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AN INVITATION TO BUDAPEST

The 17th Conference of the International Society for Fluoride Research will be held June 22-25, 1989, at the Sporthall in Budapest, Hungary. All interested scientists are welcome.

In addition to the scientific sessions and a large number of poster exhibits chaired by qualified experts as well as refreshing social programs, Hungary offers spectacular architecture, delicious cuisine, pure wines and scenic landscape.

The Organizing Committee is endeavoring to make your visit a memorable professional and cultural experience. Kindly contact, Dr. sc. med. M. Bély, National Institute of Rheumatology, Department of Morphology, H-1525 Budapest, 114. P.O. Box 54., Hungary.

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INTERNATIONAL FLUORIDATION SYMPOSIUM IN BRAZIL

On May 15-18, 1988, an international scientific symposium of considerable significance, which will undoubtedly influence the future course of debate on the risk-benefit aspects of water fluoridation, was held in Porto Alegre, Brazil. Sponsored by the Rio Grande do Sul State Section of the Brazilian Association of Sanitary and Environmental Engineers, the meeting brought together fluoride researchers representing both sides of the issue from six countries for a critical review and assessment of potential health hazards as well as the alleged enormous dental benefits of fluoridation.

Proponent speakers, in order of their presentations, included a biochemist, Jaime Cury (Brazil), and five dental researchers: Jan Ekstrand (USA, formerly Sweden), Gunnar Rolla (Norway), William H. Bowen (USA), Jack Lee (Canada), and Herschel S. Horowitz (USA).

Opponent speakers, also in order of their presentations, included two dentists, Robert Mick (USA) and John Colquhoun (New Zealand), two physicians, John R. Lee (USA) and Francisco Pompeo do Amaral (Brazil), and an applied mathematician, Mark Diesendorf (Australia). The place of a sixth opponent, biomedical researcher Albert Schatz (USA), who was unable to attend, was taken by Diesendorf, who spoke twice to restore the balance. Although most of the 400 persons who attended — mainly dentists, engineers, health administrators, and physicians — seemed to favor fluoridation, the organizers conducted the symposium with meticulous impartiality, reflecting their desire to examine the scientific differences on fluoridation.

Benefits

Both sides concurred that the prevalence of dental caries has been declining in most developed countries, including many nonfluoridated ones, over the past 20-30 years. They also agreed that tooth decay has been increasing in most developing countries over the same period, owing primarily to increased consumption of sucrose and other refined carbohydrates. But there was no agreement on the origin of the decrease in caries in developed countries.

The proponents held that the decreases were due largely to dental uses of fluoride in some form, especially topical fluorides in nonfluoridated areas. However, opponents Colquhoun and Diesendorf pointed to evidence from New Zealand as a whole, Sydney in Australia, and part of Gloucestershire in England, where the decreases had occurred before the widespread use of fluorides in any form. They proposed that changes in nutrition, oral hygiene, and possibly the immune status of the population were more likely explanations for these early decreases, although they agreed that high-concentration topical fluorides, which act directly on the external dental surfaces, would have contributed to the decreases observed during the 1970s and later.

Doubts raised by opponents about the alleged systemic dental benefits of fluoridated water were inadvertently supported by proponent Bowen. Although, according to Bowen, reduction in dental caries in rats occurred with direct exposure to fluoride in the mouth (but only at concentrations well above the "optimal" 1-ppm level advocated for humans), he stated that there was no benefit when fluoride was released slowly into the bloodstream from a body-implanted capsule.

For the most part, criticisms of studies on human populations by opponents of fluoridation were not answered by proponent speakers. Instead, they tended to focus on laboratory investigations and biochemical mechanisms for the action of fluoride on teeth. Horowitz conceded there was "some bias in the early stages of the Grand Rapids trial," but he argued that "decay reductions continued after the second dental examinations." Colquhoun observed that most of the reported spectacular caries reductions occurred mostly after one year in a much smaller group of children than in the original survey, with later reductions being much smaller or even nonexistent. DMF scores (for decayed, missing, and filled permanent teeth) of children in some age groups, after one year of fluoridation, were actually reported to be smaller than when they were a year younger. Since fluoridated water cannot turn filled teeth into unfilled ones, replant extracted teeth, or cause cavities to disappear, the reported DMF results were obviously impossible and indicated that the smaller sample of children examined after the first year was not representative of the larger original baseline group.

Critical review of past epidemiological studies, current understanding of the biochemical action of fluoride in the mouth, and various experiments on rats all seem to suggest that any anti-caries effect of fluoride is topical rather than systemic. The topical action of 1-ppm fluoride in drinking water is also likely to be much weaker than that of topical dental fluorides at concentrations of 1000 ppm or more. But if it is not necessary to swallow fluoridated water — either because its main effect is topical, or because it is relatively ineffective at 1 ppm — then the case for fluoridation, on scientific grounds, is weakened considerably.

Risks

At the conference, proponents and opponents both agreed that drinking fluoridated water during childhood produces dental fluorosis, a specific type of diffuse enamel mottling. Colquhoun cited published data showing that the prevalence and severity of dental fluorosis have increased in fluoridated areas of New Zealand and other countries. The condition reflects a disturbance of tooth formation from excessive fluoride intake during the early years of life. Thus it is a clear indication of chronic fluoride toxicity and an unavoidable side effect of water fluoridation. Colquhoun also noted that fluorosed tooth enamel is defective, being more porous and likely to become discolored or to break down, resulting in surface pitting later in life. Proponents, on the other hand, countered by citing studies indicating that prevalences of dental fluorosis had not increased. They agreed that, in any case, the condition is only a "cosmetic" effect with no significant adverse effect on health.

Both sides agreed that lifetime ingestion causes fluoride to accumulate in the bones as well as the teeth. There was also agreement that with kidney malfunction the rate of accumulation is higher. But there was disagreement on the concentrations of fluoride in drinking water which could lead to various degrees of the bone diseases known as skeletal fluorosis. Proponent Cury claimed there was no skeletal fluorosis below 8 ppm; proponent Ekstrand claimed 5 ppm was the threshold. But when confronted with the well-known classical papers by Singh and Jolly (India) and by Azar et al. (Qatar), who have each reported individual cases of skeletal fluorosis around 1 ppm, proponents stated, without citing any supporting evidence, that "other factors must have been operating", and that tropical regions like India and Qatar were irrelevant to Brazil.

Opponents emphasized the high levels of kidney disease in western countries and drew attention to reports in the medical literature of patients drinking water which contains fluoride naturally with less than 5 ppm fluoride who developed skeletal fluorosis. Furthermore, they asked whether any systematic survey for skeletal fluorosis among persons with kidney malfunction who lived in fluoridated areas has been made. (Nobody knew of any.) Opponents also suggested that skeletal fluorosis could be quite prevalent in fluoridated parts of western countries, but wrongly diagnosed as osteoarthritis.

Opponents likewise cited published papers, including a large number of clinical reports by the late George L. Waldbott and a double-blind study by Grimbergen et al., which indicate that some people suffer allergies, hypersensitivity, or intolerance reactions to 1-ppm fluoridated water or to fluoride tablets (1 mg/day). They also noted that the pro-fluoridation report of the British Royal College of Physicians had conceded that persons receiving as little as 9 mg of fluoride per day, in the course of the controversial fluoride therapy for osteoporosis, suffered similar reactions. Yet the same report also admitted that the total fluoride intake of some individuals in fluoridated regions could be as high as 12 mg/day. Proponents disregarded the published evidence of adverse health effects from fluoridated water, simply citing "authoritative" bodies, who have asserted, without performing scientific studies, that there are no ill effects from fluoride at 1 ppm.

On the subject of mutagenesis from fluoride, proponent Bowen stated that "there is not a shred of evidence." Other proponents, when presented with the 1984 reports by Tsutsui et al., which clearly demonstrated mutagenesis in human tissue cell cultures at elevated fluoride concentrations, countered that these experiments were irrelevant to water fluoridation.

On mortality in general, opponent John R. Lee pointed out that a study of death certificates in 46 U.S. cities by J.D. Erickson of the Center for Disease Control in Atlanta, Georgia, showed there was a higher mortality in fluoridated areas, after corrections were made for age, race, and sex. These differences only disappeared when further adjustments were made for population density and level of education. How arbitrary were these further, non-standard adjustments? At the end of the symposium the issue was unresolved.

Both sides agreed that bottle-fed babies drinking milk formula prepared with 1-ppm fluoridated water ingest 100 to 200 times more fluoride than infants who are breastfed, even when the mother is drinking fluoridated water. Opponents considered this fact to be grounds for concern, especially because of the possible adverse effect of these unnaturally high fluoride intakes on infants' developing immune systems. As far as anyone knew, there have been no controlled studies to investigate such a possible effect. In response, the proponents, especially the dentists, simply asserted that human breast milk is "deficient" in fluoride.

Final Outcome

In the closing session, as during the whole symposium, proponents continued to base their positions heavily on the endorsements of fluoridation by various authorities. In most cases they seemed unwilling or unable to offer specific scientific evidence to counter that presented by the opponents. Instead, they returned to endorsements or restated that all the evidence they had seen

reaffirms their belief that fluoridation is safe and effective, and that only properly qualified dental researchers (such as themselves) were capable of assessing these issues. Opponents again pointed out, as before, that the validity of scientific inquiry depends on the quality of evidence, not the opinions of vested authorities.

Finally, the proponents argued that fluoridation is especially needed by and would greatly benefit the very poor. This claim had already been countered early in the symposium by Pompeo do Amaral, who pointed out that many of Brazil's slum-dwellers do not have access to municipal water supplies. Moreover, even if they did, their health problems are the direct result of their poverty, which cannot be simply alleviated by technical fixes.

Although comprised mostly of proponents, the Commission appointed to assess the symposium did not come down on one side or the other. In its Summary Report it acknowledged that there were legitimate scientific differences about fluoridation, and it recognized "the need for more profound studies which can contribute to the searching for scientific truth which must guide any application of measures regarding public health." It also concluded that the symposium "demonstrated the importance of democratisation in dealing with social issues" and "reaffirmed the necessity for re-evaluation of postures of scientific authoritarianism or of demagogic democracy."

At the end of the symposium, speakers on both sides commended the Brazilian engineers who had initiated and organized such an outstanding event. In our view it was a major international scientific breakthrough.

Mark Diesendorf
Human Sciences Program
Australian National University
Canberra, Australia

John Colquhoun
Education Department
University of Auckland
Auckland, New Zealand

Note: The foregoing report is based in part on an earlier version by M.D. which was published recently in Social Science and Medicine (Vol. 27, No. 9, 1988, pp. 1003-1005). We thank the editor-in-chief of that journal for permission to use the material here.

MICROANALYTICAL TECHNIQUES WITH AN INVERTED PLATE-SHAPED SOLID STATE FLUORIDE ELECTRODE

by

Yu Jun* and Sun Yi-lin
Changsha, China

SUMMARY: A new plate-shaped solid state fluoride electrode utilizing soluble polymers containing electrolyte and soluble adhesive replaces the reference solution in a conventional fluoride electrode. Because of its design the electrode can be used in an inverted position: a microdrop of specimen can be placed on the sensitive membrane and the fluoride level determined. The advantage of this new electrode is that a specimen of microscopic size as small as 10 microliters can be measured.

KEY WORDS: Electrode; Fluoride; Microanalysis.

Introduction

Several years ago, methods were developed for determination of fluoride in microsamples by the hanging-drop fluoride electrode or other modification and adaptation of an electrode. However, they are somewhat cumbersome to use. In the all-solid-state fluoride electrode, the internal fluoride reference solution has been replaced by a layered structure of silver fluoride and silver wire in contact with the lanthanum fluoride crystal membrane. Although they can be inverted for determination of microsamples, these all-solid-state electrodes, too complex for most laboratories, not only need vacuum plating of a membrane, but their fabrication is also complex and time-consuming.

A new procedure of making an all-solid-state fluoride electrode which obviates these problems is described here. It utilizes some soluble polymers which contain electrolyte that replaces the reference solution in the conventional fluoride electrode. The sensitive membrane consisting of a lanthanum fluoride crystal is connected with silver-silver chloride inter-reference electrode. By means of special construction, the electrode can be used in an inverted position. A glass microreference electrode constructed from a glass tube tip pulled to the finest size was filled with a solution of agar containing 3.0 M KCl and inserted into a calomel reference electrode. It was fixed to a microadjustable elevator. A hemispherical microdrop of specimen is placed on the sensitive membrane. When the reference electrode is brought into touch with the specimen, measurements may be made.

After repeated uses, the major electrochemical characteristics of the electrode are similar to those of the conventional electrode. The most advantageous property of this new electrode is that specimens of microscopic size, as little as 10 microliters, can be used for measurements.

* From The Institute of Labor Protection, China National Nonferrous Metals Industry Company, Shu Mu Ling, Changsha, China

Materials and Methods

A plate-shaped fluoride electrode is constructed as follows:

(1) A silver-silver chloride inter-reference electrode was made by anodic oxidation of silver foil in 0.1 M HCl solution at 0.8 mA for four hours.

(2) The solid inter-electrolyte layer consisted of some soluble polymer, either polyvinyl alcohol or gelatin, and some soluble adhesive which is polyacrylic acid, polyvinyl methylether or a polyvinylpyrrolidone and polyacrylic acid mixture, and electrolyte solution which is sodium fluoride and sodium chloride. One of the soluble polymers and one of the soluble adhesives plus these electrolyte solutions were mixed in definite proportions by weight.

