 kg each) of the blast-furnace gas cleaning sludge were collected at different locations from the sludge deposit of a gas cleaning plant of a steel industry.

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Determination of Major Components of the Sludge: The major components of the samples were determined by chemical analysis following the recommended procedures (4), and the average values (in percent) were found to be as follows: Loss on ignition, 14.57%; SiO_2, 62.77%; Fe_2O_3, 3.0%; Al_2O_3, 0.70%; CaO, 4.76%; MgO, 1.7%; Sulphide, 0.99%; Sulphate, 4.49%; Chloride, 0.40%; NH_4^+, 0.31%; Na_2O, 0.42%; K_2O, 0.90%.

Fluoride Determination: 1 g finely ground and oven dried sample of the sludge matter was taken in a platinum crucible and fused with 5 g mixture of equal parts of sodium and potassium carbonates. After cooling, the fused matter was dissolved in about 100 mL of distilled water and was filtered. About 4 g ammonium carbonate was added to the filtrate and then heated on a water bath for 15 minutes to precipitate silica and aluminum, and then filtered. The filtrate was again evaporated to one fourth of its volume, and was filtered again. Thereafter, it was neutralized to phenolphthalein with sulphuric acid solution (1:3), and heated to drive off carbon dioxide. It was then diluted to 100 mL, and aliquots of it were used for the spectrophotometric determination by the zirconyl-alizarin reagent (5,6)

Results

The average concentration of fluoride (of insoluble nature) in the blast-furnace gas cleaning sludge was found to be 2.52%. No soluble fluoride was found to be associated with the sludge matter.

Discussion

The blast-furnace gas cleaning is done on a massive scale in association with steel manufacture which is a heavy industry known all over the world. The blast-furnace gas cleaning sludge is thus discharged on a sizable scale. The presence of significant amounts of fluoride in the waste requires this to be taken into consideration during the handling, disposal or reuse of this waste matter.

Acknowledgements

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References

1. Nonhebel, G.: **Gas Purification Process.** George Newnes, Ltd., London, 1964, pp. 112-119.
2. Sastry, C.R.K. and Vadivel, S.M.: **Blast Furnace Equipment.** Oxford and IBM Publishing Co., New Delhi, 1979.
3. Herbert, F. Lund: **Industrial Pollution Control Handbook.** McGraw Hill Book Co., U.S.A., 1971.
4. Vogel, A.I. (revised by Bassett, J., et al.): **Vogel's Text Book of Quantitative Analysis.** 4th ed., ELBS/Longman, England, 1986.
5. Babko, A.K. and Pilipenko, A.T.: **Photometric Analysis.** Mir Publishers, Moscow, 1976.
6. Rand, M.C., Greenberg, A.E. and Tara, M.J.: **Standard Methods for the Examination of Water and Waste Water.** 14th ed., American Public Health Association, Washington, D.C., 1975.

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DOES FLUORIDATION OF DOMESTIC WATER SUPPLIES REDUCE FRACTURE RATES IN THE ELDERLY?

by

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SUMMARY: No clear evidence exists to support the hypothesis that water fluoridation reduces osteoporotic fracture rates in the elderly.

KEY WORDS: Fluoridation; Fracture rates; Osteoporosis.

Fluoride ions are bone seekers (1), and this effect is cumulative throughout life (2). Interest in the biological aspects of fluoride arose in 1932 when industrial fluorosis was reported in cryolite workers (3) and shortly thereafter when endemic skeletal fluorosis was reported from villages in southern India (4). Apparently, fluoride is the only non-hormonal agent known to stimulate new bone formation (5). It has been used in treatment of osteoporosis since Jowsey et al. (6) demonstrated its positive effect on bone mass. Currently, a daily dose of approximately 20 mg of elemental fluoride is recommended although there are uncertainties about the optimum dose and duration of treatment (7).

Some water supplies are fluoridated at 1 ppm, resulting in a daily total fluoride intake of about 2 to 5 mg. Whether this amount of fluoride is sufficient to prevent osteoporosis in later life is questioned. If it is there might be less fractures of upper femur, vertebrae and distal forearm in the elderly. The incidence of the former continues to rise (8); it is reaching epidemic proportions (9), the financial implications of which in an aging society is obvious.

Radiological evidence of osteoporosis which has been reported by Leone and co-workers (10,11) is more prevalent in southwest U.S. areas where fluoride naturally in water supplies ranges from 0.04-0.4 ppm which is low when compared to an area with 8 ppm. A similar trend was detected in England when a low-fluoride area containing 0.2 ppm was compared to a fluoridated one at 1 ppm (12). Bone density (13-15), cancellous bone strength (16), and bone fluoride content (16-20) have also been found to be higher in areas where the water fluoride content ranges between 1 and 1.9 ppm compared to areas where fluoride levels are 0.3 ppm or less.

Is this circumstantial evidence of any clinical significance and does the fluoridation of drinking water have an effect on preventing osteoporotic fractures? Five studies found no difference in hip (21-25), wrist and vertebral (22) fracture rates in fluoridated areas compared to areas with low water fluoride. Fluoridated water contained 0.7-1.5 ppm and low fluoride water 0.3 or less with the exception of one study which failed to mention fluoride levels (25). Sowers et al (26) similarly concluded that fluoridation at 4 ppm was not associated with greater bone mass and fewer fractures. However

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this study compared an area where the water fluoride level was 4 ppm with two areas where water fluoride levels were already relatively high at 1 ppm. Bernstein et al. (27) reported a reduction in vertebral osteoporosis and fracture rates in females from areas where natural fluoride levels in water supplies were high (4-5.8 ppm) compared with low water fluoride areas (0.15-0.3 ppm). This study may be biased because elderly males had a higher incidence of vertebral collapse than females (28) and these findings cannot be extrapolated to fluoridation at approximately 1 ppm. In another controversial study, fluoridation significantly ($p < 0.001$) reduced hip fractures in men over 50 and women over 60 years of age in a fluoridated area (1 ppm) when compared to a low-fluoride area (0-0.1 ppm) between 1967-78 (29). Hip fracture rates, however, were similar in both men and women in the low-fluoride areas which suggests bias (30). These results have been widely used as an argument for fluoridation by its supporters (31-33). On the other hand, studies by Arnala (19,24,30) found no difference in fracture rates in these same areas over a similar period (1972-81). Simonen and Laitinen's data (29) were obtained from sometimes erroneous (34) hospital discharge data whereas Arnala's data came directly from hospital case reports. Furthermore, Simonen and Laitinen ignored all cases coded under sequelae of hip fractures. As the mortality from hip fractures is high, this could represent a significant omission.

