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THE PATHOGENESIS OF DENTAL FLUOROSIS

An Editorial Hypothesis

by

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Dental fluorosis is due to fluoride overdosage during the mineralization of the teeth. During the past decade new insights into the nature of the condition have been gained (1), but the exact mechanism by which dental fluorosis occurs is not yet fully understood (2). Taves and Guy (3) found that, in an area where average plasma fluoride levels were 4.2 μM/l (0.08 ppm F), there was an undesirable degree of dental fluorosis. Ericsson (4) has reported that plasma peaks of around 0.2 ppm F produce "mottling" in rat incisors; and Hodge (5) has suggested that dental fluorosis of moderate to severe degree can develop in man when plasma levels reach 0.05-0.1 ppm fluoride.

It is difficult to understand how such low concentrations of fluoride could interfere with normal cell function. Many workers have studied the in vitro effect of fluoride on cells (6-11). It seems clear that levels of at least 20 ppm F are required to affect cell growth and function; such levels are inconceivable in circulating plasma and extracellular fluid because they would be incompatible with life (12).

In 1970, Rich and Feist (13) proposed a hypothetical mechanism to explain the action of fluoride on bone. They suggested that fluoride is not evenly distributed throughout bone, rather, that it is concentrated in two specific regions, namely: 1] areas of bone formed when blood concentrations of fluoride are relatively high; and 2] the surface layer of bone immediately bordering on the osteocyte lacunae and canaliculae. After absorption of fluoride, its concentration in blood and extracellular fluid rises. Fluoride-containing extracellular fluid perfuses the osteocyte lacunae and canaliculae where presumably it is absorbed at this interphase and incorporated into the crystal lattice. Since it must pass from this region if it is to reach interlacunar bone, it can be concluded that the concentration in this region will be greater than in interlacunar bone. However, it is unlikely that fluoride would migrate deeply into interlacunar bone, since crystals in fully calcified bone are so closely packed as to partially exclude fluid and strongly impede diffusion of ions.

Accordingly, Rich and Feist postulate that fluoride is concentrated at the lacunar and canalicular surfaces. They conclude: a] Fluoride in extracellular fluid of bone is concentrated mainly in a surface layer of mineral at the border of osteocyte lacunae and canaliculae; and, b] That fluoride in extracellular fluid of bone is in a slow equilibrium with fluoride in this mineral phase.

If this hypothesis is correct, then any cells which resorb bone could be exposed to significant concentrations of fluoride during the resorptive process. This would hold for both osteoclasts and resorbing osteocytes which would be subjected to a concentration of fluoride approximately proportionate to the intensity of the resorptive process. Osteocytes however, which are entirely surrounded by surfaces on which fluoride may be concentrated, and which exist

further away from the blood stream, would be subjected to a higher concentration of fluoride upon resorbing bone than would the osteoclasts.

Bone resorption and new bone formation are processes that occur intermittently in all bones throughout the life of the individual. During the time of permanent tooth development and eruption, alveolar bone turnover is particularly rapid as tooth organs develop and grow, and their bony crypts enlarge to accomodate this growth. Figure 1 illustrates a developing tooth germ and Figure 2 represents some of the events which may occur when the crypt is being remodelled. The events illustrated in Figure 2 are self-explanatory. Any

Figure 1

Developing tooth in bony crypt.

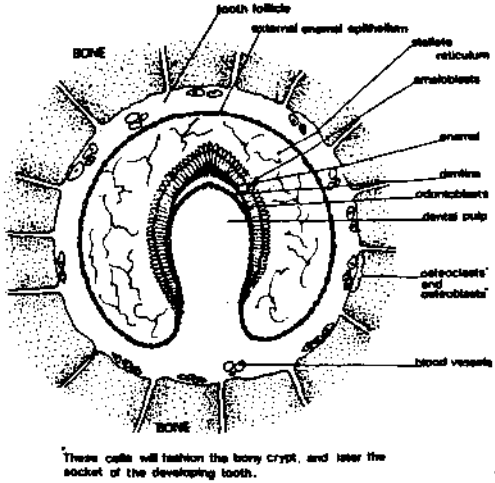
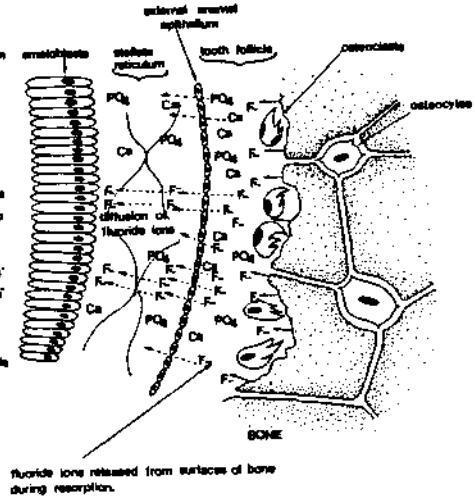


Figure 2

Resorption of bony crypt releases fluoride in bone.



(Note: in Figures 1 and 2 areas of intense stippling represent high fluoride concentrations in bone.)

fluoride concentration in bone being resorbed will be released into extracellular fluid. The local concentration could be relatively high and will depend on a] the level of fluoride in preformed bone prior to resorption; and, b] the intensity of the resorptive process. In Figure 2, fluoride is shown diffusing through the external enamel epithelium, across the stellate reticulum and into the immediate vicinity of ameloblasts.

In 1969, Weatherell (14) suggested that local rises in the extracellular concentration of fluoride might affect nearby cells. In a more recent paper, Weatherell et al. (15) showed that developing enamel not only absorbs fluoride but may raise the extracellular concentration of fluoride ion locally. Hence, the hypothesis presented in this paper is not new, but it suggests that fluoride in alveolar bone may be released during the growth of the tooth germ and expansion of the crypt. Such a mechanism may explain how concentrations of fluoride sufficient to damage cells (above 20 ppm F) could reach the vicinity of tooth-forming cells and lead to dental fluorosis.