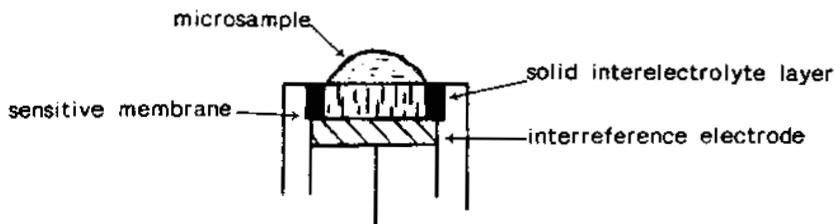
(3) To make a polypropylene thin plate, its thickness is about 1.5 mm. At the center of the plate surface, a hole has been drilled. A piece of LaF_3 crystal was fixed in this hole by a silastic adhesive.

(4) The LaF_3 crystal membrane is bound to the silver-silver chloride inter-reference electrode by inter-electrolyte. It was bound with a support, and dried under an infrared lamp at 35°C and 70% relative humidity.

The construction of the plate-shaped electrode is shown in Figure 1.

Figure 1

Inverted Electrode for Determination



A microreference electrode is constructed as follows:

(1) Heavy-wall borosilicate glass tubing, 8 mm OD, is pulled into a capillary, and a porous porcelain plug is inserted in this capillary and is fixed by sintering.

(2) The capillary tubing and glass tubing are filled with a filtered 0.2% agar containing 3.0 M KCl. The agar prevents the KCl solution from emptying out the electrode by gravity.

(3) A calomel reference electrode is inserted into the glass tubing and fixed in position.

The glass microreference electrode is attached to a retaining clip of a microadjustable elevator. The position of the retaining clip is movable.

Reagents: (1) F standard stock solution is prepared by dissolving an analytical grade NaF in deionized distilled water.

(2) The total ionic strength adjustment buffer (TISAB) has a composition of 0.22 M NaCl, 0.1 M HAC-NaAC at pH 5.00.

(3) Working F standard solution (WFSS) containing 0.05 mg fluoride per liter is prepared in 50% TISAB.

Procedures: (1) Place 5-10 microliter serum sample in a small test tube, add an equivalent volume WFSS and shake carefully.

(2) Using a micropipet, a few microliters of WFSS are dropped onto the plate-shaped fluoride electrode.

(3) Lower the glass microreference electrode body and make it touch with the sample droplet.

(4) Close the necessary electrode circuit and measure the potential.

(5) Pull up the glass microreference electrode and rinse both the reference electrode and the fluoride electrode with deionized or distilled water.

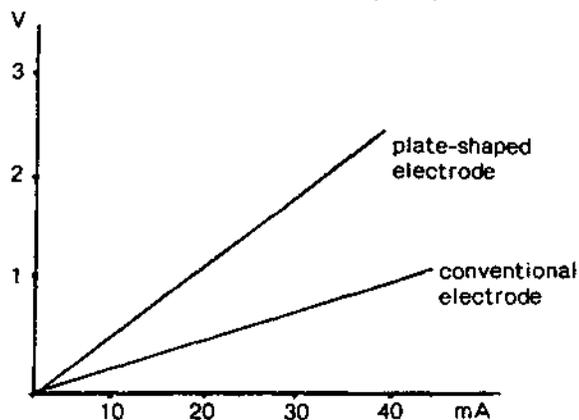
(6) Take part of the sample which had been mixed by WFSS, drop it onto the fluoride electrode and again measure the potential.

Results and Discussion

1. Electrochemical characteristics of the plate-shaped fluoride electrode: The plate-shaped fluoride electrode exhibited a linear response to fluoride ions within the concentration range 5×10^{-6} – 1×10^{-2} M in 50% TISAB, and obeyed the Nernst equation. The detection limit is 8.6×10^{-7} M which is better than the conventional electrode.

Figure 2

Voltaic Curves of the Plate-Shaped Fluoride Electrode and Conventional Electrode



The voltaic characteristics of the fluoride electrode were examined by experiment. The fluoride electrode was immersed in an electrolytic cell containing 2 M NaF solution with silver-silver chloride reference electrode, and compared with the conventional fluoride electrode under similar conditions. Figure 2 illustrates the plot of voltage vs. microamperes. Response of the fluoride electrode is similar to the conventional electrode. The plate-shaped fluoride electrode, which was constituted by a dry solid electrolyte layer, has the same mechanism of operation.

Table 1 shows the EMF reproducibility based upon repeated measurement.

Table 1
Reproducibility of the Plate-Shaped Fluoride Electrode

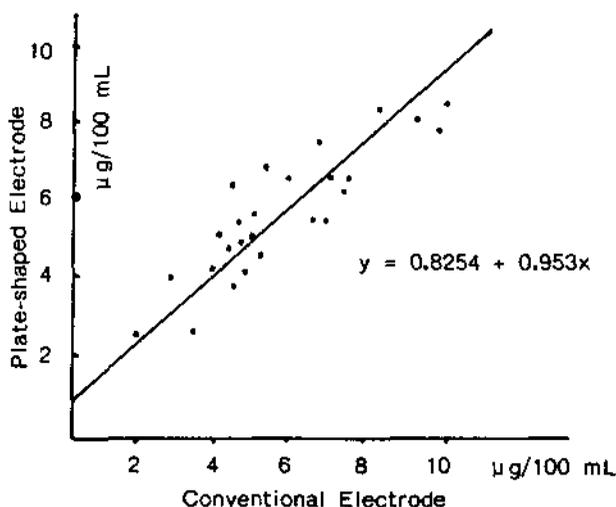
Conc. M.	First mv	Second mv	Third mv
1×10^{-3}	-63	-64	-63
1×10^{-2}	-120	-120	-121

2. Limit of Sample Volume: The experiment indicated that potentials were virtually constant even if the amount of sample volume was decreased to 5 microliters. Below 3 microliters results were not reproducible. The corresponding concentration observed was slightly higher than the actual value, or slightly lower probably because sample evaporation or leakage from the reference electrode changed the sample concentration measured.

3. Determination of Fluoride in Serum: The plate-shaped fluoride electrode was applied to the determination of forty samples of serum. All results, when compared with those of same specimens by the conventional electrode, were similar. By statistical tests, a linear regression yields a correlation coefficient of 0.935 (Figure 3).

Figure 3

Results of Serum Fluoride Measured by Two Kinds of Electrodes.



Conclusion

After continuous use for two years, the reliability of the plate-shaped electrode has been constant, proving that it is a good selective ion electrode. Compared with other modified solid-state fluoride electrodes for use with microsamples, the electrode described here is easily made and can be used with much smaller volumes of sample.

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DIFFERENCES IN SKELETAL RESPONSE TO FLUORIDE IN HUMANS AND ANIMALS: AN OVERVIEW

by

J. Franke*
Erfurt, G.D.R.

SUMMARY: From our experience with the NaF therapy for osteoporosis (158 patients with 3-5 years of treatment) 80% were good or very good responders and 20% were non-responders. Among the first group 20% of the patients reacted promptly (high alkaline phosphatase, radiologically distinct reossification of the spine after 13.3 \pm 2.8 months in comparison to 24.4 \pm 12.4 months of the average responders). In industrial fluorosis, correlation of the fluoride content in bone ash and radiological stages to exposure time (44 cases) was minimal. In addition to non-responders and average responders, a group was highly fluoride responsive (fast-responders). In animals individual as well as species differences in sensitivity to fluoride were observed. The causes for the individual reaction to fluoride are partly unknown at this time; known factors are gastric acidity, reactivity of bone cells to fluoride, urine pH, urine flow and individual renal clearance of fluoride. In renal diseases, fluoride storage in bones increased.

KEY WORDS: Fast-responders; Fluoride; Fluorosis, animals, humans; NaF therapy; Non-responders; Responders.

18 years of experience with the sodium fluoride therapy for osteoporosis has shown that patients respond differently to this treatment; some are responders and some non-responders. Of 158 patients treated 3-5 years with three different NaF preparations, pure NaF powder, strongly and mildly enteric-coated tablets, about 80% had good and very good clinical results; in only 6.3% was further intensification of complaints observed. In 13.3% complaints remained unchanged (1) (Table 1).

Distinct reossifications were detected by X-ray in 54%; in 27% reossification was uncertain. In 15% no changes in the X-ray pictures were found; 4% showed further fractures (Table 2). From these results, one can conclude that about 80-85% respond and 15-20% fail to respond. Among responders, some patients react promptly.

In the group of 82 patients treated with the mildly enteric-coated tablets (Koreberon), distinct reossification was attained (Table 3) — on an average — after 24.4 \pm 12.4 months. Of this group, 17 patients showed distinct reossification after 11-18 months (average 13.3 \pm 2.8 months) (Table 4). These patients (or 20%) are fast-responders. The alkaline phosphatase increases were mainly distinct in this group. In our therapy groups, about 20% were fast-responders, 60-65% responders, and 15-20% non-responders.

* Dept. of Orthopedic Surgery, Medical School, DDR-5010 Erfurt, Regierungsstr. 42a, G.D.R.

Table 1

Therapy Group	Painless or distinctly improved	Improved	Unchanged	Deteriorated	Total numbers	Average treatment (months)
1. pure NaF	19 (50.0%)	10 (25.7%) 75.7%	4 (10.5%)	5 (13.2%)	38	59.6 ±36.7
2. Strongly coated	17 (44.2%)	45 (39.5%) 83.7%	3 (7.9%)	3 (7.9%)	38	51.0 ±22.3
3. Mildly coated	36 (43.9%)	30 (36.6%) 80.5%	14 (17.1%)	2 (2.4%)	82	36.9 ±16.0
All Groups	72 (45.6%) 80.4%	55 (34.8%)	10 (13.3%)	10 (6.3%)	158	45.8

Table 2

Therapy Group	Distinct Reossification	Questionable Reossification	Unchanged	Deteriorated	Total numbers
1. pure NaF	14 (36.8%)	13 (34.2%)	9 (23.7%)	2 (5.3%)	38
2. Strongly coated	18 (47.3%)	12 (31.6%)	5 (13.2%)	3 (7.9%)	38
3. Mildly coated	53 (64.7%)	17 (20.7%)	10 (12.2%)	2 (2.4%)	82
All Groups	85 (53.8%)	42 (26.6%)	24 (15.2%)	7 (4.4%)	158

Table 3

Therapy Group	First Radiological Improvement (months)	Distinct Radiological Improvement (months)
mildly enteric coated (n = 82)	19.1 ±10.3	24.4 ±12.4

Table 4

	Fast-responder	Responder	Non-responder
Average time of treatment	13.3 ±2.8 months	26.5 ±12.5 months	—
Percent	17 = 20.7%	53 = 64.7%	12 = 14.6%

Variations in sensitivities to fluoride are also found in cases of industrial and neighborhood fluorosis (2-7). In two aluminum smelting workers exposed to the same fluoride influences at the same intervals of time, X-rays of one revealed severe fluorosis stage III, the other showed only the very first sign of fluorosis.

With respect to fluoride and ash content of bone in various stages of human fluorosis (5), correlations between F-exposure time and F content of bone ash were weak (Figure 1), especially in regard to the ash content of iliac crest bones (Figure 2). The average exposure time between beginning roentgenological changes and stage III fluorosis was only 3 years (Table 5). Since the fluoride concentration at the place of employment and working conditions were similar for most of the workers, strong individual differences were shown again and again in our studies (2-3,6-7,11). Apart from the non-responders and average responders, a group of highly fluoride sensitive and rapidly reacting individuals fall into Roholm's groups II or III — namely, the fast-responders.

In animals, besides individual differences in sensitivities, there are also species differences. According to Greenwood (8), Shupe and Alther (9) and Shupe and Olsen (10), cattle are the most sensitive to fluoride, followed by sheep, horses, pigs, rabbits, rats, guinea pigs, and poultry. The reason for the high sensitivity of cattle to fluoride is the negative calcium balance in dairy cows. After 2 to 3 periods of lactation, soon after calving, so-called "milk fever" with severe hypocalcemic states can develop. The other cause of this sensitivity of cattle is the prolonged period that fluorides remain in the stomach of ruminants, which provides enough time for resorption and disintegration of even highly soluble fluorides. In Figure 3, a cross section of a bovine metatarsal bone with severe fluorosis shows the extreme periosteal overgrowth: the upper right center contains the dorsal metatarsal artery surrounded by new bone formation. The original cortical bone can be seen within this overgrowth (10). This osteoporosis was caused by general intoxication by fluoride rather than an effect of fluoride on bone (clinically the animals showed signs of it).

Figure 1

Correlation Between F^- Content in Bone Ash and Duration of F^- Exposure in Patients with Industrial Fluorosis

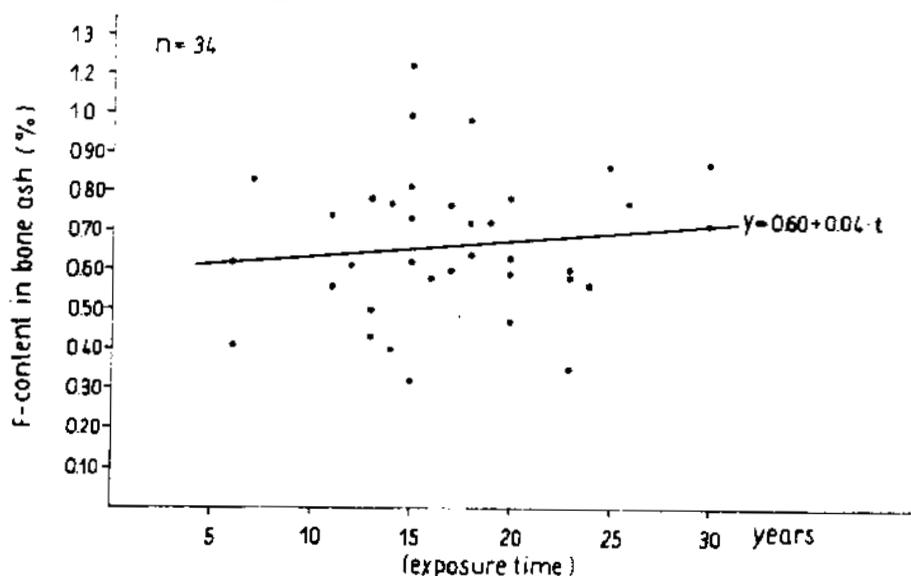


Figure 2

Correlation Between Ash Content of Bone and Duration of F^- Exposure in Patients with Industrial Fluorosis.