In view of the foregoing no unequivocal evidence exists to support the hypothesis that fluoridation of domestic water supplies reduces osteoporotic fractures in the elderly. This does not, however, mean that fluoridation has no effect on fracture rates in the elderly inasmuch as previous studies which arrived at this conclusion leave much to be desired. The study by Goggin et al. (21) was based on only 5 years of fluoridation. Korns (22) who studied the effect after 22 years of fluoridation admitted that the study was inadequate, that it was merely a preliminary work, and stressed the need for further data. Madans et al. used hospital records of patients who sustained hip fractures in 388 counties; however actual exposure to fluoride in water was not accurately known.

Conclusion

Fluoridation remains highly controversial. There is therefore a pressing need to conduct well-planned epidemiological research to determine its long term effect on osteoporosis in the elderly.

References

1. Smith, G.E.: Fluoride and Bone: An Unusual Hypothesis. **Xenobiotica**, 15:177-186, 1985.
2. Myers, H.M.: **Fluorides and Dental Fluorosis.** Basel, S. Karger, (Myers, H.M., Ed.: **Monographs in Oral Science**, Vol. 7), 1978, p. 53.
3. Møller, P.F. and Gudjonsson, S.V.: Massive Fluorosis of Bones and Ligaments. **Acta Radiol.**, 13:269-294, 1932.
4. Shortt, H.E., Pandit, C.G. and Raghavachari, T.N.S.: Endemic Fluorosis in the Nellore District of South India. **Indian Med. Gaz.**, 72:396-398, 1937.
5. Frey, H.: Fluoride in the Treatment of Osteoporosis. **Acta Med. Scand.**, 220:193-194, 1986.
6. Jowsey, J., Schenk, R.K. and Reutter, F.W.: Some Results of the Effect of Fluoride on Bone Tissue in Osteoporosis. **J. Clin. Endocrinol.**, 28:869-874, 1968.

7. Arnaud, C.D., Christiansen, C., Cummings, S.R., et al.: Consensus Development Conference: Prophylaxis and Treatment of Osteoporosis. **Br. Med. J.**, 295:914-915, 1987.
8. Boyce, W.J. and Vessey, M.P.: Rising Incidence of Fracture of the Proximal Femur. **Lancet**, 1:150-151, 1985.
9. Bulstrode, C.: Keeping up with Orthopaedic Epidemics. **Br. Med. J.**, 295:541, 1987.
10. Leone, N.C., Stevenson, C.A., Hilbish, T.F. and Sosman, M.C.: A Roentgenologic Study of a Human Population Exposed to High-Fluoride Domestic Water. A Ten Year Study. **Am. J. Roentgenol.**, 74:874-885, 1955.
11. Leone, N.C., Stevenson, C.A., Besse, B., Hawes, L.E. and Dawber, T.R.: The Effects of the Absorption of Fluoride. **American Medical Association Archives of Industrial Health** (Now changed to **Archieves of Environmental Health**), 21:326-327, 1960.
12. Ansell, B.M. and Lawrence, J.S.: Fluoridation and the Rheumatic Diseases. A comparison of Rheumatism in Watford and Leigh. **Ann. Rheum. Dis.**, 25:67-75, 1965.
13. Groot, E.H.: Water Fluoridation — Dental Caries — Osteoporosis. Old and New Views on the Fluoridation of Drinking Water. **Nutrition and Metabolism** (Basel) [Formerly, **Nutritio et Dieta**, (Basel)], 21(Suppl. 1):208-211, 1977.
14. Melman A.P.M., Houwink, B., Pot, T., Kwant, G.W. and Groeneveld, A.: Het mineraalgehalte van bot en de fluoridering van drinkwater; een vergelijkend onderzoek in Culemborg en Tiel. **Ned. Tijdschr. Geneesk.**, 117:1728-1733, 1973.
15. Sluys Veer, J. van der, Melman, A.P.M., Pot, T., Bouwink, B. and Backer Dirks, O.: Fluoridation of the Drinking Water and Bone Mineral Content, Analysed by Monochromatic (125-I) Radiation Absorptiometry. In: Kuhlencordt, F. and Kruse, H.P., Eds.: **Calcium Metabolism, Bone and Metabolic Bone Diseases**. Springer-Verlag, Berlin, 1975, p. 138-142.
16. Alhava, E.M., Olkkonen, H., Kauranen, T. and Kari, T.: The Effect of Drinking Water Fluoridation on the Fluoride Content, Strength and Mineral Density of Human Bone. **Acta Orthop. Scand.**, 51:413-420, 1980.
17. Jackson, D. and Weidmann, S.M.: Fluorine in Human Bone Related to Age and the Water Supply of Different Regions. **J. Path. Bact.**, 76:451-459, 1958.
18. Geever, E.F., McCann, H.G., McClure, F.J., Lee, W.A. and Schiffmann, E.: Fluorinated Water, Skeletal Structure and Chemistry. **Health Services and Mental Health Administration Health Report**, 86:820-828, 1971.
19. Arnala, I.: Bone Fluoride, Histomorphometry and Incidence of Hip Fracture. University of Kuopio M.D. Dissertation, Kuopio, Finland, 1983.
20. Arnala, I., Alhava, E.M. and Kauranen, P.: Effects of Fluoride on Bone in Finland. Histomorphometry of Cadaver Bone from Low and High Fluoride Areas. **Acta Orthop. Scand.**, 56:161-166, 1985.
21. Goggin, J.E., Haddon, W., Hambly, G.S. and Hoveland, J.R.: Incidence of Femoral Fractures in Postmenopausal Women. **Public Health Reports**, 80:1005-1012, 1965.
22. Korns, R.F.: Relationship of Water Fluoridation to Bone Density in Two N.Y. Towns. **Public Health Reports**, 84:815-825, 1969.
23. Maddans, J., Kleinman, J.C. and Cornoni-Huntley, J.: The Relationship between Hip Fracture and Water Fluoridation: An Analysis of National Data. **Am. J. Public Health**, 73:296-298, 1983.
24. Arnala, I., Alhava, E.M., Kivivuori, R. and Kauranen, P.: Hip Fracture

Incidence Not Affected by Fluoridation. **Acta Orthop. Scand.**, 57:344-348, 1986.
25. Iskrant, A.P.: The Etiology of Fractured Hips in Females. **Am. J. Public Health**, 58:485-490, 1968.
26. Sowers, M.R., Wallace, R.B., Lemke, J.H.: The Relationship of Bone Mass and Fracture History to Fluoride and Calcium Intake: A Study of Three Communities. **Am. J. Clin. Nutr.**, 44:889-898, 1986.
27. Bernstein, D.S., Sadowsky, N., Hegsted, D.M., Guri, C.D. and Stare, F.J.: Prevalence of Osteoporosis in High- and Low- Fluoride Areas of North Dakota. **JAMA**, 198:499-504, 1966.
28. Saville, P.D.: Osteoporosis and Fluorides. **JAMA**, 199:47-48, 1967.
29. Simonen, O. and Laitinen, O.: Does Fluoridation of Drinking-Water Prevent Bone Fragility and Osteoporosis? **Lancet**, 1:432-434, 1985.
30. Editorial: Osteoporotic Hip Fractures and Fluoridation. **Fluoride**, 19:51-54, 1986.
31. Editorial: Fluoridation Reduces Osteoporosis. **Australian Dental Journal**, October, 372-374, 1985.
32. **Fluoridation Action Report.** British Fluoridation Society, Ltd., London, January, 1986, p. 7.
33. **Fluoridation Benefits for the Elderly.** Information Sheet B2, British Fluoridation Society, London, November, 1985.
34. Arnala, I.: Fluoridation and Hip Fractures. **The Medical Journal of Australia**, 146:451, 1987.