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KENETICS OF FLUORIDE PENETRATION IN LIVER AND BRAIN

by

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SUMMARY: Our results suggest that orally administered sodium fluoride enters liver and brain. The blood-brain barrier fails to exclude the fluoride ion from nerve tissue. That fluoride ions also readily pass the placental barrier has been repeatedly demonstrated (9). Fluoride levels in brain reach a maximum approximately two hours after it has been administered, whereas accumulation in liver continues for at least three hours.

KEY WORDS: Fluoride penetration; Liver; Brain.

Introduction

To determine the effect of fluoride on brain and liver enzymes and the pharmacodynamics of the effect of fluoride, the following three questions should be answered: 1] Does fluoride penetrate into liver cells and does it pass the blood-brain barrier? 2]. What length of time is required for the fluoride concentration to reach a maximum in tissues? 3] What is the most suitable method for fluoride analysis of numerous samples within a wide range of concentrations?

Zipkin and Likins (1) showed that, in the rat, nearly 50% of the ingested fluoride is absorbed from the gastrointestinal tract within 30 minutes. A "plateau" is reached after 2 hours. Armstrong and Singer (2) studied the distribution of fluorides in muscle, liver and tendon; they observed that, after two hours, a maximum was reached which itself lasted for at least two hours. The work of Carlsson (3), on the pentration of fluoride into the brain, suggests the existence of an effective blood-brain barrier against fluoride in nervous tissue. On the other hand, Appelgren et al. (4) demonstrated (using autoradiography) that F^- penetrates into the central nervous system of the mouse.

Whereas ion-selective electrodes have been used widely for determining fluoride concentrations (5), we find that long adaptation times (up to 30 minutes) of the electrode and a lack of accuracy make this procedure unsuitable for our purposes. Therefore, the gas chromatographic method of Fresen et al. (6) for quantitative determination of fluoride in biological materials was adapted for measuring fluoride in brain and liver samples.

Materials and Methods

Male Wistar rats (250-300 g) were fed standard laboratory food (A.04 from U.A.R., Villemoisson-sur-Orge, France) and tap water. The fluoride content of

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food, as determined after $HClO_4$ digestion (5) was 10 mg/kg. The fluoride content of the water ranged between 0.0 and 0.4 mg per liter. Food was removed 24 hours prior to experiments. Rats were administered (by stomach intubation) a dose of 10, 20, 30, or 50 mg NaF/kg body weight dissolved in 0.9% NaCl. The animals were anesthetized with sodium pentobarbital (Nembutal®) i.p. at 45, 60, 90, 120, 150 and 180 min after the fluoride load (4 rats/concentration/timepoint). Blood was taken by heart puncture in heparinized tubes and the plasma was obtained by centrifugation. The animals were killed by decapitation and the brains and livers were removed and weighed. The tissues were homogenized with a Potter-Elvehjem homogenizor as 25% (w/v) suspensions in a 0.05 M TRIS-HCl buffer, pH 7.4.

Fluoride determination: 1. Gas Chromatograph: Hewlett-Packard 5710 A; Column: 20% of silicone oil DC 200/50 on Gas Chrom Q; Injection temperature: 150°C; Column temperature at start: 55°C; gradient: +5°C/min; final: 80°C for 5 min.; Detection temperature: 150°C; Carrier flow (nitrogen): 10 ml/min (as determined by the van Deemter equation); Detector: Flame ionization; Stock solution NaF: 0.221 g NaF/100 ml (equals 1 mg F^- per ml); Working solutions: 0.1-10 µg NaF/ml; Derivative and extraction solution (DES): 0.6 mg TCMS (Pierce Chemical Co., Rockford, Il, USA) + 6.1 µg isopentane (internal standard) per ml benzene; HCl 25%. 2. Procedure: 2 ml of the sample (homogenized tissue) were added to 1 ml HCl and 1 ml DES. Because of the low boiling point of the trimethylfluorosilane (TMFS) formed (16.4°C) and of the internal standard (28°C) the reaction was performed at 4°C. The tubes were mechanically shaken for 30 min, the two layers were separated by gentle centrifugation (5 min at 500 g) and 1 to 5 µl of the organic phase were injected into the GC. A set of standard solutions and a blank were analyzed at the same time.

Results and Discussion

A. THE GAS CHROMATOGRAPH (GC) DETERMINATION OF FLUORIDE as described by Fresen et al. (6) is based on the work of Bock and Semmler (7) and involves two reactions:

$$R_3\text{-SiCl} + H_2O \longrightarrow R_3\text{-SiOH} + HCl$$
$$R_3SiOH + H^+ + F^- \longrightarrow R_3\text{-SiF} + H_2O$$
$$R_3\text{-SiCl} + H^+ + F^- \longrightarrow R_3\text{-SiF} + HCl$$

(in our case: R_3SiCl = trimethylchlorosilane = TMCS). Thus, the alkylsilane is first converted by water into the corresponding silanol which in turn reacts selectively with fluoride to form fluorosilane. This compound can be extracted from the acidified reaction medium with benzene. The extracted fluorosilane is then determined quantitatively by GC.