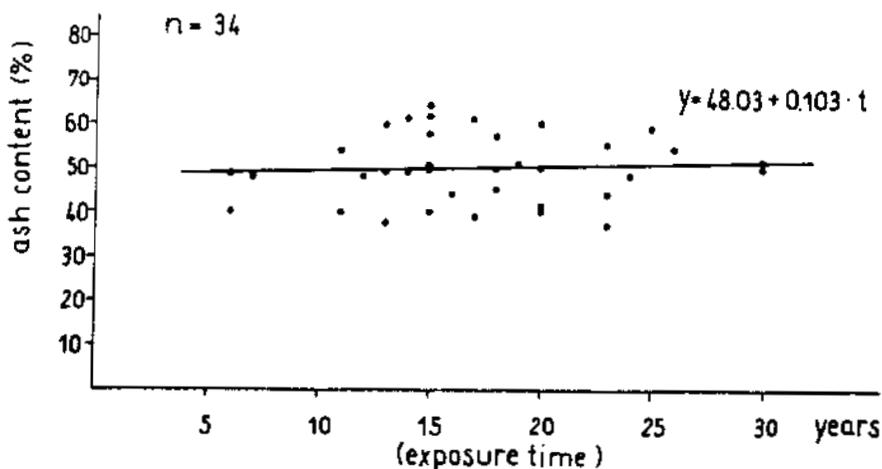
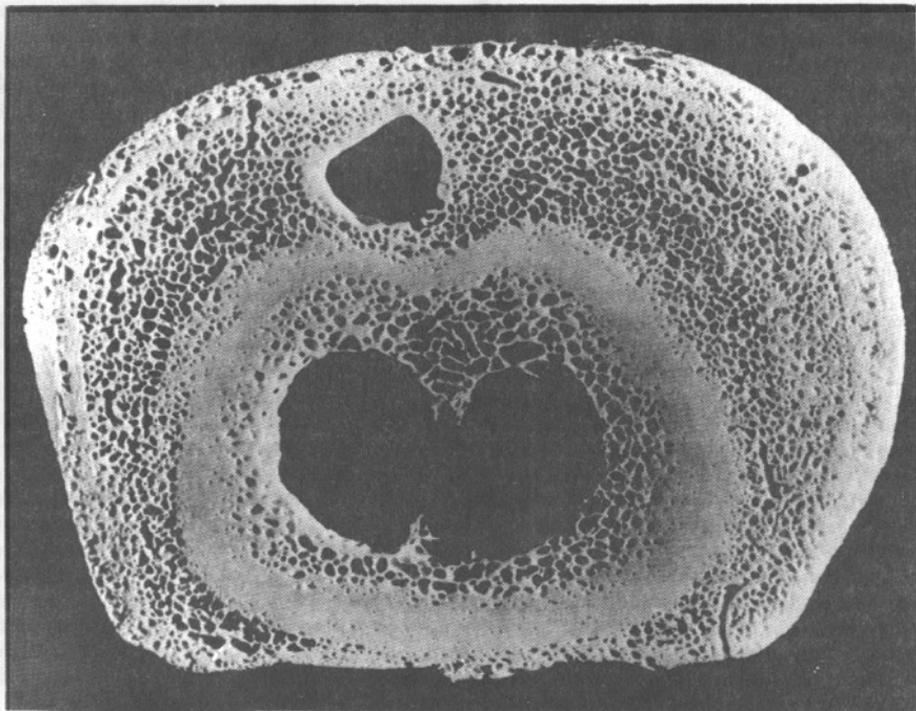


Table 5

X-ray stages	n	Age (years)	Duration of F-exposure (years)
normal	8	53.5 ±9.8	—
subtle signs	7	43.3 ±11.8	15.7 ±5.9
0-I	8	49.0 ±12.9	16.3 ±5.9
I	10	50.3 ±9.5	16.9 ±2.5
II	9	53.9 ±10.8	18.7 ±6.5
III	4	51.3 ±8.1	18.3 ±4.1
	<u>38</u>		
Fluoride contact	2	46.5 ±2.5	15
old fluorosis	4	59.8 ±5.4	17.0 ±6.8
	<u>44</u>		9.0 ±4.3 (w/o F ⁻)

Figure 3

Cross Section of a Bovine Metatarsal Bone with Severe Fluorosis [courtesy of Dr. Shupe, Logan, Utah]



Rats are markedly resistant to fluoride (2,12) (Figure 4). Young and old rats were given 3.3, 10 or 20 mg NaF/mg body weight. With the low dose after 12-15 months, histological increase in new bone formation was not visible by X-ray. The two higher doses, after 12 months had caused slight osteoporosis (Figure 5).

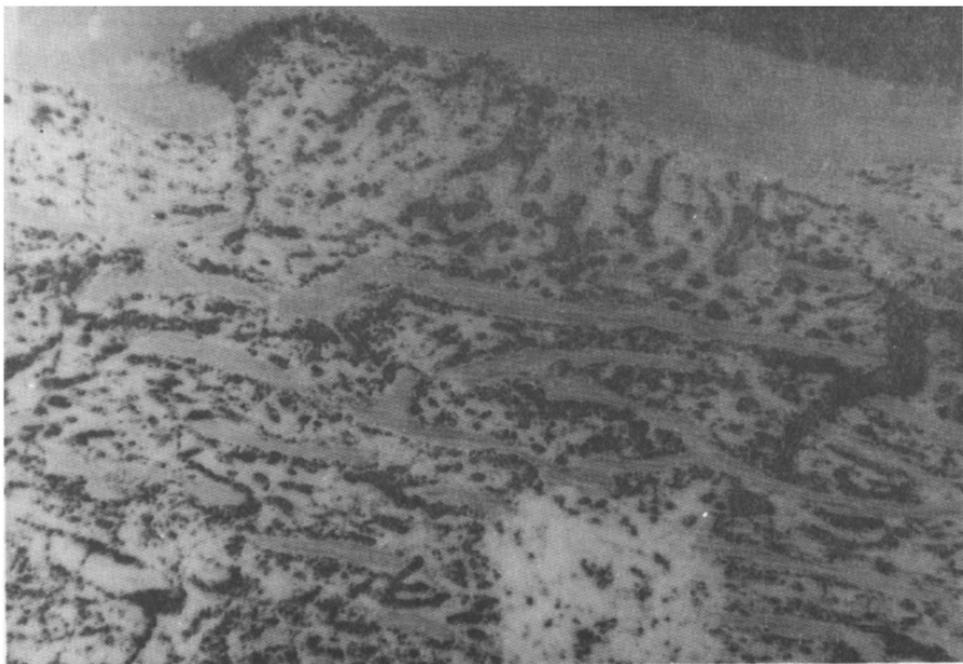
Figure 4

Histological and X-ray Results of Different NaF Doses Given to Old and Young Rats in Drinking Water.

duration	:	12-15 months	12 months	12 months
dose	:	3.3 mg/kg/day (n=16)	10 mg / kg / day (n=44)	20mg / kg / day (n=46)
		↓	↓	↓
histological results	:	small increase of bone formation	slight osteoporosis after 10 months	slight osteoporosis after 6 months
x-ray results	:	no changes	no changes	no changes

Figure 5

Vertebral Body of a Young Rat Treated with 20 mg NaF Daily for 10 Months [H.E., 1:100].



In rats also, different sensitivities to fluoride occur. During the first 3 months, some animals died, mainly old rats of the 20 mg group. Thereafter seldom did an animal die. Obviously, the fluoride-sensitive animals were eliminated after 3 months. The difference in the mortality rate between young and old animals was significant (Table 6). Young animals were more resistant to fluoride. The cause is seen in Figure 6. Young animals stored fluoride faster in their skeleton than did older ones. Old animals are more sensitive to fluoride because less fluoride is stored by bone, thereby the total organism is exposed to more fluoride.

Doubtlessly, individual reaction plays a leading role in development and severity of fluorosis. For this reason, nutritional habits, absorption capacity of fluoride through the gastrointestinal tract and renal threshold for fluoride are important. Hypoacidity or anacidity reduce F resorption. In a group of 150 aluminum smelting workers, the severity of industrial fluorosis increased

Table 6

Test Groups		Animals dead in the first 3 months	Total number of animals
Old animals	20 mg NaF/kg/day	9	21
Old animals	10 mg NaF/kg/day	5	22
Young animals	20 mg NaF/kg/day	2	25
Young animals	10 mg NaF/kg/day	0	22

Figure 6

Results of the Fluoride Determination of the Bone Ash of the 4 Experimental Groups of Rats Fed with NaF for 12 Months (Calculated Saturation Curves).

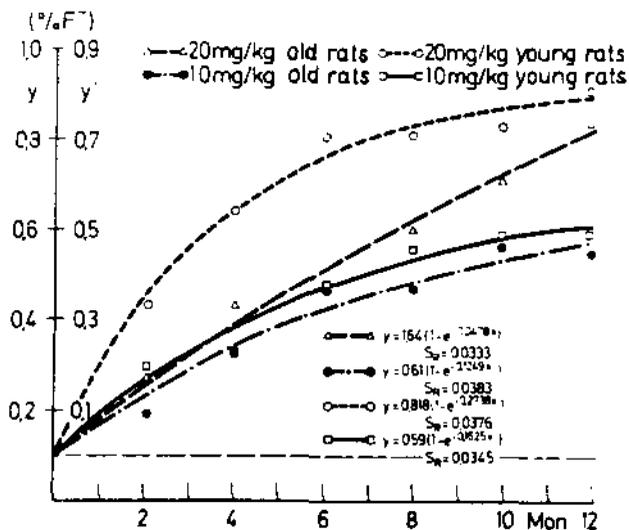


Figure 7
Correlation Between Gastric Acidity and Stage of Fluorosis

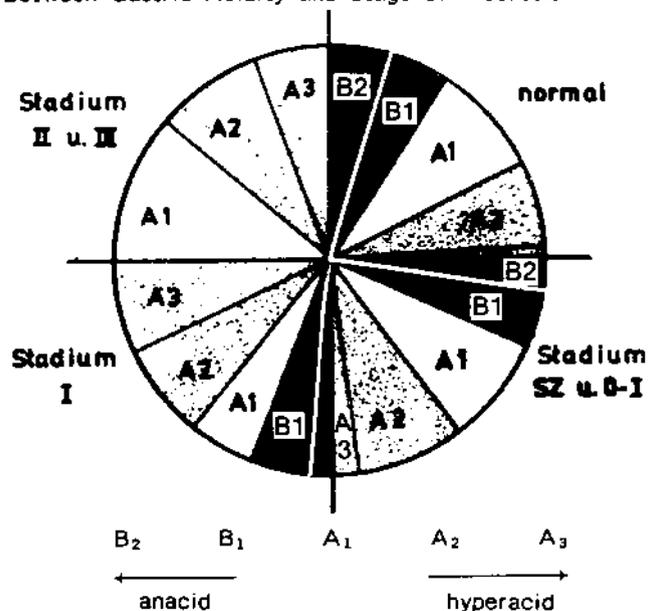
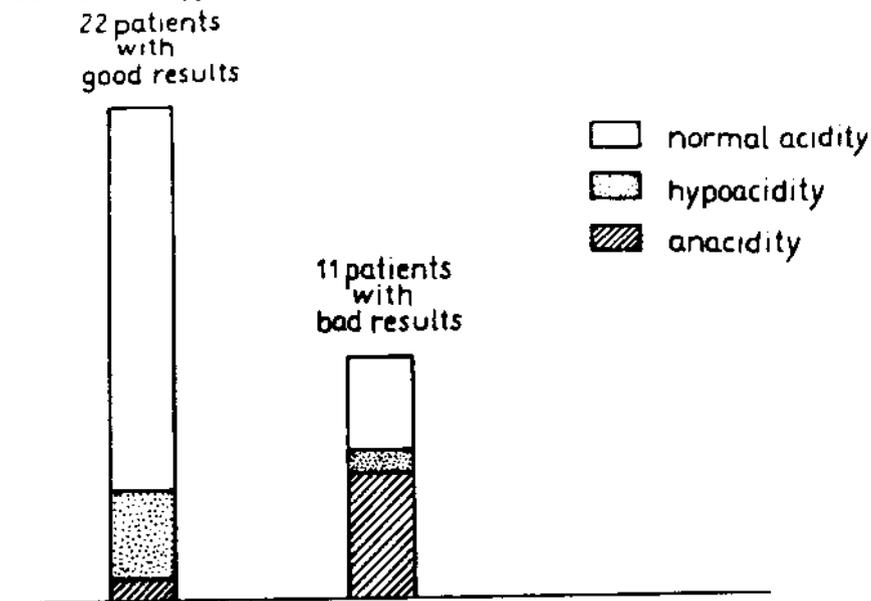


Figure 8
Results of the Determination of the Gastric Acidity in Relation to the Results of the NaF Therapy of Osteoporosis.



with the percentage of hyperacid persons, and the proportion of hypoacid or anacid persons decreased (6) (Figure 7). In fact, 8 of 11 patients treated 3-5 years with NaF without success have been hypoacid or anacid (Figure 8) (13).