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THE MOLECULAR BASIS OF DENTAL FLUOROSIS

by

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SUMMARY: Due to a marked affinity between fluoride and hydroxyapatite, fluoride is stored in hard tissues of the body. Initially, fluoride exchanges with one of the ions or polarised molecules present in the hydration shell which is considered to surround ions in the crystal lattice. Next the fluoride exchanges with an ion or group at the surface of the apatite crystal. The ionic exchange occurs between fluoride ions and hydroxide or bicarbonate ions and also with fluoride ions already present in the crystal. Finally, ions present in the crystal surface may migrate slowly into vacant spaces in the crystal interior during remineralisation.

KEY WORDS: Dental fluorosis; Mechanism of fluorosis; Mineralization; Organic phase.

Introduction

The presence of fluoride in water and food is almost universal, and therefore its intake in the diet virtually inevitable. The quantity of fluoride absorbed is usually more closely related to drinking water than to diet. A linear relationship has been reported between the fluoride concentration in drinking water and the fluoride concentration in bone (1). When a fluoride compound is dissolved in water, the fluorine exists largely as the fluoride ion, F^-. However, depending on ionic concentration and pH, the fluoride is also present in solution as HF_2^- and undissociated HF. Decrease in the pH decreases the proportion of F^- while the proportion of HF_2^- and undissociated HF increases.

Discussion

Incorporation of fluoride has been shown to alter slightly the chemical composition of tooth and bone mineral. The carbonate and citrate contents decrease slightly, whereas the magnesium level increases. The calcium:phosphate ratio, however, remains essentially unchanged.

The extent of fluoride uptake by hard tissues is dependent upon the amounts ingested and absorbed, the duration of exposure and the type, region, and metabolic activity of the tissue concerned. Consequently, there is a great disparity in fluoride levels both between individuals and between types of mineralised structures. Even within tissues which appear structurally homogeneous, concentrations may vary markedly over distances of a few microns.

Fluoride is taken up most rapidly into the tooth during the phases of growth and development. In the early stages of amelogenesis and dentinogenesis, a small amount of calcium interferes little with fluoride transport;

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the overall systemic uptake of fluoride into enamel and dentine is therefore maximal during periods of formation and calcification.

Enamel has perhaps a seemingly low average concentration of fluoride; in particular, the levels in the interior of the tissue are low. When enamel has attained a minimal degree of calcification the increasing difficulty of ionic penetration produces a concentration gradient from the surface inwards. After the tooth is fully formed fluoride is incorporated chiefly in the outer regions of the enamel acquired from fluoride present in the oral fluids and from fluoride-containing dentifrices (2).

In dentine the concentration of fluoride is 2-4 times higher than in enamel. In common with enamel, dentine fluoride is not homogeneously distributed throughout the tissue. This difference is due to the impermeability of the tissue and also the chemical trapping of the element by ionic exchange at the pulpal surface. The highest concentration occurs adjacent to the odontoblast layer where the systemic blood supply is maximal; the level then falls from the pulpal border to the amelodentinal junction.

Within Western Countries use of dentifrices containing fluoride is now widespread. Although the caries-reducing effect of the fluoride has been widely reported, its mode of action is still not understood. There appear to be three main mechanisms which act probably in combination to inhibit dental caries. Firstly, the incorporation of fluoride into the enamel crystals in the form of fluoroapatite reduces their solubility during acid attack; secondly, fluoride promotes remineralisation of early enamel lesions; and, thirdly, the presence of fluoride inhibits acid production by the plaque bacteria.

Nevertheless, it is widely recognized that excess fluoride has the potential to affect the body systems adversely (3). Fluorosis was first reported from India as early as 1937, but it is only now that the environmental and biochemical effects of fluoride are beginning to be appreciated, due mainly to modern analytical techniques for investigating the metabolism of skeletal tissues. Nevertheless, studies are now beginning to emerge which indicate that fluoride exerts a major influence on the metabolism of the organic components of enamel and dentine. Fluoride can inhibit the synthesis of proteins by ameloblasts in developing enamel. It also appears that fluoride is able to inhibit the proteases involved in the transitional phase of protein mineral interactions during enamel development.

The most detailed studies, however, have centered around the influence of fluoride in vivo and in vitro on the metabolism of the organic matrices of dentine and bone, particularly the ground-substance components. Kennedy and Kennedy (4) have shown radio-autographically that a single acute dose of sodium fluoride inhibits the incorporation of radioactive sulphate into dentinal acidic mucopolysaccharides. Walton and Eisenmann (5) suggested that the organic matrix of rat incisor dentine undergoes extensive alteration during fluorosis. The main sulphated components present in ground substance are glycosaminoglycans, which are high molecular weight anionic mucopolysaccharides that are linked to a specific protein to form proteoglycans. The proteoglycans in turn interact with collagen to form the basis of the organic matrix of all mineralised tissues. It is in this medium that calcification occurs and clearly any disturbance of metabolism of the ground-substance components would presume to have a major influence on mineralisation.

The combined studies of Embery and his co-workers at the University of Liverpool Dental School and at the Cardiff Dental School, University of Wales College of Medicine, together with those of Susheela and her colleagues at the All India Institute of Medical Sciences, New Delhi, point to a number of specific structural changes in the organic matrix of mineralized tissues which result from fluoride administration. The primary sulphated glycosaminoglycan which is present in most mineralised tissues is chondroitin 4 sulphate. It is notable, however, that in fluorotic tissue there is a increase in the sulphated glycosaminoglycan isomer dermatan sulphate (6,7). The importance of this observation is that dermatan sulphate is found in areas of bone which have unmineralised loci. It is also notable that dermatan sulphate is the primary glycosaminoglycan present in skin and in many soft tissues. It is therefore reasonable to expect that the presence of dermatan sulphate is linked very closely with the lack of ability of a particular tissue to undergo mineralisation.