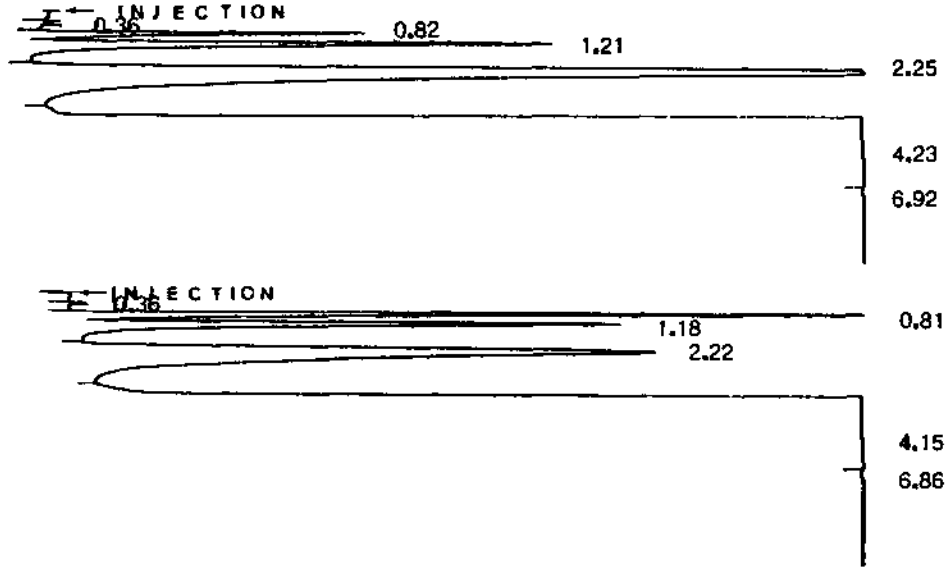
The standard solutions and other aqueous samples (serum, saliva) could be analyzed as described by Fresen et al. (6) without any further treatment. However, the high protein content of brain and liver samples disturbed the adequate centrifugal separation of the organic and the aqueous layers by forming a thick floating mass. Elimination of the proteins by trichloroacetic or perchloric acid results in an important and variable loss of F^-. Therefore, in these samples, these proteins were digested with trypsin (30 mg/sample; incubation at 37°C for 2 hrs) before the DES was added. The addition of the digestion step

broadens the application range of the method. The presence and the action of trypsin has no effect on the linearity of the F^- determination.

Figures 1a and 1b represent respectively the gas chromatograms obtained with a standard NaF solution (1 µg/ml) and for the plasma of a fluoride-treated rat (10 mg/kg). Peak 1 corresponds to the TMFS formed by the substitution in TMCS of Cl^- by F^- present in the sample. Peak 2 is the internal standard (isopentane), while peak 3 is the excess of TMCS. The solvent (benzene) eluates hereafter as a broad peak and does not interfere with the analysis.

Fig. 1.a. and 1.b.

GC chromatograms



(1.a.) Standard NaF solution (1 µg/ml). (1.b.) Plasma sample of NaF-treated rat.

Table I

Amount Added (µg F^-)	Liver + F^- mean (µg)	Liver + F^- recovery (%)	Brain + F^- mean (µg)	Brain + F^- recovery (%)
0.00	0.000		0.000	
0.05	0.046	92.0	0.045	90.0
0.20	0.190	95.0	0.193	96.5
0.50	0.481	96.2	0.493	98.0
1	1.030	103.0	0.970	97.0
2	1.990	99.5	1.985	99.2
5	4.830	96.6	4.980	98.0
10	9.700	97.0	9.650	96.5

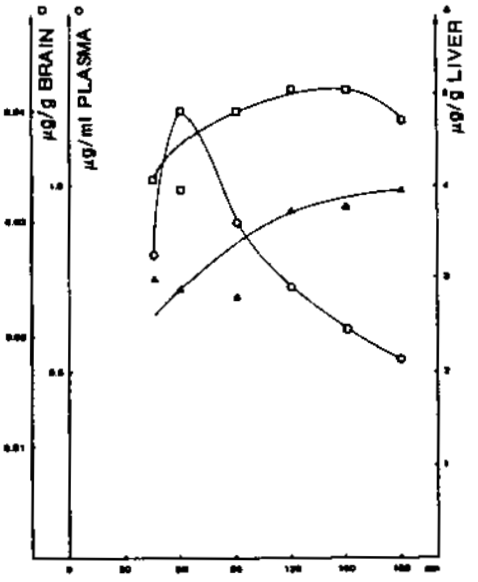
To check the recovery, different amounts of fluoride (ranging from 0.01 to 10 µg) were added to 5 rat liver and 5 rat brain samples before homogenation. These samples, together with a set of standards were carried through the entire procedure. The results of this experiment are shown in Table I.

Thus, the GC method of Fresen et al. for the quantitative determination of fluoride, once adapted for protein-rich samples by including an enzymatic digestion step, proved to be reproducible, sensible and accurate. Very recently, Retief et al. (13) showed, in a comparative study, the accuracy of the Fresen method.

B. Pharmacokinetics of F^-: The 50 mg/kg dose proved to be lethal for at least 75% of the animals within 30 minutes (range: 5-30 min). With 30 mg/kg respiration difficulties and convulsions were observed in all rats. Figure 2 represents the data obtained from rats treated with 10 mg NaF/kg body weight. In contrast to the results of Carlsson (3), the fluoride ion is able to cross the blood-brain barrier and to penetrate into the brain, where its concentration reaches a maximum two hours after ingestion. Penetration into the liver is slower, but greater amounts are taken up. Although the lower values at 60 min are not as marked for the the other doses, uptake in the brain and liver might be biphasic. The F^- concentration in the liver and in the brain rises with the dose of F^- administered (see Figure 3 and Table II).

Figure 2

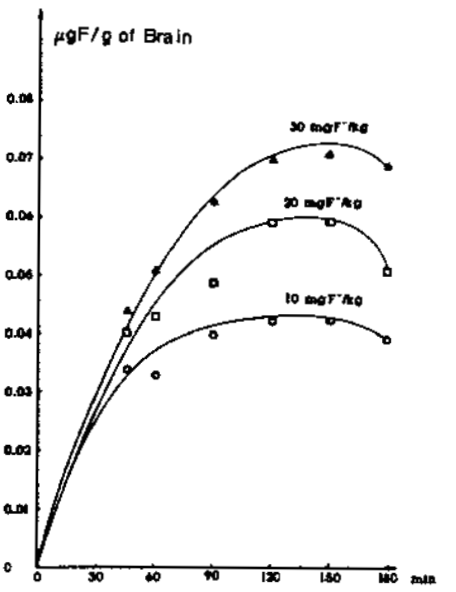
Kinetics of Penetration of F^- in Plasma, Liver and Brain



Rats treated with 10 mg NaF/kg.

Figure 3

Relationship between Dose and F^- Concentration in Brain.