Whitford and Pashley (14) who confirmed this finding, found in rat experiments that fluoride absorption from the stomach was at least 50% faster due to a pH 2.1 buffer compared with absorption due to pH 7.1 buffer. Likewise, fluoride renal clearance depends on the pH and on the volume of urine. With increasing urinary volume and with alkaline urinary pH, F-excretion increases (15-17). Consequently, the nature and composition of nutrition influence fluoride absorption from the gastrointestinal tract and renal fluoride reabsorption. Also, in patients with severe kidney diseases, fluoride excretion is diminished. Under these circumstances fluorosis can develop more rapidly than in healthy persons (18-20).

Another possibility is the reaction of bone cells to fluoride. In a female patient with severe osteoporosis showing further fractures, a control biopsy six years after therapy began, showed no histological reaction to fluoride. A fluoride value of 0.7% in ash indicated a fluorosis stage I to II. This case demonstrated that, in some individuals, the osteoblasts do not respond to fluoride.

Conclusion

It has been observed that both individual and species differences in sensitivity to fluoride occur. Known causes of these differences are pointed out. However, the many unknown factors which influence the individual response to fluoride demand further research.

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A STUDY OF EQUILIBRIUM BETWEEN IONIC FLUORIDE AND NONIONIC FLUORIDE IN SERUM OF MONKEYS WITH SKELETAL FLUOROSIS

by

Caishuang Li*, Yubin Tan, Xueping Liang, Jiyuan Fan
Tianjin, China

SUMMARY: In order to evaluate the effect of non-ionic fluoride on fluoride equilibration, we have made a model of monkey skeletal fluorosis, to examine equilibrate changes of ionic fluoride and non-ionic fluoride in serum during fluorosis (giving monkey NaF 10 mg/kg, daily, orally). Non-ionic fluoride decreased slightly at an early stage, whereas ionic fluoride increased. However when osteofluorosis became severe, non-ionic fluoride decreased gradually to the minimum value which suggested that the non-ionic fluoride might be a factor in maintaining the equilibrium of serum fluorides.

KEY WORDS: AIF molecular absorption spectrophotometry; Dynamics equilibrium; Ionic fluoride; Non-ionic fluoride; Skeletal fluorosis; Total fluoride.

Introduction

When fluoride accumulates, the level of fluoride in blood increases as well as the fluoride level of tissue, organ and bone. Serious over accumulation will induce skeletal fluorosis. In order to understand the pharmacologic and toxicologic effect of fluoride on human health, it is necessary to study the dynamics of fluoride in human blood. For this purpose, this experiment has been designed. Most scientist recognize two existing forms of blood fluoride (1-4): ionic and non-ionic fluoride. Also at least two forms of fluoride exist in blood NMR analysis (5-6). Many scientists have reported about blood fluoride, but little concern has been given to non-ionic fluoride probably because: 1. methods to determine non-ionic fluoride easily are lacking. First the blood must be ashed, then ISE (Ion Specific Electrode) or other methods used which is complicated and inaccurate. 2. In the past, it was believed that only ionic fluoride had biological activity whereas non-ionic fluoride had none. Therefore non-ionic fluoride has not received sufficient attention.

In order to evaluate the effect of fluoride on human health, the two forms (ionic and non-ionic fluoride) should be studied simultaneously. To this effect, skeletal fluorosis has been induced in the monkey for systematic observation of ionic and non-ionic fluoride changes during the process.

Method

Nine monkeys were separated into two groups, four for control, five for test. The test group was given daily 10 mg/kg NaF, by oral administration until skeletal fluorosis had emerged. During this period, blood was taken inter-

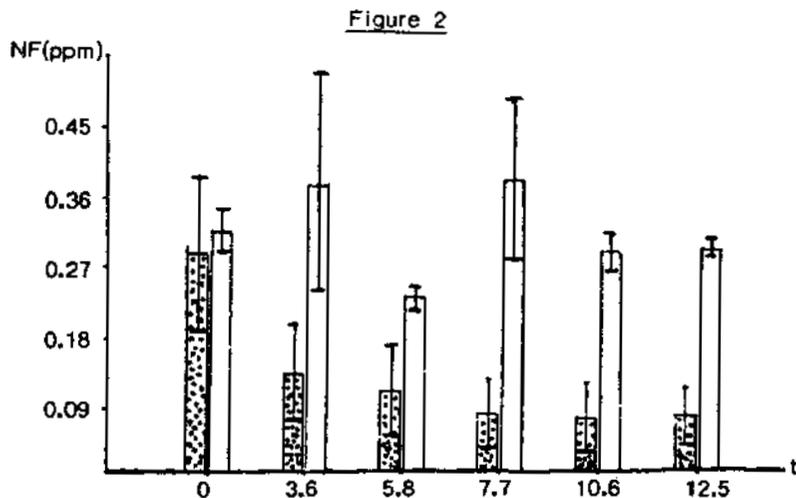
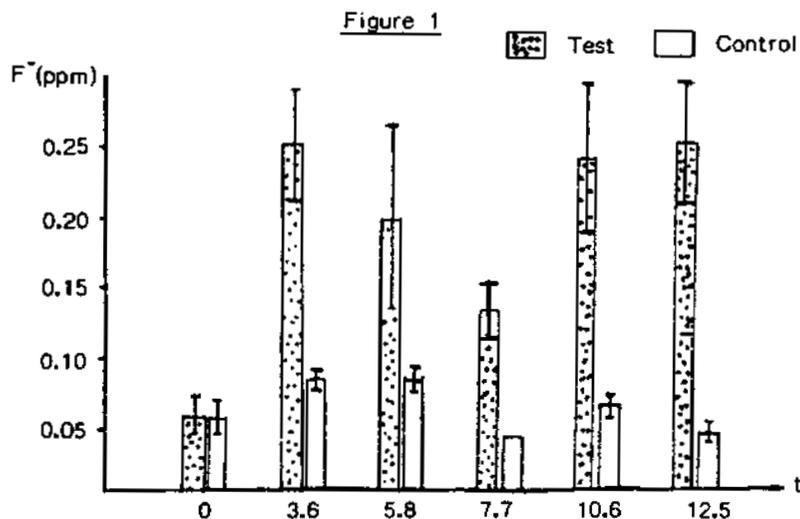
* Associate Professor Caishuang Li, The Institute of Endocrinology, Tianjin Medical College, Tianjin, China.

mittently to estimate ionic fluoride with ISE, total fluoride with AIF molecular spectrophotometer (total fluoride minus ionic fluoride, i.e. non-ionic fluoride).

Results

Non-ionic fluoride decreased when ionic fluoride increased during the 3.6 month observation; and non-ionic fluoride continued to decrease during 5.8, 7.7, 10.6, 12.5 months until the minimum value was reached (Figures 1-4).

Relation of F^- , NaF, TF, F^-/TF Concentration to Time (Month) in Monkey Serum



Fluoride

Figure 3

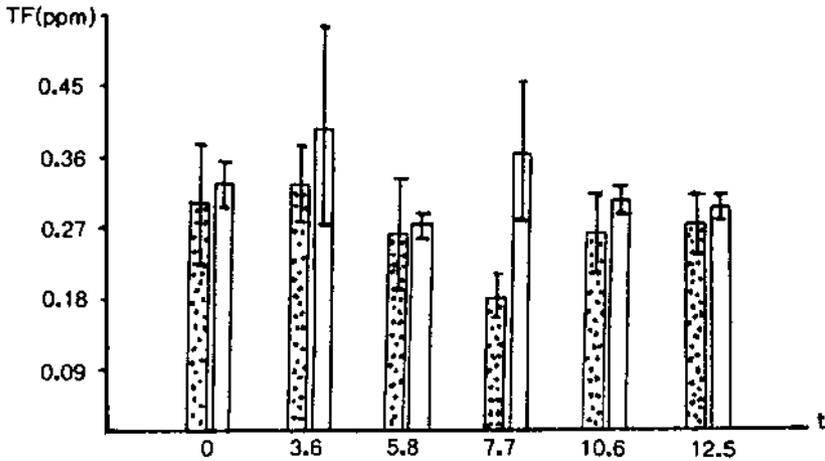
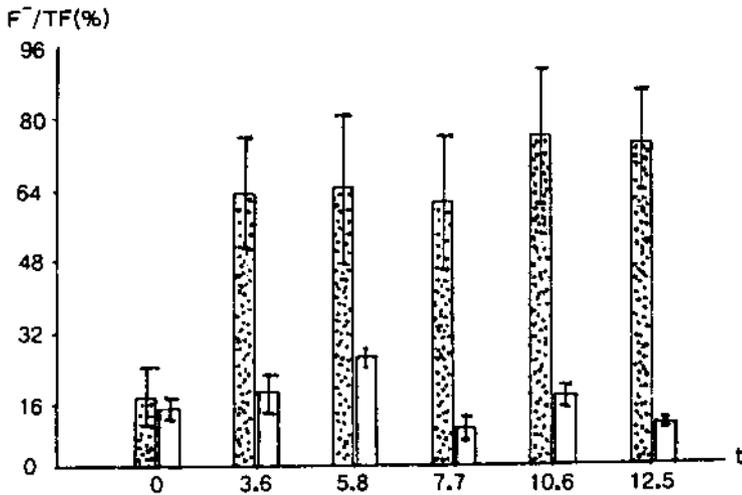


Figure 4



Discussion

Two forms of fluoride (ionic and non-ionic) exist in blood of healthy humans, where there is an equilibrium between them. This equilibrium may be destroyed in skeletal fluorosis. Initially, whether non-ionic fluoride has any biological activity has not been considered. However the study of changes in serum ionic and non-ionic fluoride during fluorosis revealed that serum ionic fluoride concentration increases as the degree of poisoning advances, in conformity with previous reports. On the contrary, total fluoride decreases

when ionic fluoride increases to a relatively high level which means that non-ionic fluoride decreases and tends to go toward zero (since $TF = F^- + NF$). The question arises as to why non-ionic fluoride decreases when ionic fluoride increases. Are serum non-ionic fluoride and fluoride poisoning related? Is non-ionic fluoride connected with serum protein? Considering that NF may combine with serum protein, whether NF has any relation with serum protein deserves attention. Research regarding the connection between non-ionic fluoride and serum protein has not as yet been completed.

Some of the monkeys collected chondropathy after four months of the experiment. In order to proceed without interruption, vitamin D and calcium were administered to all animals following which serum ionic fluoride decreased which showed that vitamin D and calcium are useful in alleviating fluorosis. In human fluorosis the decrease of serum non-ionic fluoride was minimal. Possibly the animals were not observed long enough, or the number of cases was insufficient. For an adequate answer further follow-up is needed.

Conclusion

Regarding the increase in ionic fluoride as well as the decrease in serum non-ionic fluoride in skeletal fluorosis of monkeys, the results show that the two fluoride forms in blood interact. Therefore, two forms of fluoride in blood should be considered simultaneously in diagnosis and therapy.

Acknowledgement

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HYDROXYPROLINE AND URINARY FLUORIDE IN RATS REPEATEDLY EXPOSED TO INHALED PHOSPHORITES

by

W. Czarnowski and J. Krechniak*
Gdańsk, Poland

SUMMARY: Rats were insufflated intratracheally for 12 weeks with phosphorites containing 2% fluoride. Urinary fluoride, which was significantly elevated during the period of dosing, failed to return to control values within 2 weeks after exposure ended. This result may indicate the possibility of fluoride accumulation in workers exposed to phosphorites. Elevated pulmonary hydroxyproline and weight of lung indicate that phosphorites display a moderate fibrogenic activity.

KEY WORDS: Intratracheal insufflation; Rats; Phosphorites; Pulmonary hydroxyproline; Urinary fluoride.

Introduction

From the standpoint of occupation toxicology, phosphorites can be considered both biologically active dusts as well as fluorine containing materials.

We ascertained previously (1) that urinary fluoride in rats, given single oral or intratracheal doses of phosphorites, was significantly elevated for several days. Moreover, urinary fluoride in workers employed in transport of phosphorites was distinctly increased compared to controls.

In the present study we investigated the influence of subacute intratracheal exposure of rats to phosphorite dusts on urinary fluoride as well as on pulmonary and urinary hydroxyproline levels. The aim of this study was to investigate the possibility of fluoride accumulation in workers employed in fertilizer plants and to evaluate the biological activity of phosphorite dusts.

* From Department of Toxicology, Medical Academy, Gdańsk, Poland.

Materials and Methods

Experimental Design: Male Wistar rats weighing 220 ± 20 g were given, intratracheally, Jordan phosphorite which contains about 2 percent fluoride. Only the fraction containing particles 0.06 mm or less in diameter was used as a suspension of 50 mg in 0.5 mL of saline per rat. Control animals were given 0.5 mL of saline.

Phosphorites were insufflated weekly for 12 weeks. Thereafter half the animals were killed; their lungs were examined to determine the pulmonary hydroxyproline content. The remaining rats were left for another 2 weeks to control urinary fluoride and hydroxyproline levels during the post-exposure period.

Urinary samples were collected after termination of the rapid phase of fluoride excretion (1). Animals were placed in metabolic cages 48 h after every dosing and 24 h portions of urine were collected.

Determination of Fluoride and Hydroxyproline: Urinary fluoride was determined by means of fluoride-specific electrode (Aquajon, model B 002) and Ag/AgCl reference electrode with a double jacket. The ion potential was measured by an N-512 Elpo pH meter. Before measurement, samples were diluted with equal volumes of pH 7.0 citric buffer (2). Calculations were based on a response factor from a standard curve prepared daily.

Hydroxyproline in urine and lung were determined colorimetrically according to Stegeman (3). Before measurement samples were subjected to acid hydrolysis.

Significance was determined by Student's t-test.