Any derangement in ground-substance metabolism would therefore be of great importance during the impairment of mineralisation in dental fluorosis. The work of Embery and Smalley (8) has also shown that the glycosaminoglycans and the proteoglycans present in the developing rat incisor undergo a reduction in molecular size and charge in the fluorotic state. Additionally, fluoride is also known to inhibit the incorporation of radiosulphate into chondroitin-4-sulphate by isolated odontoblasts.

The influence of fluoride on connective tissue metabolism in the tooth is also not restricted to changes in the ground substance, since Susheela and her co-workers have observed that the collagen structure is also altered during fluorosis. For example, during fluoride ingestion there is a reduction in collagen content and a reduction in uptake of proline and lysine into mature collagen. Fluoride also reduces the rate of hydroxylation of proline and lysine in bone collagen, and there is evidence concerning a reduction in the collagen cross-link precursors. Additionally, the work by Embery's group has shown that protein conponents appear in fluorotic urine which are not present in the normal state and may be evidence of prognostic indicators of ensuing fluorosis.

Conclusion

It appears that fluoride affects the skeleton and the mineralised tissues of the body in a very marked way, not only the inorganic phase of mineralised tissues, but also the organic phase which is altered quite dramatically in the presence of fluoride. The ground-substance components and the fibrous proteins of mineralised tissue undergo a marked alteration in structure and metabolism during fluorosis. The combined studies indicate that new compounds appear in the presence of fluoride which are normally detected in skin and other soft tissues where mineralisation does not occur.

In spite of research carried out in the past years, neither a reliable prognostic test for early detection of fluorosis nor a treatment for the disease is currently available. Tests for early detection of fluorosis and therapeutic measures are likely to emerge when the nature of fluoride action on calcified and non-calcified tissues is understood in precise scientific terms. Although a great many people who are exposed to fluoride become fluorotic, not all of them become fluorotic to the same extent, in which case there could be some inhibitory factor which is peculiar to the latter individuals. Indentifi-

cation and characterization of such factors may be of immense value in future preventive measures.

In Western nations we may be causing our own problems in relation to fluoride through dietary constituents such as tea, through use of fluoride-containing dentifrices, and from the increasing use of fluoride tablets and gel treatment in young people. In women showing indications of osteoporosis, the administration of fluoride is one of the preventive measures used to strengthen the inorganic phase of bone. Thus we may be causing a small-scale problem in certain individuals in relation to the effects of fluoride on skeletal tissues even in modern countries like our own.

References

1. Zipkin, I., McClure, F.J., Leone, N.C. and Lee, W.A.: Fluoride Deposition in Human Bones After Prolonged Ingestion of Fluoride in Drinking Water. **Public Health Rep.**, 73:732-740, 1958.
2. Weatherall, J.A., Deutsch, D., Robinson, C. and Hallsworth, A.S.: Assimilation of Fluoride by Enamel Throughout the Life of the Tooth. **Caries Res.**, 11:85-111, 1977.
3. Susheela, A.K., Koacher, J., Jain, S.K., Sharma, K. and Jha, M.: In: Fluoride Toxicity, pp. 78-90, **Proc. 13th Conf. Internat. Soc. Fluoride** Res., New Delhi, 1985.
4. Kennedy, J.S. and Kennedy, G.D.S.: Autoradiographic Studies on the Influence of Fluoride on Sulphate Metabolism. **J. Dent. Belge.**, 1:63-68, 1959.
5. Walton, R.E. and Eisenmann, D.R.: Ultrastructural Examination of Dentine Formation in Rat Incisors Following Multiple Fluoride Insertions. **Archs. Oral Biol.**, 20:485-488, 1975.
6. Jha, M. and Susheela, A.K.: In vivo Chondrogenesis and Histochemical Appearance of Dermatan Sulphate in Rabbit Cancellous Bone. **Differentiation**, 22:235-236, 1982.
7. Embery, G. and Stanbury, J.B.: The Metabolism of Proteoglycans and Glycosaminoglycans in Dental Fluorosis. In: **Fluoride Toxicity**, pp. 65-77, Proc. 13th Conf. Internat. Soc. Fluoride Res., New Delhi, 1985.
8. Embery, G. and Smalley, J.W.: The Influence of Fluoride Administration on the Structure of Proteoglycans in the Developing Rat Incisor. **Biochem. J.**, 190:263-272, 1980.

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THE EFFECT OF GEOCHEMICAL FACTORS ON THE PREVALENCE OF DENTAL DISEASES FOR PREHISTORIC INHIBITANTS OF THE STATE OF MISSOURI

by

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(Abstracted from Am. J. of Physical Anthropology, 75:1-14, 1988)

The objective of this study was to determine whether the prevalences of periodontal diseases, corronal caries, and root caries of prehistoric inhabitants of Missouri varied among various geochemical regions of the state. Burial sites were located in different geochemical regions, and data on dental caries and alveolar bone loss were collected from 179 of the best-preserved skeletal remains of the Late Woodland (A.D. 400-900) and Mississippian (A.D. 900-1700) periods. Significant differences in caries and alveolar bone loss were found between several regions. Since fluoride concentration in surface waters in these regions did not differ significantly, differences in prevalences of dental diseases may be due to variations in geochemical and dietary factors other than fluoride.

KEY WORDS: Carbohydrates; Caries; Epidemiology; Fluoride; Periodontal disease; Trace elements.

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CARIES PREVALENCES AMONG GEOCHEMICAL REGIONS OF MISSOURI

by

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(Abstracted from Am. J. Physical Anthropology, 78:79-92, 1989)

During 1983-1984 the Missouri Bureau of Dental Health conducted a large-scale caries survey of life-long resident second- and sixth-grade children in rural communities in various geochemically defined regions of Missouri. Although caries prevalences varied significantly between certain regions, there were no significant differences overall between children drinking fluoridated water (mostly 0.8-1.2 ppm) and nonfluoridated (below 0.8 ppm) water.

Among the 3,388 sixth graders examined, the mean caries score (average number of decayed and filled teeth plus the number of missing permanent

teeth per child) was 1.93 in the fluoridated (natural or artificial) areas and 1.98 in the nonfluoridated areas. The average regional percentage of these children with caries (or fillings) was virtually identical: 62.1% in the fluoridated areas and 62.5% in the nonfluoridated areas. The same overall similarity in the percentages of children with caries was also observed among the 3,431 second graders: 65.1% in the fluoridated areas and 65.2% in the nonfluoridated areas.