Our data, for plasma samples, correspond with those of Patz et al. (8). In our experiments the absorption of fluoride by the liver is slower than that described by Armstrong and Singer (2). The sharp rise in plasma F^- concentra-

tions corresponds with previous observations obtained using ^{18}F (10). The levels in the plasma of fluoride-treated rats (10^{-5} M) are of the same order of magnitude as those for which in vitro activity of certain enzymes is significantly reduced (11,12).

Table II

Time	45 min			60 min			90 min		
Dose (mg/kg)	Plasma	Brain	Liver	Plasma	Brain	Liver	Plasma	Brain	Liver
10	0.80	0.034	3.00	1.20	0.033	2.90	0.90	0.040	2.80
20	1.40	0.040	5.60	1.80	0.043	5.60	1.45	0.49	6.00
30	1.60	0.044	7.10	2.20	0.051	7.90	2.10	0.063	9.30
	120 min			150 min			180 min		
	Plasma	Brain	Liver	Plasma	Brain	Liver	Plasma	Brain	Liver
10	0.74	0.042	3.75	0.62	0.042	3.80	0.54	0.039	3.97
20	1.20	0.056	6.20	1.04	0.056	6.50	0.79	0.051	6.64
30	1.90	0.70	9.60	1.60	0.071	10.12	1.40	0.069	11.36

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THE FLUORINE CONTENT OF RICE GROWN IN VARIOUS DISTRICTS IN JAPAN

by

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SUMMARY: In Japan, fluoride levels of rice, grown in various districts as well as those within a prefecture, differed. In unpolished rice, fluoride ranged from 0.44 to 2.84 ppm; in polished rice, from 0.21 to 1.57 ppm. Fluoride in polished was above 0.7 ppm in a coalfield zone, in an area around hot springs, in one near volcanoes, and in the vicinity of aluminum plants. For analyzing the fluoride content of rice, the ashing-microdiffusion method proved to be preferable to others.

KEY WORDS: Fluoride content; Japan; Rice.

Introduction

Since fluoride toxicity is related to total daily fluoride intake, the fluoride content of foods, which constitute the major source of fluoride in daily life, is important. Total daily fluoride intake varies in each country of the world (1). It is believed to be higher in Asia than in Europe because fluoride levels in foods are higher (2,3). A comparative study of the F content of rice, produced in various districts of Japan, the principle food in Asian countries, is presented.

Method

Fourteen samples of unpolished (N = 14) and 45 of polished rice (N = 45) grown in 26 prefectures of Japan were collected in 1983 and 1984. For comparison, rice produced in China (in 1983) was employed. For F determination, the sample was washed, dried and crushed to 80 mesh or less. To 1 g of crushed sample, CaO was added and ashed at 550°C for 24 hours. The ashed sample was introduced into Conway's cell, $HClO_4$ added, saturated with Hexamethyldisiloxane (HMDS) and micro-diffusion performed (60°C, 3 hours) (4); after adding Total Ionic Strength Adjustment Buffer (TISAB), determination was made with ion-electrodes, twice for each sample and averaged. The above-described method was used for analysis of F content of rice (Table 1), because, when determinations were made without ashing, all figures obtained were low, suggesting insufficient separation of F from the sample. In the ashed sample, the figure obtained by the conventional distillation method (5) and that by the micro-diffusion method are quite similar (Table 1). However, for treatment of many samples or for analyzing foods containing a lower fluoride content, the micro-diffusion method was preferred because it is simple, rapid, and requires only a small sample.

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Results

Variations were found in F levels of rice grown in various districts of Japan as well as in rice produced within the same prefecture (Table 2). The F content in unpolished rice ranged from 0.44 ppm to 2.84 ppm. In polished rice, on the other hand, it ranged from 0.21 ppm to 1.57 ppm (Figure 1). Rice produced in the following districts contained 0.7 ppm F or more: a coalfield zone (0.70, 0.72, 0.95 ppm: Kurade, Fukuoka Prefecture), an area around hot springs (0.86 ppm: Takeo, Saga Prefecture), an area near aluminum plants (1.28, 1.33 ppm: Ohmuta,

Table 1

F content in polished rice by each analytical method.

Pre-treatment method	Values (ppm)
Non-Ashing	
1. Direct	0.25 ±0.04
2. Micro-diffusion	0.19 ±0.05
Ashing (550°C)	
3. Direct	0.30 ±0.05
4. Distillation	0.38 ±0.05
5. Micro-diffusion	0.37 ±0.04

(M ±SD)

Table 2

F content of rice grown in various districts of Japan.

Prefecture	F content (ppm)
Unpolished Rice	
Hokkaido	0.80
Fukushima	0.65
Ibarangi	0.45
Gunma	0.54
Nagano	0.51
Hyogo	0.44
Nara	0.56
Shimane	0.48
Tokushima	0.70
Saga	1.17
Kumamoto	0.97, 0.94
	2.84
Kagoshima	1.75
Polished Rice	
Hokkaido	0.34, 0.42
Aomori	0.25
Akita	0.24, 0.28
	0.29, 0.39
Yamagata	0.37, 0.45
Saitama	0.35
Chiba	0.39

Prefecture	F content (ppm)
Polished Rice	
Shizuoka	0.48
Niigata	0.26,0.31
	0.36, 0.51
Ishikawa	0.51
Nagano	0.31
Aichi	0.63
Osaka	0.47
Hyogo	0.21, 0.23
	0.32, 0.34
	0.46, 0.49
Wakayama	0.27, 0.41
Tottori	0.39
Fukuoka	0.35, 0.70
	0.72, 0.95
Saga	0.46, 0.86
Nagasaki	0.51
Kumamoto	1.17, 1.26
	1.28, 1.33
	1.43, 1.57
Kagochima	0.51, 0.56
	0.59

Fukuoka Prefecture) and an area around volcanoes (1.17, 1.26, 1.43, 1.57 ppm: around Mt. Aso, Kumamoto Prefecture). Furthermore, rice with 0.5 ppm or more was produced in other specific areas such as in the vicinity of aluminum plants (0.51 ppm: Naoetsu, Niigata Prefecture) and where 2,000-7,000 g/m²/year volcanic ash descended annually (0.56, 0.59 ppm: Tarumi, Kagoshima Prefecture).