Results and Discussion

Multiple intratracheal insufflations with phosphorites may cause increased absorption and accumulation of fluoride in the organism. Exposure of rats to fluoride was evaluated by urinary fluoride determination. Control animals were given only saline (Table 1).

Differences between exposed and control animals were significant from the second week of dosing to the end of the experiment. The levels of significance were between $p < 0.001-0.05$.

During the 2 weeks post-exposure period urinary fluoride in exposed animals decreased slightly but failed to return to control values. It seems that fluorides, accumulated during subacute exposure to phosphrites were not completely excreted from the body. This may indicate the possibility of similar accumulation of fluorides in workers employed in fertilizer production and transport.

Urinary hydroxyproline was determined in this experiment because the level of this amino acid in urine is known to change in different dust-induced diseases (4) and endemic fluorosis (5). However, no significant differences in urinary hydroxyproline were found between exposed and control animals (Table 2).

Table 1
Urinary Fluoride in Rats ($\mu\text{g}^-/24$ hr/animal)

Time (weeks)	1	2	3	4	6	7	8	9	10	11	12	14	
Exposed animals (n = 20)	\bar{X}	47.4	67.4	75.5	81.3	72.4	89.4	66.2	71.9	77.9	77.3	78.5	73.7*
	\pm S.D.	6.2	6.8	10.8	13.9	10.3	38.6	14.1	12.1	25.5	11.4	17.6	9.7
Control animals (n = 16)	\bar{X}	48.9	57.0	60.6	47.4	44.0	47.3	51.3	44.5	44.6	44.2	44.0	43.3**
	\pm S.D.	6.5	8.0	11.5	9.9	6.5	8.8	13.6	5.5	6.4	5.6	4.8	5.5
p		<0.01	<0.01	<0.001	<0.001	<0.001	<0.01	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001

Table 2
Urinary Hydroxyproline in Rats ($\mu\text{g}/24$ hr/animal)

Time (weeks)	1	2	3	4	6	7	8	9	10	11	12	14	
Exposed animals (n = 20)	\bar{X}	269.2	268.3	258.0	213.3	210.2	192.5	140.6	151.9	129.0	146.0	120.0	181.3*
	\pm S.D.	43.7	46.5	39.7	60.4	48.1	31.3	35.2	57.7	34.6	37.2	58.8	40.1
Control animals (n = 16)	\bar{X}	358.9	266.9	253.3	199.9	210.9	213.3	161.6	189.1	171.0	168.0	165.0	180.9**
	\pm S.D.	65.4	74.5	40.7	29.1	41.7	37.9	43.9	44.8	34.8	20.1	36.4	47.8

\bar{X} = Mean Value \pm S.D. = Standard Deviation n = number of animals p = level of significance
* n = 10 ** n = 8

Figure 1
Body Weight of Rats

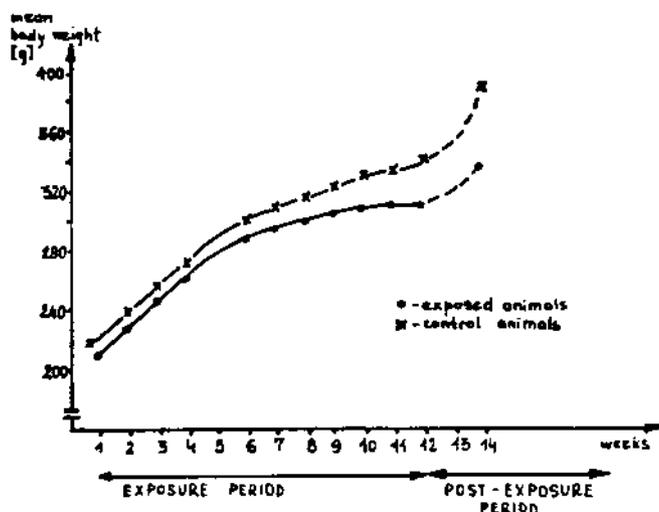


Table 3
Weight of Lung and Pulmonary Hydroxyproline in Rats

Time	After Last Insufflation			
	48 hr		2 weeks	
	Exposed (n = 9)	Control (n = 8)	Exposed (n = 9)	Control (n = 8)
Weight of wet lung (g) $\bar{X} \pm S.D.$	3.59 \pm 0.49 p < 0.001	2.29 \pm 0.79	3.81 \pm 0.73 p < 0.001	2.20 \pm 0.70
Weight of dry lung (g) $\bar{X} \pm S.D.$	0.78 \pm 0.09 p < 0.001	0.44 \pm 0.12	0.73 \pm 0.16 p < 0.001	0.35 \pm 0.08
Pulmonary hydroxyproline (mg/lung) $\bar{X} \pm S.D.$	0.57 \pm 1.04 p < 0.001	5.09 \pm 0.61	9.02 \pm 1.55 p < 0.001	4.78 \pm 0.72

Body weight of exposed animals decreased significantly from the 9 week dosing (Figure 1). During the post-exposure period mean body weight in both groups of rats increased considerably; however, significant differences remained between exposed and control animals. Some restraint in the growth of rats may be caused both by phosphorite dusts and by the procedure of insufflation.

Lung hydroxyproline is commonly accepted by industrial toxicologists as a criterion in evaluation of fibrogenic activity of dusts (6). Also the weight

of lungs gives some information on this issue. In this experiment the weight of both wet and dry lungs in exposed animals was significantly elevated compared with controls (Table 3). Similarly pulmonary hydroxyproline in exposed rats was significantly higher than in control animals (Table 3). So there is evidence that phosphorite dusts increase the content of collagen in lungs. Phosphorites may be classified as dusts of moderate or weak fibrogenic activity (7). Manganese dioxide, vanadium pentoxide and some welding fumes display similar activity. However, action of phosphorites is more fibrogenic than iron oxide or titanium dioxide (8).

Conclusions

1. Mean urinary fluoride level in rats exposed to phosphorites was significantly higher than in controls.
2. Fluorides, accumulated as a result of multiple dosing with phosphorites, were not completely excreted with urine during the 2 week post-exposure period.
3. The brand of phosphorites examined caused a significant increase in the weight of lung and pulmonary hydroxyproline which indicates that phosphorites can be considered dusts of weak or moderate fibrogenic activity.

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EFFECTS OF FLUORIDE POLLUTION ON CALCIUM AND MAGNESIUM CONTENT OF MANDIBLES (LOWER JAWS) OF WILD GAME

by

E. Dąbkowska and Z. Machoy*
Szczecin, Poland

SUMMARY: Our investigation aimed to show the manner by which changing conditions of the natural environment, polluted by industrial emission, influences the mineral composition of the deer's masticatory system. Fluoride accumulation in bone tissue is accompanied by a simultaneously rise in calcium and magnesium content.

KEY WORDS: Bone; Deer; Environment; Mineral composition.

Introduction

Free-living forest animals are, for many reasons, less frequently the subject of toxicological investigations compared with experimental and breeding animals. The main source of environmental pollution by fluoride compounds, to which game in Poland is exposed, stems from industrial emissions of large factories, since, in this country there are neither endemic areas nor natural waters high in fluoride. Industrial emissions are harmful to vegetation of the forests and also to the game found therein. It has been reported that, under circumstances of high fluoride, the physical condition of adult animals deteriorates; they weigh less; their antlers are more poorly developed, and their tusks are more fragile. These findings prompted us to analyze the mineral composition of the mandibles from deer living near a large industrial plant located in a forest region, which processes phosphorites and apatites.

Materials and Methods

The subject of investigation was the mandibles of deer (*Cervus elaphus* L) that were killed in the years 1982 and 1983, and provided by the Hunters' Circles from various regions of Western Pomerania in Poland. The control group was comprised of mandibles of the same animal species from the region of Eastern Poland in vicinity of the National Park at Białowieża. At the beginning, the animal's age was estimated on the basis of attrition affecting the grinding surface of premolar and molar teeth. Next a dental drill was utilized for collecting powdered bone material from the mandible surface 5 cm away from the jaw curvature angle. Four 10 mg samples were collected from each animal and were analyzed for fluoride determination while the other two samples were used to determine concentrations of calcium and magnesium. These samples for fluoride evaluation were first dissolved in perchloric acid while the samples for calcium and magnesium determinations were dissolved in nitric acid, according to the method of Dąbkowska (1). Fluoride concentrations were determined with an ion-specific electrode; calcium and magnesium by atomic spectrophotometry.

* Direct correspondence to Z. Machoy, Pomeranian Medical Academy, Department of Biochemistry, Szczecin, Poland.

This paper represents the results concerning the two most hazardous areas which are downwind from an industrial factory. The analyzed jaws came from Forest district administration Trzebież (T) - 23 heads and Forest district administration Międzyzdroje (M) - 19 heads. The control group was made up of 14 jaws from Białowieża (B) which was separated from the emission source by some hundred kilometers in the eastern (upwind) direction. The results were subjected to statistical analysis. Student's t-test was used for testing the significance of differences between the two means. To graphically demonstrate the relationship between the fluoride amount in mandibles and the animal's age, a rectilinear regression equation was calculated.

Results and Discussion

Figure 1

Relationship Between the Level of Fluorides in Jaws and Animal Age (Rectilinear Regression Equation)

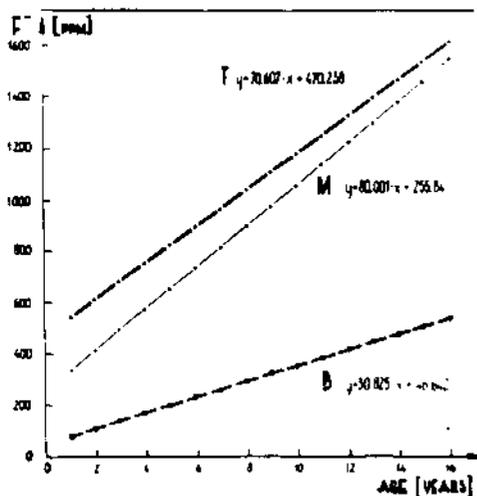


Table 1 depicts the results of fluoride, calcium and magnesium content in the mandibles that were supplied by the 3 forest district administrations: T and M as well as the control one B. The mandibles from the control group show the lowest content of fluoride, calcium and magnesium. In order to establish the relationship between the amount of fluoride and the animal's age, a rectilinear regression equation was introduced (Figure 1). If accumulation of fluoride in mandibles were uniform, the three lines would be parallel to each other. This obviously is not the case. The straight line T, which is the area directly next to the industrial plant, shows the greatest angle of inclination (tg). In this area the fluoride accumulation in the mandibles with the increase in the animal's age is the fastest which is expected. Since deer do not wander far away from their feeding places and lairs, they would have taken in the most fluoride.

In fact, young deer in the area of Forest District Administration Międzyzdroje (M) showed a higher fluoride level in their mandibles, but as they grew older the fluoride accumulation slowed. The forests of the control area (B) are, in accordance with Polish data, least exposed to emission hazard. Hence the animals inhabiting these areas were the best group for comparison. The recorded fluoride content elevation (B and M) does not match the value characteristic of fluorosis reported by world literature (2). Calcium is found to be the principle component of the bone; however, magnesium appears in it in small amounts. The increase in fluoride content in the mandibles shows a direct correlation with a higher quantity of calcium and magnesium in them (Table 2). The presence of magnesium in bone has been mentioned in the analyses by Hendricks and Hill (3). Bone magnesium is in the inorganic component. Animal experiments have shown that variation in dietary intake produce changes in the bones as well as in other tissues. The mutual interactions of calcium and magnesium in the bones has been discussed (4). In body fluids,

Table 1

Content of Fluoride, Calcium and Magnesium in Jaws of Animals from the Three Analyzed Forest District Administrations Trzebież (T), Międzyzdroje (M) and Białowieża (B). Forest District Administration B serves as Control, (F⁻ in ppm; Ca and Mg in g/kg).

n	Trzebież (T)			Międzyzdroje (M)			Białowieża (B)		
	F ⁻	Ca	Mg	F ⁻	Ca	Mg	F ⁻	Ca	Mg
1	587.15	270.65	4.94	276.45	274.86	4.02	99.59	215.07	4.07
2	507.30	278.77	5.63	627.00	399.58	4.32	90.53	240.69	4.12
3	969.00	270.65	5.05	553.80	382.17	6.09	224.12	263.42	4.62
4	552.90	264.22	4.92	484.50	303.78	8.32	100.15	234.60	3.97
5	376.20	281.23	4.97	969.00	336.90	8.57	177.40	243.78	4.57
6	525.40	297.18	4.59	1026.00	306.34	7.66	190.95	248.36	4.72
7	1025.00	280.41	5.11	500.50	236.09	6.54	364.95	258.73	6.54
8	1000.00	295.34	5.02	1197.00	234.57	6.64	185.20	265.90	4.82
9	974.70	329.78	6.07	855.00	265.98	5.93	191.50	248.36	3.97
10	552.90	577.29	5.43	490.20	237.32	6.04	156.09	234.57	3.91
11	587.15	338.87	6.14	490.20	201.20	3.81	171.00	248.44	4.02
12	376.20	313.80	5.83	541.50	262.63	5.53	125.40	216.54	3.01
13	507.40	309.76	5.73	552.90	246.11	3.51	128.80	230.01	3.51
14	524.00	355.01	5.02	1000.00	277.14	13.38	213.80	280.41	4.97
15	524.00	363.70	4.77	522.50	282.22	7.25			
16	533.35	326.61	6.64	774.50	309.93	7.05			
17	843.60	396.65	4.77	870.00	309.92	7.05			
18	1197.30	291.32	6.99	985.00	269.20	4.52			
19	1938.00	338.69	5.83	1005.00	292.66	3.21			
20	592.80	291.32	6.49						
21	627.00	316.48	4.57						
22	991.80	293.66	5.33						
23	957.60	310.62	4.62						
Mean	750.90	321.39	5.41	722.16	285.71	6.29	172.82	244.93	4.34
S.D.	344.25	63.41	0.68	251.07	48.34	2.29	67.59	17.76	0.80

Table 2

Differences between Mean Contents of Fluoride, Calcium and Magnesium in Jaw Bones Significant ($p < 0.05$) or Not Significant (NS) differences. Value of "t-test."