Overall, among the 1,806 sixth graders in the fluoridated areas, 1,206 of them, or 66.78%, had caries (or fillings). Among the 1,582 sixth graders in the nonfluoridated areas, 1,016 of them, or 64.22%, had caries (or fillings). A similar pattern was also observed for the second graders: 1,305 out of 1,883, or 69.30%, had caries in the fluoridated areas, and 1,041 out of 1,548, or 67.25%, had caries in the nonfluoridated areas.

Within the seven geochemical regions for which the requisite data were recorded, the distribution of higher and lower caries scores and proportions of children with caries was about equally divided between the fluoridated and nonfluoridated groups, and, in some cases, the figures were essentially identical.

Also compared with the regional differences were caries prevalences among earlier (A.D. 400-1700) inhabitants (from skeletal remains; cf. C.F. Hildebolt et al.: Am. J. Phys. Anthropol., 75:1-14, 1988). Where the same surface waters are still used for drinking, an overall concordance was found between regional, coronal caries patterns of the earlier and contemporary populations. — A.W.B.

KEY WORDS: Epidemiology; Fluoride; Geochemical factors; Trace elements; Water fluoridation.

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CIRCULATING LEVELS OF SIALIC ACID AND GLYCOSAMINOGLYCANS: A DIAGNOSTIC TEST FOR ANKYLOSING SPONDYLITIS

by

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(Abstracted from Annals of the Rheumatic Diseases, 47:833-837, 1988)

Circulating levels of sialic acid (N-acetylneuraminic acid) and glycosaminoglycans (GAGs) were measured in 69 patients with spinal disorders of orthopedic interest. Osteoporosis and osteoarthrosis showed a decrease in serum sialic acid (SA) levels, but the mean SA/GAG ratio demonstrated no change

from control values. Idiopathic backache showed no difference in any of the parameters studied compared with control values. Although ankylosing spondylitis and osteofluorosis were remarkably similar in clinical and radiological features, mean ratio between the two conditions differed with statistical significance from controls suggesting that the SA/GAG ratio can be used as a diagnostic test in ankylosing spondylitis.

In a control group of eight men and two women aged 23-55 years [mean (S.D.) 32.9 years (10)], serum sialic acid concentrations ranged from 648.0 to 812.7 mg/L [mean (S.D.) 722.2 (67.1)] and their GAG concentrations from 165.0 to 225.0 mg/L [mean (S.D.) 194.6 (18.4)]. The ratio SA/GAG ranged from 3.0 to 3.97 [mean (S.D.) 3.73 (0.42)].

In seventeen patients with ankylosing spondylitis (16 male, one female) ranging in age from 16 to 50 years [mean (S.D.) 31 (8.2)] the sialic acid concentration in serum increased ($p < 0.01$); whereas GAG content did not differ significantly from controls, SA/GAG ratio increased significantly ($p < 0.01$).

In twenty osteoporosis patients (19 female, one male) aged from 32 to 70 years [mean (S.D.) 53 (11.9)], serum sialic acid concentration showed a significant decrease ($p < 0.001$), but the GAG concentration and the SA/GAG ratio did not differ significantly from controls.

Sixteen patients had osteoarthrosis (four male, 12 female) who were included in this group, aged from 40-60 years [mean (S.D.) 50.0 (6.0)]. Decrease in serum sialic acid concentration was significant ($p < 0.001$). The slight decrease in GAG concentration was not statistically significant and the ratio of SA to GAG remained unaltered.

Ten patients with ideopathic backache (eight male, two female) were 18 to 40 years old [mean (S.D.) 31.5 (8.2)]; sialic acid and GAG concentrations in serum did not differ significantly; the SA/GAG ratio was also unchanged compared with controls.

In six patients with osteofluorosis (four male, two female) aged 19 to 60 years [mean (S.D.) 37.3 (15)] sialic acid content in serum decreased significantly ($p < 0.001$) whereas the GAG concentration increased slightly. The increase in GAG concentration was not statistically significant. The decrease in SA to GAG ratio was highly significant ($p < 0.001$) compared with controls.

KEY WORDS: Ankylosing spondylitis; Glycosaminoglycans; Idiopathic backache; Osteoarthrosis; Osteofluorosis; Osteoporosis; Sialic acid.

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FLUOROSIS – EARLY WARNING SIGNS AND DIAGNOSTIC TEST

by

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(Abstracted from the **Bulletin of Nutrition Foundation of India**, Vol. 10, April, 1989)

Since early 1930, when dental fluorosis was first detected in India, it has been considered a disease affecting exclusively teeth and bones. Regarding skeletal fluorosis, radiographs only reveal late characteristics of the disease namely interosseous membrane calcification, enhanced bone density and bone mass. A blood test for differentiation of fluoride toxicity from other bone disorders is now available.

Early warning signs of fluoride intoxication in subjects residing in endemic areas are nausea, loss of appetite, gas formation and nagging pain in the stomach, chronic diarrhea, chronic constipation, persistent headache. The gastro-intestinal system is one of the body systems most sensitive to adverse effects of fluoride. An individual may manifest one or a few of the above complaints. Case histories are now available which establish the correlation of fluoride toxicity with gastro-intestinal problems. Unusual fatigue, loss of muscle power, weakness and pain, excessive thirst and frequent urination, depression, tingling sensation in fingers and toes constitute additional early complaints related to the neuromuscular system.

Teeth, although not discolored, tend to fall out; an individual may become edendulous at an early age or have allergic manifestations which, although nonspecific in subjects living in fluorosis-endemic areas, should arouse suspicion. Prompt intervention in response to these early warning signs (i.e. changing to safe drinking water) has provided considerable relief in these cases within a short span of time. Biochemical abnormalities in two important matrix constituents, namely sialic acid (SA) and glycosaminoglycans (GAG) constitute a diagnostic test for fluorosis. Circulatory levels of SA and GAG (designated as SA/GAG test), are reduced to almost 30 percent of normal in fluoride toxicity and fluorosis.

The test is useful in distinguishing fluorosis from ankylosing spondylitis, a similar clinical condition. Unlike in fluorosis in which SA/GAG values are depressed, in ankylosing spondylitis they are significantly elevated. They are not significantly changed in arthritis, osteoporosis and spondylosis.

To avoid the misdiagnosis of fluorosis and ankylosing spondylitis this blood test should be routine in hospital laboratories in endemic fluorosis areas.

KEY WORDS: Diagnosis; Early signs; Fluorosis; Glycosaminoglycans; India; Osteoporosis; Sialic acid; Symptoms.