F in rice produced in China, analyzed for comparison (Table 3), was 1 ppm or more in unpolished rice; in polished rice 0.4 ppm or more. Thus the range of F in rice produced in Japan is high in comparison, especially that of unpolished rice.

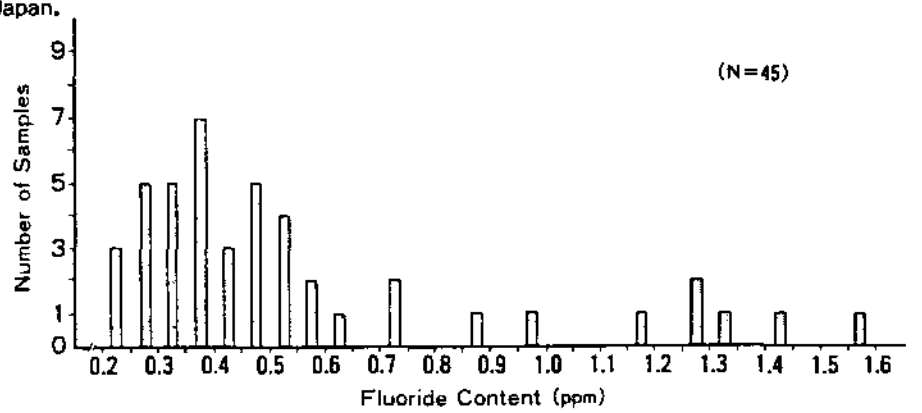
Table 3

F content of rice grown in China.

Area	F content (ppm)
Unpolished Rice	
China	1.08, 1.37 1.74, 1.76
Polished Rice	
Gui zhou	0.43
Yun nan	0.48
Guang xi	0.48
Guang dong	0.42

Figure 1

Distribution of fluoride content in polished rice grown in various districts in Japan.



Discussion

The fluorine content in unpolished and polished rice produced in Japan reported by others (6-11) to be 0.89-10 ppm and 0.19-17 ppm respectively indicates wide differences from one research worker to another. According to data obtained in our study unpolished rice contained 0.44-2.84 and polished rice 0.21-1.57. Fluoride content is affected by atmosphere, soils, river water and other environmental conditions as well as cultivation methods among which are use of phosphatic fertilizers and agricultural chemicals. Especially in rice produced in the environs of aluminum plants have high fluoride levels been reported (10 ppm or more in unpolished rice and 5 ppm or more in polished rice) (9). Moreover in our study, fluoride content varied according to districts; rice high in fluoride came from the coalfield zone, probably caused by environmental and/or cultivation conditions previously mentioned.

According to our calculation, the average daily fluoride intake of Japanese people from polished rice, the major food, would be 0.04-0.34 mg, because in

1984 the average daily intake of rice is estimated to be 214 g. In districts, where the fluoride level was high, the fluoride content in crops other than rice would be high also which resulted in considerable differences in total daily fluoride intake between districts. In China, data are now available (12,13), establishing the relationship between high fluoride foods and fluorosis, although it has not yet been reported to this extent here in Japan (7). In our experiments a high fluoride level was detected in several specimens of rice produced in China.

Conclusion

The ashing-micro-diffusion method, together with the ashing-distillation method, was effective for analyzing rice. Considerable differences in F levels of rice between various districts were observed by this method. F levels in rice were unusually high in a coalfield zone, in special areas around hot springs, aluminum plants and volcanoes. Regarding foods other than rice and total F intake, considerable differences between various districts are also anticipated.

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THE TOXICITY AND STRUCTURE OF VARIOUS AROMATIC FLUORIDES

by

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SUMMARY: The toxicity of 24 fluorinated aromatic compounds in rats has been investigated. The median lethal doses (LD_{50}) of 4-fluorophenol, 2-fluorophenol, 4-trifluoromethylbenzaldehyde and 2-chloro-4-fluorophenol were 336, 450, 662 and 1000 mg/kg, respectively. Compounds which had the LD_{50} of 1 to 3 g/kg were 2- and 3-fluorobenzaldehyde, 2- and 3-fluorobenzoyl chloride, 4-trifluoromethylbenzyl alcohol, 4-trifluoromethylbenzal chloride. Compounds which had the LD_{50} of 3 to 5 g/kg were 4-fluorobenzoyl chloride, 2- and 3-fluorobenzoic acid, 2-trifluoromethylbenzaldehyde, 4-trifluoromethylbenzoyl chloride, 2-trifluoromethylbenzyl alcohol. 4-fluorobenzoic acid, 2-, 3- and 4-fluorotoluene, 2- and 4-trifluoromethylbenzoic acid, 2-trifluoromethylbenzoyl chloride, hexafluoroparaxylene had LD_{50} of more than 5 g/kg. In fluorobenzene derivatives, there was no relationship between toxicity and the orientation of fluorine. In benzotrifluoride derivatives, the 4-orientation was more toxic than the 2-orientation. Taking account of the LD_{50}, the symptoms of poisoning, body weight changes, the toxicity of fluorinated compounds was dependent on that of the parent non-fluorinated compounds.

KEY WORDS: The LD_{50} value; Aromatic fluorides; Structure; Toxicity

Introduction

The demand for intermediates in producing fluorides has been increasing recently, because fluorinated products are known in many cases to have lower toxicity and higher physiological activity than the corresponding non-fluorinated compounds. However, the toxicity of these intermediates is unknown. In this study, we investigated the acute toxicity of orally administered fluorinated aromatic compounds in rats.

Methods

The investigated compounds were 2- and 3-fluorobenzaldehyde; 2-, 3- and 4-fluorobenzoyl chloride; 2-, 3- and 4-fluorobenzoic acid; 2- and 4-fluorophenol; 2-, 3- and 4-fluorotoluene; 2-chloro-4-fluorophenol; 2- and 4-trifluoromethylbenzaldehyde; 2- and 4-trifluoromethylbenzoyl chloride; 2- and 4-trifluoromethylbenzyl alcohol and hexafluoroparaxylene; 4-trifluoromethylbenzal chloride.