Elements	Mean Values			Differences between B & T		Differences between B & M		Differences between T & M	
	B	T	M	"t"	p	"t"	p	"t"	p
F ⁻ (ppm)	172.82	750.90	722.16	7.631	< 0.001	8.849	< 0.001	0.305	NS
Ca (g/kg)	244.93	321.39	285.71	5.314	< 0.001	3.285	< 0.005	1.967	< 0.05
Mg (g/kg)	4.34	5.41	6.29	4.221	< 0.001	3.324	< 0.005	1.559	NS

these elements reveal antagonistic properties. As is apparent from Table 2, differences in levels of these elements in bones (evidenced between the studied groups T and M, and the control group B) are statistically significant. In her densitometric studies, Dąbkowska has shown that there is a higher degree of mandible mineralization in animals living in areas polluted by emissions that contain fluoride compounds (1). For condyloid process and for coronoid process, the differences were statistically significant at $p < 0.001$, and for the point of the greatest curvature in the jaw angle at $p < 0.01$. This is consistent with the finding that fluoride increases the crystallinity and produces greater bone density (5).

Conclusions

1. The comparison of the mineral composition in mandibles of animals from areas polluted by industrial emissions containing fluoride compounds, and that from the control area points to a progressive increase in fluoride accumulation.
2. The mandible fluoride level depends on the individual's age. In the area polluted by emissions the distance from the source of emission and the direction of the wind are important.
3. The mandible fluoride accumulation is accompanied by increased content of calcium and, to a lesser extent, of magnesium.

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EFFECT OF FLUORIDE IN EXCESS ON LIPID CONSTITUENTS OF RESPIRATORY ORGANS IN ALBINO RABBITS

by

Shashi*, J.P. Singh and S.P. Thapar
Patiala, India

SUMMARY: Total lipids and their various fractions namely, triglycerides, free fatty acids, cholesterol and phospholipids of respiratory organs (lungs and trachea) of rabbits, were analyzed following subcutaneous injections of NaF for 100 days. The level of total lipids and neutral lipids decreased during fluoride intoxication. Triglycerides and free fatty acids showed overall decrease in fluoridated rabbit respiratory organs compared to controls. Cholesterol content of trachea was reduced in low fluoride groups and increased in high fluoride groups in both sexes. In lungs, the level of cholesterol fell in fluorotic groups. Phospholipids showed no significant change in the lung of males whereas in females the amount was significantly elevated. In female trachea, the concentration of phospholipids increased in the high fluoride group. In trachea of males the increase in concentration of phospholipids in 10 and 20 mg F⁻ groups was significant.

KEY WORDS: Free fatty acids (FFA); Phospholipids (PL); Sodium fluoride (NaF); Total lipids (TL); Triglycerides (TG).

Introduction

Several studies have suggested that fluoride influences the metabolism of lipids. In rats, lipid metabolism has been altered by toxic levels of fluoride (1). At non-toxic levels, fluoride has been shown to influence serum lipid metabolism in rabbits (2,3). Fluoride also influences metabolism of total lipids, triglycerides and cholesterol in the liver of rabbits (4). Fluoride poisoning induced changes in respiratory organs in the form of pulmonary edema and congestion in various experimental animals (5). However, research regarding the biochemical effects of fluoride on these organs is lacking. To study the effect of fluoride on lipid metabolism, biochemical parameters such as total lipids, neutral lipids and their fractions, triglycerides, free fatty acids, and cholesterol as well as phospholipids in lungs and trachea of rabbits provided with varying degrees of sodium fluoride, were examined. The results are reported herein.

Materials and Methods

White albino rabbits of both sexes, obtained from Kaila Scientific Corporation, Agra, were given normal diet and water ad libitum. The animals were divided into four groups of 10 each. One group, given 1 cc distilled water/kg body wt. served as control. The remaining animals, given subcutaneous injections of NaF solution in the concentration of 5, 10, and 20 mg/kg body wt., were sacrificed after 100 days under general anesthesia. The lungs and trachea

* Department of Zoology, Punjabi University, Patiala-147 002, India.

of control and fluoridated animals were removed and kept in chloroform and methanol 2:1 v/v for extraction of lipids (6). The known weights of tissues, homogenized in chloroform:methanol 2:1 v/v were kept for 24 hours at room temperature. The contents were filtered through the sintered glass funnel (G-3). Extracts were then purified from the non-lipid contaminants by adding 0.85% sodium chloride solution. Total lipids were dried over anhydrous sodium sulfate and weighed; the results gave the amount of total lipids gravimetrically.

Separation of Neutral Lipids: The Freeman and West (7) method with slight modification was used for preparation of silica gel G thin layer plates (20x20 cms) for chromatography. The plates, activated at 110°C for 90 minutes, were developed in a solvent system n-hexane:diethyl ether:acetic acid glacial 90:10:1 (v/v) up to 17 cms from the point of origin. The developed plates were air dried and placed in sealed chambers saturated with iodine vapors which provided yellow spots. After identifying and marking the different spots, they were put into extracting solvent (n-hexane:diethyl ether 1:1 v/v). Pooled extracts, evaporated to dryness under reduced pressure, were taken in known volume of chloroform:methanol (1:1 v/v). Extracts containing different fractions were used for colorimetric analysis. Quantitative analysis of triglycerides was done according to the Vanhandle and ZilverSmith method (8); estimation of free fatty acids as described by Chakrabarty *et al.* (9); cholesterol by the Stadtman (10) colorimetric method. Phospholipids were estimated according to Ames (11) statistical analysis; results shown as mean \pm S.D. with Student's t-test used for statistical analysis.

Table 1

Effect of Fluoride on the Total Lipid Content of Rabbit Respiratory Organ

Tissue	Concentration of fluoride mg/kg body wt.	Total Lipids	
		mg/g wet wt. (Mean \pm S.D.)	
		Male	Female
Lung	0	77.131 \pm 1.943	73.505 \pm 4.958
	5	63.876 \pm 2.165***	49.466 \pm 2.907***
	10	51.720 \pm 4.630*** ⁺	50.055 \pm 8.453***
	20	47.540 \pm 3.493***	49.354 \pm 3.208***
Trachea	0	377.930 \pm 22.913	344.728 \pm 17.778
	5	321.428 \pm 48.790*	273.000 \pm 53.740**
	10	150.900 \pm 7.570*** ⁺	145.833 \pm 19.283*** ⁺
	20	86.805 \pm 7.186*** ⁺	84.900 \pm 20.690*** ⁺

Four experiments in each group.

SD denotes Standard Deviation of the Mean

p-Values as Compared to the Control.

* p < 0.05 ** p < 0.02 *** p < 0.001

p-Values Indicate Comparison of Treated Groups with Each Other
⁺p < 0.001 ⁺p < 0.01

Results

Total Lipids: Table 1 shows the effect of fluoride on total lipids of lung and trachea of males and females. The concentration of TL decreases in both organs in all treated rabbits compared to controls.

Neutral Lipids: a) Lungs: Neutral lipids and their various fractions in lungs during fluorosis are shown in Table 2. The level of neutral lipids fell rapidly in both sexes. The amount of TG and cholesterol decreases significantly in lungs of all treated rabbits. In males FFA significantly increased in Group B, compared to controls; in the remaining experimental groups, FFA shows depletion.

b) Trachea: Table 3 indicates the level of neutral lipids and their fractions in trachea of rabbits of both sexes. The amount of neutral lipids in both sexes fell in fluorotic rabbits. The level of TG decreased significantly ($p < 0.001$) in Groups B, C and D compared to Control A. The level of FFA fell to 3.920 mg/g w.w. in high fluoride group compared to controls (85.130 mg/g w.w.). In females the decrease, 87.270 mg/g w.w. vs 6.590 mg/g w.w. is highly significant ($p < 0.001$). The cholesterol content declined significantly ($p < 0.05$) in Group B and rose in male Groups C and D. On the other hand, in females the concentration of cholesterol fell in Group B and C, and rose in the high fluoride group.

c) Phospholipids: The changes in the amount of phospholipids in respiratory organs of control and experimental rabbits, are presented in Table 4. In male, lung increase and decrease in PL is statistically insignificant. In female, the PL content decreased in the low fluoride group and increased significantly in high fluoride groups. In male trachea, the level of PL increased significantly in Group C and Group D. In female, the amount of PL increase in Groups B and C, was statistically insignificant. In Group D, the PL rapidly increased compared to controls (12.121 mg/g w.w. vs 31.547 mg/g w.w.).

Discussion

A high fluoride intake results in various biological changes including alterations in lipid metabolism. Vatassery et al. (12) recorded that in guinea pigs provided with a diet containing 18% fat and 0.25 ppm fluoride for 3, 6, 9 and 13 weeks, total lipids in serum increased in high fluoride group between 9 and 13 weeks. According to Soni et al. (13), 5.0 mg/kg NaF increases lipid peroxidation in rat lung whereas 20.0 mg/kg NaF decreases it.

During current rabbit experiments, TG content was depleted in trachea during fluorosis. Triglycerides underwent hydrolysis by a hormone-sensitive lipase to form free fatty acids and glycerol. Fluorides are known as strong inhibitors of various enzyme systems like lipase, bone phosphatase, esterases. Lipase, a hydrolyzing enzyme is particularly susceptible to the inhibitory action of fluoride in amounts as low as 1 part in 5 million (14). This type of inhibition of lipase may result in an increased level of TG in lungs during chronic fluoride intoxication. Fluoride affects the endogenous metabolism of triglycerides. In guinea pigs, fed with excess dietary fat and given high fluoride intake, the level of triglycerides decreased, whereas in animals receiving a low fat diet and high fluoride intake the level of serum triglycerides increased (15).

Table 2
Effect of Fluoride on the Neutral Lipids and Their Fractions in Lung of Rabbit
(Data are Mean \pm S.D. in Each Group)

Constituent	Concentration of Fluoride				
	Group A (0)	Group B (5)	Group C (10)	Group D (20)	
Neutral Lipids (mg/g w.w.)	m	54.540	38.581	30.170	24.270
	f	48.005	22.270	18.958	15.624
Triglycerides (mg/g w.w.)	m	40.330 \pm 0.140	23.560 \pm 0.250**	18.890 \pm 0.120** ⁺⁺⁺	14.300 \pm 0.160** ⁺⁺⁺
	f	33.945 \pm 0.117	10.410 \pm 0.240**	10.858 \pm 0.276** ⁺	8.290 \pm 0.190** ⁺⁺⁺
Free Fatty Acids (mg/g w.w.)	m	10.940 \pm 0.250	11.960 \pm 0.630*	8.520 \pm 1.555** ⁺⁺⁺	8.500 \pm 0.092**
	f	11.550 \pm 0.091	8.660 \pm 0.092**	6.190 \pm 0.276** ⁺⁺⁺	5.690 \pm 0.108** ⁺⁺⁺
Cholesterol (mg/g w.w.)	m	3.270 \pm 0.051	3.071 \pm 0.045**	2.760 \pm 0.290* ⁺	1.470 \pm 0.025** ⁺⁺⁺
	f	2.510 \pm 0.031	3.200 \pm 0.400**	1.910 \pm 0.550** ⁺⁺⁺	1.644 \pm 0.110** ⁺⁺⁺

m = male f = female

p-Values as Compared to the Control: * p < 0.01 ** p < 0.001

p-Values Indicate Comparison ⁺ p < 0.05, ⁺⁺ p < 0.01, ⁺⁺⁺ p < 0.001.

Table 3
 Effect of Fluoride on the Neutral Lipid, Triglycerides, Free Fatty Acids and Cholesterol Content
 of Rabbit Trachea
 (Data are Mean \pm S.D. in Each Group)

Constituent	Concentration of Fluoride			
	Group A (0)	Group B (5 mg)	Group C (10 mg)	Group D (20 mg)
Neutral Lipids (mg/g w.w.)				
m	287.796	107.444	81.247	53.311
f	274.744	161.958	82.027	58.905
Triglycerides (mg/g w.w.)				
m	201.818 \pm 2.571	96.944 \pm 1.924**	72.578 \pm 0.435***	44.878 \pm 0.419***
f	185.747 \pm 9.796	144.848 \pm 2.419**	69.368 \pm 0.301***	58.095 \pm 1.649***
Free Fatty Acids (mg/g w.w.)				
m	85.130 \pm 3.415	10.031 \pm 0.130**	5.052 \pm 0.364***	3.920 \pm 0.288***
f	87.270 \pm 4.125	16.270 \pm 0.477**	9.041 \pm 0.659***	6.590 \pm 0.091***
Cholesterol (mg/g w.w.)				
m	0.848 \pm 0.293	0.470 \pm 0.091*	3.619 \pm 0.659***	4.513 \pm 0.137***
f	1.727 \pm 0.128	0.840 \pm 0.096	0.955 \pm 0.174**	4.220 \pm 0.481***

m = male f = female

p-Values as Compared to the Control: • p < 0.05, ** p < 0.001

p-Values Indicate Comparison in Treated Groups with Each Other:
 † p < 0.01, †† p < 0.001

Table 4

Effect of Fluoride on the Phospholipid Content of Rabbit Lung and Trachea

Tissue	Concentration of fluoride mg/kg body wt.	Phospholipids mg/g wet wt. (Mean \pm S.D.)	
		Male	Female
Lung	0	23.890 \pm 3.120	25.500 \pm 0.190
	5	25.296 \pm 4.222	25.071 \pm 0.160*
	10	21.550 \pm 3.047	31.100 \pm 0.198** ⁺⁺
	20	23.200 \pm 0.120	33.730 \pm 0.343** ⁺⁺
Trachea	0	8.735 \pm 0.100	12.121 \pm 1.262
	5	9.259 \pm 1.061	13.848 \pm 1.601
	10	11.477 \pm 0.272** ⁺⁺⁺	12.309 \pm 0.188 ⁺
	20	29.333 \pm 11.015* ⁺⁺⁺	31.547 \pm 1.031** ⁺⁺⁺

p-Values as Compared to the Control.