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EFFECT OF FLUORIDATED SUCROSE ON RAT CARIES

by

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(Abstracted from **Caries Res.**, 22:232-236, 1988)

The present study was designed to test the effect of frequent pulses of low fluoride levels on rat caries when supplied in a standarized cariogenic rat diet containing 67% sucrose (MIT-200). The test diets were variants of Diet MIT-200 in which the sucrose component had been fluoridated with NaF solution resulting in total concentration of 0 (control), 2, 3, 5, 10, or 20 ppm fluoride in the final diets. Rats received one of the test lots 17 times daily in a programmed feeding machine beginning at age 22 days, and were inoculated with Streptococcus mutans at age 23, 24, and 25 days. After 5 weeks, the rats were sacrificed and their mandibular molars scored for number and severity of sulcal buccolingual and proximal caries. Frequent daily pulses of as little as 2 ppm fluoride in dietary sucrose were effective in significantly ($p < 0.01$) reducing buccolingual rat caries.

KEY WORDS: *Cariogenic diets; Fluoride pulses; Rat caries; Test diets.*

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EFFECT OF SUBACUTE DOSAGE OF FLUORIDE ON MALE MICE

by

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(Abstracted from **Toxicology Letters**, 44:21-29, 1988)

In mice to which a sublethal concentration (one-tenth LD_{50}) of fluoride (F) (5.2 mg F/kg body weight) was administered daily for 35 days, both body weight gain as well as food and water consumption decreased. Moreover red blood cell counts decreased significantly whereas white blood cell counts increased Albumin, total protein, cholesterol, glucose and alkaline phosphatase activity in serum declined.

Body weight of control mice increased steadily until day 22. In mice administered fluoride weight gain failed to change considerably until day 19, after which body weight declined. A true comparison of body weight of fluoride-administered mice with that of control animals was low at day 1. However, on day 19 body weight of control animals reached that of fluoride-administered mice. No statistically significant relationship was seen between

the weight of the animal and food and water consumption in fluoride-administered mice. However, in control animals the relationship was significant. In fluoride-administered mice, on the other hand, blood cell counts (at the end of 35 days) showed a significant decrease in RBC ($p < 0.05$) and an increase in WBC ($p < 0.05$). In these mice also, decrease in lymphocytes ($p < 0.05$) and hemoglobin level ($p < 0.05$) and increase in monocytes, basophils and eosinophils ($p < 0.05$) was significant. Albumin, total protein, cholesterol, glucose, and alkaline phosphatase in serum of fluoride-administered mice ($p < 0.05$) also decreased significantly. Kidneys of fluoride-administered mice accumulated 3.5 times more fluoride than the control animals: brain, intestine and liver accumulated 2 times and stomach 1.5 times more fluoride than controls.

KEY WORDS: Blood cell; Fluoride; Fluoride accumulation; Mice; Serum chemistry; Soft Tissue.

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FLUORIDE REDEPOSITION AND RETENTION DURING BONE TURNOVER IN LACTATING RATS

by

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(Abstracted from J. Dent. Res., 67:1213-1216, 1988)

The ability of adult skeleton to retain fluoride (F) during calcium stress was assessed in lactating rats that received 50 ppm F in water from weaning to 11 weeks of age after which they received a low-F intake and were mated. At delivery, nine dams were killed as a baseline group: 20 dams were fed a low-F diet plus distilled water during lactation. Half of the rats were subjected to additional stress namely a low-Ca intake to stimulate bone resorption. F loss was determined during lactation. Bone turnover was measured by loss of previously incorporated tritiated tetracycline (3H-TC); changes in bone Ca and F contents were compared with changes in 3H-TC content. The extent of bone resorption ranged from 16.5% in the humerus of the adequate-Ca group to 77.1% in vertebrae of Ca-deficient dams. Loss of bone F was greatest in animals with greatest loss of 3H-TC. Once F was resorbed from bone, only a relatively small portion was redeposited (0-31.4%). The low extent of F redeposition appears to be related to a low Ca deposition in lactating rats.

KEY WORDS: Bone resorption; Calcium deposition; Fluoride retention; Lactating rats.

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MEASURING LENGTH OF EXPOSURE TO FLUORIDATED WATER

by

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(Abstracted from **Community Dent. Oral Epidemiol.** 16:131-134, 1988)

Previous studies have measured subjects' exposure to fluoridated water in two ways, namely the number of years exposure to fluoridation and a dummy variable indicating the fluoridation status of the subjects' present community. The former assumes that fluoride concentrations of water supplies are constant across the years, the latter assumes that subjects have never changed their residence. A newly developed measure of lifetime fluoridation exposure (LFE), which contains residence history and fluoride levels, may reduce these sources of error.

The number of years measure and lifetime fluoridation exposure (LFE) both seem to be valid measures of fluoridation exposure. LFE also appears to be fairly insensitive to measurement error due to inaccurate recall of residence histories. Measuring fluoridation exposure with a dummy variable is not recommended.

KEY WORDS: Fluoridation; Preventive dentistry; Residence history.

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FLUORIDE THERAPY IN OSTEOPOROSIS: ACUTE EFFECTS ON PARATHYROID AND MINERAL HOMOEOSTASIS

by

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(Abstracted from **Clin. Sci.**, 75:143-146, 1988)

Acute metabolic effects of sodium fluoride therapy were studied in 41 osteoporotic patients who received large calcium supplements (33 of whom underwent simultaneous metabolic balance studies). Mean serum calcium fell transiently within 24-48 h by 0.03 ±0.07 (S.D.) mmol/L ($p < 0.001$). In a subgroup, ionized calcium fell and biologically active parathyroid hormone (bio-PTH) rose more than fivefold ($p < 0.01$). Urine calcium rose after an insignificant fall. Significantly positive pretreatment calcium and phosphorus balances failed to change overall during the first 8 days of treatment. However, when balances in two groups analyzed relative to serum changes in

patients whose serum levels changed least — sodium fluoride increased fecal calcium ($p < 0.025$) and phosphorus ($p < 0.01$) and reduced calcium balance ($p < 0.01$): a mean balance difference between the two groups of 2.1 mmol daily ($p < 0.001$) occurred. Very small changes in serum levels therefore indicate marked metabolic responses: sodium fluoride which stimulates bio-PTH activity must also enhance mineral uptake from circulation into tissue(s). By separate and opposing action(s), it inhibits intestinal calcium and phosphorus absorption, predominantly in those whose serum levels remain stable. These effects may be relevant to long-term therapeutic results.

KEY WORDS: Calcium absorption; Fluoride therapy; Mineral homeostasis; Phosphorus balance; Osteoporosis; Parathyroid hormone.