Compounds were obtained from Central Glass Co., Ltd. (Tokyo, Japan). All compounds were evaluated for purity using gas chromatography and were found to more than 99% pure.

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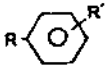
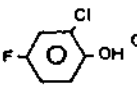
7-week-old Wistar rats (SPF) were divided into 4 to 7 groups with 5 to 10 rats for each substance. Experimental conditions were as follows: temperature, 23° ±1°C; humidity, 55 ±5%; lighting, 12 hours (6:00 am–18:00 pm). Rats were acclimated to this environment for one week before tests. 2-, 3- and 4-fluorobenzoyl chloride; 2- and 4-trifluoromethylbenzaldehyde; 4-trifluoromethylbenzyl alcohol were suspended with polyethylene glycol and 4-fluorophenol was dissolved with distilled water prior to use. The suspension and the solution of fluorides were administered by stomach tube in volumes of 0.5-1.5 ml/100 g body weight in rats. Other compounds were oils. Since the administration of oily compounds was in a small quantity, these were administered by using a microcylinder through a stomach tube, followed by 1 ml polyethylene glycol through a stomach tube. The symptoms of poisoning were observed hourly for 8 hours following the administration and the number of animal deaths and the poisoning symptoms were noted and measured body weight for 7 days. The median lethal dose (LD_{50}) was calculated by the method of Litchfield and Wilcoxon as the standard of mortality to 72 hours following the administration.

Results and Discussion

Table 1 shows the LD_{50} of the investigated compounds. The LD_{50} of 4-fluorophenol, 2-fluorophenol, 4-trifluoromethylbenzaldehyde and 2-chloro-4-

Table 1

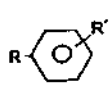
LD_{50} (g/kg) and Structure of Investigated Compounds

 R′	2-F	3-F	4-F	2-CF_3	4-CF_3
R= CHO	1.9 (1.5-2.3)**	1.3 (0.8-2.0)	N.D.*	3.6 (2.7-4.6)	0.66 (0.54-0.81)
COCl	2.6 (1.8-3.7)	2.4 2.0-3.0)	3.2 (2.9-3.5)	8.2 (7.0-9.5)	3.2 (2.7-3.8)
COOH	4.0 (2.9-5.6)	3.0 (2.8-3.2)	5.0<	5.0<	5.0<
OH	0.45 (0.39-0.52)	N.D.	0.34 (0.28-0.40)	N.D.	N.D.
CH_3	5.1 (4.4-5.9)	5.0 (4.4-5.7)	7.0 (6.0-8.2)	N.D.	N.D.
CH_2OH	N.D.	N.D.	N.D.	4.1 (3.2-5.3)	1.7 (1.4-2.0)
	1.0 (0.9-1.3)	10<	1.5 (1.3-1.7)		

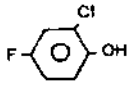
* N.D.: No Data ** 95% Confidence Limits

Table 2

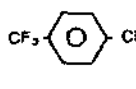
Acute Symptoms During 8 Hours Following Administration



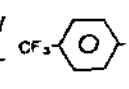
R' =	2-F			3-F			4-F			2-CF_3			4-CF_3		
	M*	C**	W***	M	C	W	M	C	W	M	C	W	M	C	W
R=CHO	↓	+	+	↓	+	+	N.D****			↓	−	+	↓	+	+
COCl	↓	−	+	↓	−	+	↓	−	+	↓	−	+	↓	−	+
COOH	↓	−	+	↓	−	+	↓	−	+	↓	−	−	↓	−	−
OH	↓	+	+	N.D.			↓	+	+	N.D.			N.D.		
CH_3	↑	−	−	↑	−	−	↑	−	−	N.D.			N.D.		
CH_2OH	N.D.			N.D.			N.D.			↓	−	+	↓	−	+



M	C	W
↓	+	+



M	C	W
↓	−	+



M	C	W
↓	−	+

* M : Movement change (↑ = increase, ↓ = decrease)

** C : Convulsion

*** W : Weakness

**** N.D. : No Data

fluorophenol were less than 1 g/kg. The LD_{50} of other compounds were 1.3 g/kg to 8.2 g/kg and hexafluoroparaxylene was more than 10 g/kg. In fluorobenzene derivatives, there was no relationship between the toxicity and the orientation of fluorine. In benzotrifluoride derivatives, the 4- orientation was more toxic than the 2- orientation. It is thought that there is less steric hindrance between functional groups and trifluoromethyl radicals in the 4-orientation, so that the toxicity of functional groups appears more strongly.

Table 2 shows the symptoms during 8 hours following the administration. The change of movement was compared with the control state. The existance of convulsion are indicated by +,-. Weakness was determined by the inability to move from the prone state by clop stimulation of back of rats. With almost all compounds, a decrease of movement and weakness was observed around the dosages of the LD_{50}. However, in Fluorotoluene, movement increased after dosage. The symptoms of poisoning shown in Table 2 disappeared within 3 days in almost all of the compounds. In fluorobenzaldehyde, convulsions were observed at high doses. In fluorobenzoylchloride, CNS depression was observed. In fluorophenol, violent convulsions were observed soon after administration, and the animals died immediately after administration in high doses. At lower doses tremors appeared, and the symptoms disappeared within 3 days. With fluorobenzoic acid, CNS depression, diarrhea, and decrease of body temperature were observed. With fluorobenzaldehyde and fluorobenzoylchloride, CNS depression was observed. These symptoms were very similar to those of the analogous non-fluorinated compounds.