* p < 0.01

** p < 0.001

p-Values Indicate Comparison of Treated Groups with Each Other

+ p < 0.05

++ p < 0.01

+++ p < 0.001

Fluorides are known to cause a decrease in the active ion transport at the cell membrane and an increase in the membrane permeability of cells because of its inhibition of pyrophosphatase activity (16,17). This inhibition also interferes with fatty acid oxidation (18). Low concentrations of fluoride inhibit the carnitine independent oxidation of fatty acids by tissue homogenates (19). Fluoride also inhibits O₂ uptake during fatty acid oxidation, which gives rise to accumulation of pyrophosphate in inner mitochondrial matrix. The accumulation is due to the fact that fluoride inhibits an internally localized inorganic pyrophosphatase and the inner mitochondrial membrane is impermeable towards pyrophosphate. Fluorides also inhibit the enzyme acyl-CoA synthetase involved in fatty acid oxidation. Thus the observed decrease in the free fatty acids of lung and trachea of male and female rabbits may be due to the inhibition of these enzymes. Present experiments show a significant elevation of phospholipids during chronic fluoride intoxication in both organs which might result from a defect in the lipoprotein metabolism involving either the failure of the lipid to couple the protein moiety or the release of the lipoprotein from the liver into the plasma.

Conclusions

From these experiments, it is concluded that fluoride poisoning significantly influences the metabolism of lipids in respiratory organs of rabbits of both sexes. The decrease in the level of total lipids and neutral lipids may be due to the inhibition of lipases by fluoride. The increase in phospholipids is due to reduced lipoprotein metabolism.

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CHILD DENTAL HEALTH DIFFERENCES IN NEW ZEALAND

by

John Colquhoun*
Auckland, New Zealand

(Abstracted from Community Health Studies XI:85-90, 1987)

The School Dental Service in New Zealand provides regular dental care to 98 percent of 5-13-year-old school children and 68 percent of preschool children. In addition to having comprehensive child dental care, New Zealand is also one of the most fluoridated countries in the world; over a half of its population is drinking artificially fluoridated water. Official statistics for the six main population areas suggest that child dental decay differences are small and are not tied to the presence or absence of water fluoridation. These differences could be related to demographic, especially socio-economic factors.

In an earlier study the author presented evidence which suggested that, when allowing for socio-economic differences, child dental health is now better in non-fluoridated areas.

The present study, utilizing officially collected School Dental Service statistics, by fluoridation status, from all Health Districts of New Zealand, compares the most populous, largely urban areas. Socio-economic information, on three pairs of communities claimed to have benefited from fluoridation, reinforce the author's previous findings. In other words, when similar population areas are compared, no obvious dental health benefits related to fluoridation are detected.

Respecting economic status, in Christchurch average income is lower than that in Auckland and Wellington; usually in lower-income areas dental health is poorer. The two fluoridated areas with income levels comparable to non-fluoridated Christchurch are mostly scored poorer. When considering the socio-economic variable, child dental health appears to be better in the non-fluoridated area.

Two recent New Zealand studies claim that, when the socio-economic background is closely examined, benefit resulting from fluoridation, although small, is significant. These studies are based on very small samples of primary teeth - as few as 12 5-year-olds; there was more decay, not less, among fluoridated-area upper class 7-year-olds.

In New Zealand regular six-monthly fluoride applications are provided to all children in non-fluoridated areas (use of fluoride tablets is also recommended), and to only selected children in fluoridated areas, while use of fluoride toothpaste is encouraged everywhere. It is uncertain how extensively fluoride tablets have been consumed in non-fluoridated areas. The present author found, during an investigation in the central Auckland areas, that less than 5 percent in its lower-income, non-fluoridated area were regular ingesters.

Some recent studies suggest that the decline in dental decay in developed countries commenced before the widespread use of fluoride. Lack of a clear

difference in caries scores between fluoridated and non-fluoridated areas, plus increased fluoride-induced mottling, raises the question of the cost-benefit of fluoridation in contemporary society. The latter question has also been raised in Britain.

In conclusion the author comments that, from available data, one cannot be certain whether uses of fluoride, or other environmental factors, have contributed more to the decline in dental decay in New Zealand. Indeed, Danish studies show that a preventively-oriented School Dental Service can achieve levels of child dental health equal to those purported to result from fluoridation.

KEY WORDS: Children; Dental caries; Fluoridation; New Zealand; School Dental Health Service.

REPRINTS: Education Department, University of Auckland, Private Bag, Auckland, New Zealand.

FLUORIDE UPTAKE AND FLUORIDE RESISTANCE IN ORAL STREPTOCOCCI

by

S. Kashket and R.J. Preman
Boston, Massachusetts, USA

(Abstracted from J. Dent. Res. 64:1290-1292, 1985)

Fluoride uptake was examined in the highly F-sensitive *Streptococcus salivarius* strain 25975, the F-resistant mutant Flr103 and the relatively insensitive *S. sanguis* H7PR3.

F was taken up and concentrated in streptococcal cells when these were incubated in buffers at pH 5.5 or 7.0. Both strains of *S. salivarius* took up F, but the final concentration of intracellular F ($[F^-]_{in}$) was greater in strain Flr103 than in strain 25975. Uptake of F by *S. sanguis* H7PR3, a relatively F-insensitive strain, was similar to that for strain Flr103. F uptake in all instances was greater in buffer at pH 5.5 than at 7.0. The pH remained essentially unchanged during the course of the experiments.

In general, the finding that F-resistant cells retained more F than did cells that are sensitive to the inhibitor leads to the conclusion that F resistance is not necessarily attributable to the exclusion of F from the cells.

KEY WORDS: Oral streptococci; Cellular F uptake; Cell resistance.

REPRINTS: Forsyth Dental Center, 140 The Fenway, Boston, MA 02115, USA.

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INFLUENCE OF TYPE AND LEVEL OF DIETARY PROTEIN ON
FLUORIDE BIOAVAILABILITY IN THE RAT

by

Carol D. Boyde and Florian L. Cerklewski*
Corvallis, Oregon, USA

(Abstracted from Journal of Nutrition 117:2086-2090, 1987)

The purpose of this study was to determine whether the type or level of dietary protein influences fluoride bioavailability — defined in this study as absorption and use — in the young growing rat under usual dietary feeding conditions. Our results suggest that food fluoride, such as that originating from preparation of foods with fluoridated water, will be less bioavailable for an individual ingesting a high-protein diet than for one fed a low-protein diet.

Foods can make a significant contribution to total fluoride intake especially for younger age groups when they are prepared with fluoridated water. Fluoridation of public water supplies in the USA has influenced fluoride content of foods. Absorption of fluoride from foods is about 50-80% in contrast to essentially complete absorption in the absence of food.

Rats fed high protein-containing diets gained significantly more weight ($p < 0.001$) than did rats fed low protein-containing diets. When diets contained lactalbumin as the diet source rats tended to eat less and grow more slowly than rats fed diets containing casein. Both food intake and growth were unaffected by dietary fluoride level.

The percentage of absorbed fluoride that was retained was significantly higher ($p < 0.001$) in rats fed diets containing 10 mg/kg fluoride than in rats fed diets containing 2 mg/kg fluoride regardless of the type or level of protein. Rats fed diets containing either protein source at 360 g/kg retained significantly less ($p < 0.001$) absorbed fluoride than rats receiving 120 g/kg diet regardless of dietary fluoride level because decreased fecal fluoride excretion was accompanied by increased urinary fluoride excretion. Femur fluoride concentration, however, was significantly less ($p < 0.001$) in rats fed either protein source at 360 g/kg diet than at 120 g/kg diet regardless of the dietary fluoride level. Enhanced fluoride absorption in rats fed a high protein-containing diet, however, failed to increase skeletal uptake of fluoride.

We conclude that femur fluoride did not increase in rats fed a high protein-containing diet because the amount of absorbed fluoride that was actually retained was depressed by high protein as a direct result of enhanced urinary fluoride excretion. Fluoride reabsorption simply did not keep pace with increased glomerular filtration rate expected of a high protein diet.

KEY WORDS: Bioavailability; Fluoride; Protein.

REPRINTS: Florian L. Cerklewski, Department of Foods and Nutrition,
College of Home Economics, Oregon State University, Corvallis,
OR 97331, USA.

DIETARY FLUORIDE INTAKE OF 15-19-YEAR-OLD MALE ADULTS
RESIDING IN THE UNITED STATES

by

L. Singer*, R.H. Ophaug* and B.F. Harland
Minneapolis, Minnesota, USA

(Abstracted from J. Dent. Res. 64:1302-1305, 1985)

The average daily dietary fluoride intakes of 15-to-19-year-old males were estimated from the analysis of 24 Food and Drug Administration "market basket" food collections made from 1975 to 1982. Males of this age group residing in fluoridated (> 0.7 ppm) cities had an average daily dietary fluoride intake of 1.85 mg/day when the diet provided an estimated caloric intake of 11.72 megajoules (2800 calories). In nonfluoridated cities, with < 0.3 ppm in drinking water, the average dietary fluoride intake was 0.86 mg/day. Beverages and drinking water contributed an average of 75 ± 2% of the daily dietary fluoride intake.

Approximately two-thirds of the daily fluoride intake (66-80%) is obtained from beverages and water (Composite XII). Interestingly, Composite XII (beverages and water) sometimes had a fluoride concentration higher than that in the municipal water; the high fluoride levels found in Composite XII are largely accounted for by brewed tea.

Our estimates of dietary fluoride intakes for the young male adult 15 to 19 years of age are similar to those cited above and to a mean daily fluoride intake of 2.11 mg reported for adults 21 to 81 years of age in Foncheng, China, a fluoridated community.

KEY WORDS: Adult males; Dietary fluoride; Fluoride intake in U.S.A.

REPRINTS: *Biochemistry Program, School of Dentistry, University of Minnesota, 18-104 Moos Tower, 515 Delaware St. SE, Minneapolis, MN 55455, USA.

CARIES INHIBITION OF MIXED NaF₂PO₃F DENTIFRICES
CONTAINING 1,000 AND 2,500 F: 3-YEAR RESULTS

by

Louis W. Ripa*, Gary S. Leske, Francine Forte, Andre Varma
Stony Brook, NY, USA

(Abstracted from J. of the Am. Dental Assoc., 116:69-73, 1988)

The purposes of this report are to present the final results after 3 years use of two test products and an active control, and to determine whether

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significant differences exist in the caries increments among the three groups.

The initial study population included 3,785 children in grades 5 through 7 (mean age 11.7 years) from two school districts on Long Island, NY, which are less than 20 miles apart (fluoride in water, $F < 0.1$ ppm). After baseline examinations for caries, the children were stratified according to age, gender, and initial caries score and were randomly assigned to one of three dentifrice groups. The two experimental dentifrices differed from the active control in both their fluoride system and their abrasive.

Group 1: control group. The children used a conventional 0.76% $\text{Na}_2\text{PO}_3\text{F}$ dentifrice (1,000 ppm F). The abrasive was originally insoluble sodium metaphosphate. Without the investigators' knowledge, it was changed to dicalcium phosphate hydrate during the study to keep the control dentifrice formulation consistent with the simulated marketed dentifrice that had also changed its abrasive system.

Group 2: Used an experimental dentifrice containing 0.38% $\text{Na}_2\text{PO}_3\text{F}$ (500 ppm F) and 0.11 NaF (500 ppm F) for a total fluoride concentration of 1,000 ppm in a silica abrasive system.

Group 3: Used an experimental dentifrice containing 0.95% $\text{Na}_2\text{PO}_3\text{F}$ (1,250 ppm F) and 0.28% NaF (1,250 ppm F), a total fluoride concentration of 2,500 ppm in a silica abrasive system.

Dentifrices were identically packaged in plain white tubes except the subject's name and code number appeared on a plain label. Participating siblings in the same household were assigned to the same dentifrice group.

Examinations were conducted by two examiners experienced in clinical field trials who had been calibrated often in other studies. No statistically significant differences were found in caries increments between groups. No added benefit occurred from increasing the fluoride concentration to 2,500 ppm in a mixed dentifrice.

In this study the average child had an annual DMF increment of 1.2 surfaces, irrespective of the dentifrice group which is low but consistent with the trend of declining caries incidence in American schoolchildren.

KEY WORDS: Caries inhibition; Fluorophosphate dentifrices; School-age children.

REPRINTS: Dr. Ripa, State University of New York at Stony Brook, Stony Brook, NY 11794, USA.

COMMUNITY HEALTH EFFECTS OF A MUNICIPAL WATER SUPPLY HYPERFLUORIDATION ACCIDENT

by

Lyle R. Petersen, Diane Denis, David Brown,
James Hadler* and Steven D. Helgerson
Hartford CT, USA

(Abstracted from Am. J. Public Health, 78:711-713, 1988)

This report describes the public health effect of hyperfluoridation of public water supplies in a small, unidentified suburban residential Connecticut community.