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THE MACROSCOPIC AND SCANNING ELECTRON-MICROSCOPIC APPEARANCE AND MICROHARDNESS OF THE ENAMEL, AND THE RELATED HISTOLOGICAL CHANGES IN THE ENAMEL ORGAN OF ERUPTING SHEEP INCISORS RESULTING FROM A PROLONGED LOW DAILY DOSE OF FLUORIDE

by

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(Abstracted from **Archs. Oral Biol.** 33:361-373, 1988)

Fluoride was used in sheep to test the hypothesis that diffuse opacities in enamel result from a chronic, mild disturbance to ameloblast activities. According to histological examination, ameloblasts remained in only 4 of the 7 teeth. Their regression and formation of cementum adjacent only to labial enamel were progressing abnormally.

The normal pattern of ameloblast regression in sheep requires further careful study involving a greater number of sheep. Nevertheless, even in the few teeth examined, wherein cells were still present, regression failed to proceed in an orderly fashion following fluoride dosing. Cementogenesis, where related to amelogenesis, was obviously disturbed.

KEY WORDS: Amelogenesis; Cementogenesis; Enamel opacities; Fluoride; Sheep.

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A METHOD TO ASSAY FLUORIDE IN FOODS, BEVERAGES AND DIETS

by

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(Abstracted from Caries Res., 22:210-216, 1988)

Foods and beverages containing sugars, frequently consumed as snacks between meals, contribute to dental caries in different proportions depending on such factors as carbohydrate content, textural properties, and mineral composition (calcium, phosphate and fluoride concentration). A method to assay fluoride in foods and beverages involves (1) an acid hydrolysis at 110 degrees C in a closed vial with a valve for dispensing reagents and (2) estimation of total Acid Hydrolyzed fluoride with a specific ion electrode.

Foods, beverages, and diets were analyzed for acid-hydrolyzable and free fluoride. The simplicity of the method eliminates fluoride contammination and is accurate and reproducible in determining fluoride in foods and beverages.

KEY WORDS: Fluoride assay; Fluoride in beverages; Food fluoride;

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THE INFLUENCE OF DENTIST SUPPLY ON THE RELATIONSHIP BETWEEN FLUORIDATION AND RESTORATIVE CARE AMONG CHILDREN

by

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(Abstracted from Med. Care, 26:907-917, 1988)

Of 985 children aged 9 to 14 who were insured in Washington state using dental claims from 1982 to 1985, almost two thirds with continuous fluoridation exposure lived where the number of persons per dentist was smallest. Compared to other children, these received more diagnostic and preventive services and had the highest probability of receiving restorative care. Among children who received restorations, those in this group received the fewest while the last results reflects expected reductions in caries due to fluoridation the other results may reflect providers' response to less tooth decay and increased competition for patients.

KEY WORDS: Dental services; Fluoride exposure.

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DIFFUSION OF FLUORIDES IN HUMAN DENTAL ENAMEL IN VITRO

by

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(Abstracted from Arch. Oral Biol. 31:811-814, 1986)

Findings supporting the concept that dental enamel can behave as an ion-selective membrane with certain molecular-sieve effects are reported. Diffusion rates for fluoride (F) from solutions of sodium fluoride (NaF), sodium fluorosilicate (Na_2SiF_6), and sodium monofluorophosphate (Na_2PO_3F) containing 0.1 percent F (1000 ppm) in physiological saline were determined at 23°C in a two-chamber diffusion cell divided by a 300-μm section of human premolar enamel. Mean diffusion coefficients, D ($cm^2s^{-1}x10^9$), of the three F compounds in three enamel sections were estimated at 48-hour intervals over a three-week period: NaF (4.56 ±1.58), Na_2SiF_6 (2.62 ±1.23), and Na_2PO_3F (1.68 ±0.71). Although complexed F diffused more slowly than uncomplexed, the faster diffusion of F from Na_2SiF_6 (pH 3.4) than F from Na_2PO_3F (pH 6.4) suggests diffusion as undissociated HF from Na_2SiF_6.

KEY WORDS: Fluoride diffusion; Human enamel.

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CARIES PREVALENCE AND LENGTH OF RESIDENCY IN FLUORIDATED AND NON-FLUORIDATED COMMUNITIES

by

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(Abstracted from Caries Res., 22:311-315, 1988)

To determine the relation of the caries experience of Canadian schoolchildren with length of residence in non-fluoridated Camrose (0.23 ppm) and adjacent fluoridated Wetaskiwin (1.08 ppm), 115 sixth grade children (mean age 11.94 ±0.65 yrs.) were examined in Camrose and 89 in Wetaskiwin. Mean DMFT and DMFS values were similar in both non-fluoridated and fluoridated communities, namely DMFT of 2.39 and 2.65 and DMFS of 3.40 and 3.54, respectively. For children with 5-year residence, DMFT values were 2.43 and 2.26 and DMFS values 3.35 and 2.79, respectively, for non-fluoridated Camrose and fluoridated Wetaskiwin. Although the fluoridated community had 17% less surfaces with caries, differences between the fluoridated and nonfluoridated communities were not statistically significant. Within the fluoridated

community, differences in DMFT and DMFS between children resident less than 5 and greater than 5 years were statisically significant (DMFT p less than 0.05; DMFS p less than 0.01).

When comparing regions where communities are adjacent, with and without water fluoridation, and when making decisions on fluoride supplementation levels for children who have changed residency to such communities, this type of information should be taken into account.

KEY WORDS: Canada; Dental caries; Fluoridation; Residency

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SHORT-TERM EFFECTS OF FLUORIDE AND STRONTIUM ON BONE FORMATION AND RESORPTION IN THE MOUSE

by

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(Abstracted from **Metabolism** 35:547-551, 1986)

The present investigation was undertaken to compare the short-term effects of fluoride and strontium on bone-forming and bone-resorbing cells in vivo and to determine the mechanisms whereby these elements increase bone density. In the mouse, the influence of low doses of sodium fluoride and strontium chloride on the mineral and bone metabolism was evaluated by analytic methods and dynamic bone histomorphometry.

Body growth was identical in all groups of mice as judged by the final body weight after treatment with NaF (21.1 ±1.8 g), $SrCl_2$ (22.4 ±1.8 g), or NaF + $SrCl_2$ (23.9 ±1.7 g) compared to control (21.8 ±1.6 g). Similarly, tibia length was identical in mice treated with NaF (16.0 ±0.4 mm), $SrCl_2$ (16.0 ±0.5 mm), or NaF + $SrCl_2$ (16.1 ±0.3 mm) compared to untreated animals (15.8 ±0.3 mm).

Serum phosphate tended to fall in treated mice; in the group treated with NaF + $SrCl_2$ the fall was significant.

After four weeks of treatment with NaF alone, the osteoblastic surface had increased significantly, 21.1% compared to controls. This effect was associated with a comparable increase in osteoid surface, osteoid thickness, and matrix apposition rate (MaAR) which were enhanced by 20.1%, 29.2%, and 36.3% respectively.