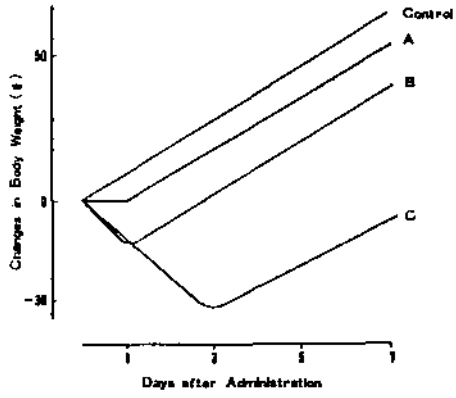


Figure 1

The pattern A, B and C in body weight changes of rats administered with approximate LD_{50} dosages. Pattern A: slight decrease in body weight. Pattern was similar to control. Pattern B: body weight decreased for 1 or 2 days. Pattern was similar to control. Pattern C: body weight decreased for 3 days. Increasing ratio of body weight was low.

Figure 1 shows 4 patterns of body weight changes found with those animals which were administered dosages near the LD_{50}. 2-trifluoromethylbenzyl alcohol, and 2-trifluoromethylbenzoyl chloride followed pattern A. 3- and 4-fluorotoluene, 2- and 3-fluorobenzoic acid, 2- and 3-fluorobenzaldehyde, 2- and 4-fluorobenzoyl chloride, 4-trifluoromethylbenzoyl chloride, and 2-trifluoromethylbenzaldehyde followed pattern B. 3-fluorobenzoyl chloride, 2-fluorotoluene, 2-chloro-4-fluorophenol, 2- and 4-fluorophenol, 4-trifluoromethylbenzyl alcohol, 4-trifluoromethylbenzal chloride, and 4-trifluoromethylbenzaldehyde followed pattern C. In pattern A, it is thought that there were few effects to living systems by fluorides. In pattern B, it is supposed that the body weight decreases primarily by gastrointestinal impairment because the decline of body weight after dosage is recovered in one day. In pattern C, some organic injury, for example, hepatic or renal insufficiency may be a result, for the severe decrease of the body weight continued for a few days and the increasing ratio of body weight was low. Pathological investigations are necessary to investigate these questions in detail.

Except for 2- and 4-fluorophenol and trifluoromethylbenzoyl chloride and 2-chloro-4-fluorophenol, the LD_{50} of fluorides investigated in this study was low. It is estimated that the toxicity of fluorinated compounds was similar to those of the parent non-fluorinated compounds.

In the Litchfield and Wilcoxon method, more than 10 animals are necessary in a group. In this study, we investigated LD_{50} in 10 rats in the 2- and 4-fluorophenol; 2- and 4-trifluoromethylbenzaldehyde; 2- and 4-trifluoromethylbenzoyl chloride; hexafluoroparaxylene and in 5 rats in other compounds. In 2-trifluoromethylbenzyl alcohol, 3-fluorobenzaldehyde, 2-fluorobenzoyl chloride, the ratio of the width of confidence limits to the LD_{50} was 50-91%. The confidence of the LD_{50} of the 5 compounds was low. It was less than 50% in other compounds.

We cannot appreciate the true toxicity of the compounds by the comparison of the LD_{50} alone in these experiments. It should be considered to appreciate overall the degree of symptoms and the body weight changes, etc. For example, in this study, 2-fluorotoluene had a high LD_{50} value; however, severe body weight changes were observed. There was no relation among the LD_{50}, poisoning symptoms and the degree of body weight changes. The deaths observed within the period of about 72 hrs reveal that the compounds intensely

affect the cardiovascular or respiratory systems. The lasting decrease of body weight was a sign of the toxicity to some parenchymatous organs (1). Histological investigations are necessary to evaluate the toxicity of these fluorides in detail.

Reference

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STUDIES ON FLUORIDE UPTAKE BY SOFT TISSUES OF AN EDIBLE MUDSKIPPER, BOLEOPHTHALMUS DUSSUMIERI (CUVIER AND VALENCIENNES) OF DUMAS COAST, GUJARAT

by

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SUMMARY: Mudskippers, B. dussumieri were exposed to a fluoride effluent containing 5, 50 and 80 ppm fluoride. Intestines accumulated maximum amounts of fluoride, followed by muscles and liver. All tissues showed a considerable drop in fluoride content after 240 hrs. It is presumed that these 3 tissues have the capacity to exclude fluoride from the cells.

KEY WORDS: Fluoride effluent; Mudskipper; Fish; Boleophthalmus dussumieri; Liver; Intestine; Muscles.

Introduction

Few reports on the toxicity of effluent from a fluorine industry situated at Bhestan (District Surat, Gujarat State) to aquatic organisms are available. Hatching was delayed when the eggs of a freshwater fish, Catla catla were exposed to various concentrations of the effluent (1). Fry of C. catla showed changes in metabolites and minerals on exposure to the effluent from the Surat fluorine industry (2). Mudskipper, Boleophthalmus dussumieri exposed to 40 and 80 ppm fluoride concentrations of the effluent for 288 hr showed increase in blood glucose, changes in clycogen content and SDH activity in liver and muscles (3). High fluoride accumulation was observed in a freshwater prawn, Machrobrachium rosenbergii, when they were exposed to 3, 6, 9, and 12 ppm fluoride concentrations of the effluent for 48 hr (4).

To understand the effect of fluoride effluent from the Surat fluorine industry on mudskippers, B. dussumieri, accumulation of fluoride in liver, intestine and muscles was considered in the study.

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Materials and Methods

The mudskippers, B. dussumieri, collected from the Dumas coast of South Gujarat, were brought to the laboratory and acclimated in sea water for 7 days [photoperiod, 12 hr D/N; temp., 25 ±2°C; salinity, 27%; pH 8.05 ±0.1]. They were fed with commercial fish food twice a day.