At the treatment plant, hydrofluorosilicic acid (H_2SiF_6) is injected into the water supply. At approximately 3:00 pm on March 11, 1986, an inadvertently opened valve began to divert hydrofluorosilicic acid that normally would have been injected into water supplying the community and distant metropolitan areas, solely into the small community's water supply. Hyperfluoridated water would have reached the domestic taps at approximately 6:00 pm (beginning of exposure period, time = 0 hours). At +1 hours (7:00 pm) residents began notifying water company personnel that the water tasted abnormal and turned blue on contact with soap, and of itching and gastrointestinal symptoms. At +1 and +4 hours (7:00 and 10:00 pm), household tap water samples revealed fluoride and copper concentrations > 40 times normal (fluoride 42-51 ppm [normal 1.0 ppm], copper 25-41 ppm [normal 0.03 ppm]).

At +10 hours (4:00 am), a sample of water from a water main had fluoride and copper concentrations of 50 ppm and 0.03ppm, respectively. The water mains were then flushed. Beginning at +12 hours (6:00 am), residents were told not to drink or bathe in the water and to discard ice or beverages made with tap water.

The exposure period was considered to have been +0 to +12 hours. The quantity of ingested hyperfluoridated water was estimated as the number of glasses of tap water consumed during the exposure period plus the number of glasses of beverages made from tap water during that period. The latent interval was defined as the time from consumption of the last glass of hyperfluoridated water to the illness onset. A person was considered dermally exposed to the hyperfluoridated water if he or she bathed or showered at home during the exposure period. Information concerning 321 persons was gathered from 86 (68 percent) of the 127 households. Gastrointestinal symptom histories were obtained for 312 persons, 55 (18 percent) of whom were cases. Symptoms included abdominal cramping (66 percent), nausea (62 percent), headache (49 percent), diarrhea (42 percent), vomiting (13 percent), diaphoresis (12 percent), and fever (4 percent). The main duration of gastroenteritis symptoms was 5.5 hours with a range of 1 to 60 hours. No person sought medical evaluation for gastroenteritis symptoms. Of the 160 persons who drank water, 52 (33 percent) had gastroenteritis compared to only two (1.4 percent) of the 141 persons who did not drink water (relative risk = 23; 95 percent confidence intervals = 5.7, 92.4).

Of the 300 persons whose skin irritation histories were obtained, 30 (10 percent) reported unusual itching, with a duration of 2 to 62 hours. Of these 30 persons, 12 reported skin rash. Persons dermally exposed to hyperfluoridated water in a shower or bath were 2.7 times as likely to have reported itching as unexposed persons (96 per cent CI = 4, 5.3). Persons who reported itching, but who had not bathed or showered, had other water exposures such as dish-washing; their itching was localized to the area of water contact.

Low-dose fluoride ingestion causes nausea, vomiting, abdominal cramping, and diarrhea. Outbreaks have occurred in water supplies with levels from 30 to > 1000 ppm. Symptoms occur with a 5 mg ingested dose. Both copper and fluoride may have had additive effects in this outbreak. However, the infrequency of vomiting and of occurrence of symptoms < 30 minutes after water ingestion suggested fluoride toxicity.

KEY WORDS: Abdominal symptoms; Connecticut; Dermatological effects; Fluoridation accident; Gastrointestinal symptoms; Hyperfluoridation; Overfluoridation.

REPRINTS: James L. Hadler, MD, MPH, Epidemiology Section, State of Connecticut Department of Health Services, 150 Washington St., Hartford, CT 06106, USA.

FLUORIDE-INDUCED HYPERKALEMIA: THE ROLE OF Ca^{2+} -DEPENDENT K^+ CHANNELS

by

Charles C. Cummings and Michael E. McIvor
Philadelphia, PA and Baltimore, MD, USA

(Abstracted from Am. J. of Emergency Medicine, 6:1-3, 1988)

Acute fluoride poisoning is associated with sudden cardiac death by an unknown mechanism. Because F^- binds to Ca^{2+} to cause marked hypocalcemia, lowered serum Ca^{2+} concentrations have been thought to be a major underlying factor in the ventricular irritability of F^- -toxic patients. However, correction of the hypocalcemia does not prevent sudden death. Paradoxically, while decreasing extracellular Ca^{2+} levels, *in vitro* studies have shown F^- increases intracellular Ca^{2+} , which is thought to trigger Ca^{2+} -dependent K^+ channels and produce a K^+ efflux. The K^+ efflux may be important clinically, as patients with F^- overdose can exhibit hyperkalemia shortly before cardiovascular collapse. In erythrocyte suspensions, we found that propanolol, which increases the sensitivity of the Ca^{2+} -dependent K^+ channels, exacerbates the efflux, and quinidine, which blocks the channel, prevents the efflux. In six dogs, 35 mg/kg of sodium fluoride given intravenously produced intractable ventricular fibrillation within 140 minutes. Four dogs given 200 mg of quinidine sulfate with the sodium fluoride developed no ventricular arrhythmias. The

data indicate that F^- -induced hyperkalemia is important in sudden cardiac death following acute fluoride toxicity and that this hyperkalemia is mediated by Ca^{2+} -dependent K^+ channels.

Author's Abstract

KEY WORDS: Ca^{2+} -dependent K^+ channels, Fluoride, Hyperkalemia, Quinidine.

REPRINTS: Dr. McIvor, Division of Cardiology, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21205, USA

MUTAGENIC ACTIVITY OF FLUORIDES IN MOUSE LYMPHOMA CELLS

by

William J. Caspary*, Brian Myhr, Linda Bowers, Douglas McGregor
Colin Riach and Alison Brown
Research Triangle Park, NC, USA

(Abstracted from Mutation Research, 187:165-187, 1987)

The L5178Y mouse lymphoma cell forward-mutation assay was used to test for the mutagenic activity of sodium and potassium fluoride at the thymidine kinase locus. Mutants were detected by colony formation in soft agar in the presence of trifluorothymidine. Mutagenic and toxic responses were observed in the concentration range of 300-600 $\mu\text{g/mL}$ with both sodium and potassium fluoride. Approximately 3-fold increases in mutant frequency were observed in concentrations in the 500-700 $\mu\text{g/mL}$ range that reduced the relative total growth to approximately 10% in the absence or presence of a rat-liver S9 activation system. A sample of 30% sodium fluoride-70% sodium bifluoride (NaHF_2) induced a similar mutagenic response but was more toxic with respect to the fluoride concentration. A specificity for fluoride ions in causing mutagenesis was indicated by the fact that much higher concentrations of sodium or potassium chloride were necessary to cause toxicity and increases in the mutant frequency. The possible involvement of chromosomal changes was signaled by the predominant increase in the small colony class of mutants.

— Author's Abstract

KEY WORDS: Chemical mutagenesis; Fluoride; -In vitro; Mouse lymphoma cells; Thymidine kinase locus; Trifluorothymidine resistance.

REPRINTS: Dr. William J. Caspary, Cellular and Genetic Toxicology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, Research Triangle Park, NC 27709, USA.

FLUORIDE CONCENTRATION IN DECIDUOUS ENAMEL IN HIGH- AND LOW-FLUORIDE AREAS

by

Y Iijima* and T. Katayama
Morioka, Japan

(Abstracted from Caries Res. 19:262-265, 1985)

Fluoride concentrations were determined in surface enamel of exfoliated deciduous molar teeth from schoolchildren who had resided continuously since birth in areas where water naturally contained fluoride varying from 0.32 to 3.18 ppm and in an area with less than 0.1 ppm.

Fluoride concentrations in enamel surface from the fluoridated areas were significantly higher than those in enamel from the low fluoride (0.1 ppm) area. An increase in fluoride concentration in drinking water resulted in an increase in the fluoride content of the outermost enamel, which frequently reached over 10,000 ppm fluoride in the two higher-fluoride areas (3.18 and 1.74 ppm). The results confirm previous findings that fluoride accumulates preferentially in the outer layers of deciduous enamel.

The distribution of fluoride within a depth of about 50 μ M from the enamel surface emphasizes the marked decrease in fluoride concentration from the enamel surface to the interior. The differences between inner and outer enamel is smaller in the area with less than 0.1 ppm fluoride in the drinking water.

KEY WORDS: Enamel, deciduous; Enamel, fluoride content of; Fluoride, systemic; Water fluoridation.

REPRINTS: Y. Iijima, Department of Preventive Dentistry, School of Dentistry, Iwate Medical University, Morioka, Japan.

COMMUNITY WATER FLUORIDATION IN LENINGRAD AND MOSCOW

by

Richard A. Abrams
Milwaukee, WI, USA

(Abstracted from Community Dent. Oral Epidemiol., 16:129-130, 1988)

The fluoride content of 44 water samples obtained between 1983 and 1986 in the cities of Leningrad and Moscow, USSR, were analyzed blindly outside the Soviet Union.

August 1, 1964, the Presidium of the Supreme Soviet of the USSR passed a decree directing water fluoridation in those instances where the natural water fluoride concentration was less than 0.5 ppm. Samples were taken by dentists as well as tourists from potable water sources in public areas, e.g. hotels, museums, schools, government buildings, restaurants, as well as private residences. All samples were collected in sample bottles and were analyzed for fluoride content in both Sweden and the United States. Neither the Swedish nor the American government agency performing the water analysis was aware of the origin of the samples being tested. Only three water samples from Moscow had a level of fluoride 0.8 ppm or above. All other samples had fluoride levels of less than 0.3 ppm. The highest fluoride concentration found in Leningrad was 0.1 ppm. Studies in Leningrad over a 10-year period showed a decrease in decay among children drinking fluoridated water since birth. The Palace of Congresses in the Kremlin had the highest fluoride level (1.0 ppm).

KEY WORDS: Fluoridation; Fluoride concentrations; Soviet Union.

REPRINTS: Division of Community Dentistry, School of Dentistry, Marquette University, 604 N. 16th Street, Milwaukee, WI 53223, USA.

FLUORIDE IONS INCREASE COLLAGENASE PRODUCTION BY RABBIT SYNOVIAL FIBROBLASTS

BY

J. Jendryczko and M. Drozd
Katowice, Poland

(Abstracted from Biomed. Biochim. Acta 44:341-343, 1985)

Treatment of rabbit synovial fibroblasts with fluoride ions (10^{-4} or 10^{-3} M) stimulates the production and secretion of latent collagenase.

To assess membrane perturbation, the activity of 5'-nucleotidase in the presence of fluoride ions was studied; 5'-nucleotidase was generally inhibited in the 90-94% range; it did not decrease at higher fluoride ion concentrations.

In untreated cells, collagenase was 0.09 ± 0.04 (U/min mg of cell protein).

In cells, treated with 10^{-3} M NaF, it was 0.21 ± 0.05 ; in cells treated with 10^{-2} M NaF, 0.39 ± 0.08 .

Thus, fluoride is taken up by cultured rabbit synovial fibroblasts and it increases production of collagenase by these cells.

KEY WORDS: Collagenase production; Rabbit synovial fibroblasts.

REPRINTS: A. Jendryczko, Department of Biochemistry and Chemistry, Silesian Medical School, 40-752 Katowice, Poland.

Fluoride

FLUOROSIS AND CARIES PREVALENCE IN A COMMUNITY DRINKING ABOVE-OPTIMAL FLUORIDATED WATER

by

Jonathan Mann*, Munder Tibi and Harold D. Sgan-Cohen
Jerusalem Israel

(Abstracted from *Comm. Dent. Oral Spidemiol.*, 15:293-295, 1987)

The present study was to assess the prevalence and severity of dental caries and dental fluorosis in a community with 5 ppm fluoride naturally in water. In all teeth groups, the predominant contributor to DMFS was the decayed surfaces or DS (decayed untreated) component. The second molar was second in caries; its DMFS score was 2.21. In molars (1st and 2nd) DMFS scores were higher than in central, lateral and canine anterior teeth. These differences reached statistical significance ($p < 0.001$), by the t-test.

In first molars, the teeth most susceptible to caries, the prevalence of fluorosis at 5 ppm was 100%; of 182 adolescents, 53 demonstrated mild fluorosis, 83 moderate, 46 severe. A statistically significant positive relation was observed between caries prevalence and fluorosis. In boys, fluorosis levels were significantly higher than in girls. The more severe the fluorosis, the higher the DMFS scores; statistical significance, employing analysis of variance, was $p < 0.001$.

The majority of subjects with mild fluorosis were girls (36 of the total 53, or 68%) whereas the majority of subjects with severe fluorosis were boys (38 of the total 46, or 83%). The direct relation between fluorosis and sex was statistically significant ($p < 0.001$), employing chi-square. All subjects demonstrated caries, predominantly in first and second molars. In the mild fluorosis subgroup more mandibular than maxillary molars were carious. This difference between the subgroups was statistically significant ($p < 0.05$), employing chi-square.

Within the present population, caries prevention significantly decreased with increasing severity of fluorosis. Prolonged exposure to excessive fluoride may damage enzyme involved in processes related to enamel mineralization. This damage creates a porotic tooth, susceptible to caries, as debris and plaque are entrapped in hypoplastic areas. Severe fluorosis was present among 42.2% of the boys and only 8.6% of the girls. No differences in oral hygiene were evident between boys and girls. Occlusal caries predominated.

To control for environmental post-eruptive effect, further research will be focused on a younger age group; teeth soon after eruption will be observed. In further research, more detailed and accurate recording of dietary and other oral factors will also be emphasized.

KEY WORDS: Dental caries; Dental fluorosis; Drinking water; Israel

REPRINTS: J. Mann, Department of Community Dentistry and Oral Hygiene, Hebrew University - Hadassah Faculty of Dental Medicine, P.O.B. 1172, Jerusalem 91010, Israel.

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, double-spaced with generous margins. Measures are given in metric system (SI).
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Fiske, C.H. and Subba Row, Y.: The Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, 66:375-400, 1925.

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