After four weeks of combined treatment with NaF + $SrCl_2$ the osteoblastic surface, the osteoid surface, and MaAR rose to the same extent (+17.2%, +19.8%, and +28.1%, respectively) as in mice treated with NaF alone whereas

the osteoid thickness was further increased (+43.2% v +29.2%, $p < 0.005$).

Treatment of mice with Sr for four weeks reduced the number of bone resorbing cells, whereas such effect was not observed in rats after nine weeks of treatment. The observed decrease in the number of active osteoclasts may be related to some inhibition of cellular calcium transport induced by Sr. On the other hand, bone formation was much less stimulated after short-term supplementation in mice than after long-term treatment in rats. Osteoid surface increased slightly while the osteoclast number decreased; trabecular bone density remained unaffected. The bone phosphorus content increased whereas serum phosphate levels decreased in mice treated with F and Sr.

These data demonstrate that F supplementation produces a rapid stimulatory effect on bone matrix synthesis in vivo at a dose that does not affect bone mineralization. Our data clearly show that F at low dosage level rapidly stimulates the bone matrix formation rate and that this effect can be attributed to increased osteoblastic population.

Increased trabecular bone density occurs after a short period of treatment with NaF, a finding that has been only reported in different species after long-term exposure to F.

In summary, we have shown that the mechanisms whereby F and Sr influence bone density are different due to their distinct early effects on bone formation and resorption. In contrast to long term treatment, four weeks of oral Sr supplementation decreased the number of active osteoclasts whereas osteoid formation was slightly increased.

These data indicate that fluoride exerts a direct and rapid effect on bone-forming cells. Whether fluoride also produces a rapid stimulatory effect on osteogenic mammalian cells and bone matrix production in vivo has not been investigated.

KEY WORDS: Bone formation; Bone resorption; Fluoride and bone; Mice; Osteoblast stimulation; Strontium and bone.

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INHIBITION OF ENAMEL DISSOLUTION RATES BY SODIUM FLUORIDE AND AMINE FLUORIDES IN THE PRESENCE OR ABSENCE OF STRONTIUM IONS

by

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(Abstracted from **Pharm. Actahelv.** 61:30-32, 1986)

Dissolution rates of enamel pellets were determined in acidic solution containing sodium fluoride and amine fluoride in the presence and absence of strontium ions.

There was no significant difference in the dissolution rates between sodium and amine fluoride at the low concentration (25 ppm). However, at the higher level (100 ppm), the dissolution rate of enamel in the presence of amine fluoride was about one-fifth that of sodium fluoride. Supplementation of $SrCl_2$, at 10^{-2} or 10^{-3} M concentration to the buffer solution decreased significantly the dissolution rate of enamel pellets in the presence of sodium fluoride only, whereas no additional dissolution rate decreases were observed in the presence of amine fluoride.

The decrease in dissolution rate was probably due to the resulting CaSrF--hydroxy-apatite complex. No synergistic effect of $SrCl_2$ on reduction of the dissolution rate of the enamel pellets was observed in the presence of the anine fluoride. Additional dissolution rate decrease effect may be attributed to the adsorption layer of the long chain amino group on the enamel surface thus preventing a Ca-Sr exchange at the enamel surface.

KEY WORDS: Amine fluoride; enamel dissolution, inhibition of; Sodium fluoride; Strontium fluoride.

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DENTIFRICE USAGE AMONG DANISH CHILDREN

by

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(Abstracted from J. Dent. Res., 67:1114-1117, 1988)

Dentifrice usage by 179 Danish children approximately 3, 7, 9, and 16 years old was assessed by measurement of aggregated quantities used at home during a two-week period as well as tooth-brushing habits. Mean daily usage with the same brand of dentifrice increased from 1.1 g among 3-year-olds to 1.5, 2.3, and 3.4 g among 7-, 9-, and 16-year-olds, respectively.

The relationship between the amount of dentifrice used and the diameter of the orifice was significantly positive. The length of ribbon of paste squeezed out per brushing, however, was similar, regardless of tube orifice diameter. Considering that young children swallow an average of 15 to 30% of the dentifrice which they use for brushing, it is obvious "that a notable number of the 3- and 7-year-olds can be expected to ingest fluoride from 1000 to 1500-ppm-F dentifrices in quantities exceeding recommended daily doses."

KEY WORDS: Denmark; Dentifrice ingestion; Fluoride dentifrices.

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Interpretive Review:

THE FALLACIOUS FAITH IN FLUORIDE

by

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Abstracted from J. Nat. Acad. Research Biochemists, 8:11:1086-1091, 1988)

Studies regarding the recent decline in tooth decay in the United States show similar decreases in both fluoridated and non-fluoridated areas. In some communities the pre-fluoride decline had already attained most of the improvement that would later be credited to fluoridation. According to 24 studies, considerable reductions in the incidence of dental caries occurred in unfluoridated areas, namely Australia, Denmark, Holland, New Zealand, Norway, United Kingdom, and the United States. The magnitudes of these reductions are comparable to those observed in fluoridated areas and attributed to fluoridation.

Some children have sound teeth without fluoridation and some have very decayed teeth even though they consume fluoridated water. Dental caries were practically non-existant in the Pacific Islands until the introduction of refined foods. After only five years, the incidence of decay in children was common; in adults between 40 and 50 years of age it had increased 50 percent. Unrefined foods protect teeth and refined foods destroy enamel. With white flour or refined sugar, enamel is dissolved but enamel remains healthy with whole grain flour or unrefined sugar. Several studies showed that raw milk also had a "similar protective action on the teeth."

By interfering with collagen production, fluoride interferes with the body's normal regulation of collagen mineralization. Teeth can lose their translucent appearance and develop white chalky portions or dark staining. Parts of the teeth fracture off, pits result, and even parts of the teeth break away. This condition known as mottling – yellow, brown and black stains along with pits, crevices and broken tips – has been known since the beginning of modern dentistry as caused by fluoride. There is also some evidence that topical fluoride treatments can demineralize tooth enamel and promote tooth decay.

KEY WORDS: Caries decline; Collagen interference; Fluoridation; Fluoride review; Topical fluorides.

REPRINTS: (Editor) National Academy of Research Biochemists, Inc., 137 W. Chapman Ave., Ste. 2, Fullerton, CA 92632, USA

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INSTRUCTIONS TO AUTHORS

Fluoride, the official journal of the International Society for Fluoride Research (ISFR) is published quarterly (January, April, July, October). Its scope is the publication of papers and reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference are published 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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1. **General** – No precise limit is given on the length of the paper; it should be written concisely in English, submitted in two copies, doublespaced with generous margins. Measures are given in metric system (SI).

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