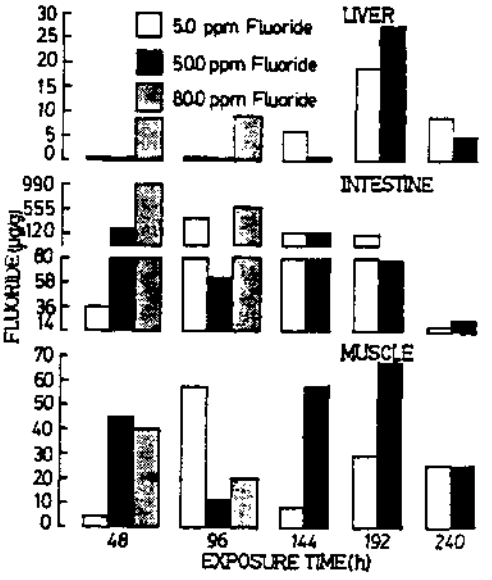
The effluent collected from the discharge point (468 ppm F) of the fluorine industry situated at Bhestan (District Surat) was diluted with an appropriate volume of sea water to obtain 5, 50 and 80 ppm fluoride solutions. Thirty fish (16.0-18.5 cm in length and 15.0-20.0 g in weight) were exposed to media containing 5, 50 and 80 fluoride in polypropylene containers. The fluoride content in the effluent concentrations was measured with a fluoride electrode (Orion Research, Inc., USA). The same number of fish were maintained in sea water and treated as control. The exposure media were changed daily. Three fish each were removed from 5 and 50 ppm fluoride at the end of 48, 96, 144, 192 and 240 hr. From 80.0 ppm fluoride media, samples were taken at the end of 48 and 96 hrs only, since the majority of fish exposed to this concentration died at the end of 120 hr. The fish were immediately sacrificed; liver, intestine and muscles were isolated. They were pooled separately and dried in an oven at 80°C until a constant dry weight was obtained. Fluoride content in the isolated tissues was estimated according to Wright and Davison (5).

Results and Discussion

Considerable fluoride accumulation was observed in liver, intestine and muscles of the mudskipper exposed to 5.0, 50.0 and 80.0 ppm fluoride. The fluoride content in liver, intestine and muscles of fish from the control group was 0.5, 2.0 and 3.5 ppm, respectively. Fluctuations in fluoride content in muscles and intestines were similar; however the intestines accumulated a higher amount of fluoride than the former. Liver accumulated the least (Figure 1). Gastric lumen, unlike other tissues, can absorb even undissociated HF molecules. Intestine, in the absence of inhibitory cations, can absorb fluoride ions which generally exceed 90% (6). In the present study, at the end of 240 hrs, all tissues showed a drop in fluoride content. Initial exposure (48 hr) to 5.0 ppm fluoride showed a comparatively low fluoride accumulation in tissues. However, when exposure duration increased, the tissues accumulated considerable fluoride, sometimes as much as those

Figure 1

Fluoride accumulation in soft tissues of B. dussumieri exposed to different concentrations of fluoride effluent.



exposed to 50.0 ppm fluoride. At the end of 240 hrs, fluoride content in tissues dropped. It is obvious, from the present study, that soft tissues have the capacity to eliminate fluoride ions. Such elimination of fluoride ions has been reported in mammalian tissues (7).

Conclusion

Thus it can be stated that the higher the infiltration of fluoride ions, the faster the elimination. This mechanism is not triggered actively when penetration of fluoride ions is slow. Therefore, prolonged exposure of midskipper to low fluoride levels is as toxic or even more toxic than short term exposure to high fluoride levels.

Acknowledgements

The author thanks the late Dr. Kiran M. Desai (Department of Biosciences, South Gujarat University, Surat) for suggestions and Dr. Pankaj Hiradher (Department of Biosciences, South Gujarat University, Surat) for comments on the manuscript.

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DRY DEPOSITION OF FLUORIDES ON LIME PAPERS

by

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SUMMARY: Lime papers have been used to determine the dry deposition of fluorides which is higher under shorter exposure periods, due to a loss of reactivity of the calcium hydroxide. In reaction with CO_2, calcium carbonate is formed on the lime papers, which is less reactive than calcium hydroxide. Losses in reactivity have been studied by comparing different integration periods.

Deposition rates based on experiments with 28 day exposure have been compared with deposition rates based on exposures of lime papers during 4 corresponding consecutive weeks. The decrease in reactivity, higher at low deposition rates, amounted to approximately 50%. At higher deposition rates, reactivity dropped to almost 25%.

The good correlation between deposited amounts of fluorides on lime papers and emission concentrations in ambient air, indicates that the ambient fluoride concentration is the most important factor determining the deposition rate of fluorides on lime papers. However, large differences are possible due to climatic conditions of which wind speed must be the most important parameter. The deposition velocities for 28 and 7 days were 10.1 and 13.6 mms^{-1}, respectively.

KEY WORDS: Fluoride, Dry deposition, Lime papers, Deposition velocity.

Introduction

For many years lime papers have been used as an inexpensive and easy method to detect the occurrence of fluorides in ambient air. The limitation of the method is that one cannot directly derive ambient air concentrations from it. Measurements with lime papers, the so-called "fluoride load" determinations (1), are in a more recent point of view "dry deposition" measurements on artificial surfaces.

Filter papers impregnated with calcium hydroxide are mainly used (2-6). Other research workers use sodium hydroxide (7-10) or sodium formate (11). The use of calcium hydroxide has the disadvantage that there is a loss of reactivity in function of time, because of the reaction of ambient CO_2 with calcium hydroxide whereby calcium carbonate, a neutral salt is formed (12). Sodium Hydroxide and sodium formate lose their reactivity more slowly (forming sodium carbonate which has an alkaline reaction) but sodium fluoride which results is highly soluble and under high air humidity, volitilization of HF is possible as shown by Davison (13). Sodium hydroxide papers accumulate lower

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amounts of fluoride under the same circumstances than calcium hydroxide papers (14) probably due to the volatilization effect.

In this paper, the accumulation of fluoride by lime paper is studied as a function of time and a comparison with ambient air concentrations has been carried out. Lime papers are preferable for accumulating gaseous fluorides, the most phytotoxic compounds in ambient air. Particulate fluorides have a much lower accumulation rate as shown by Israël (4). In the polluted area where this work was carried out, a maxium of 10 percent of particulate fluorides was present.

Materials and Methods

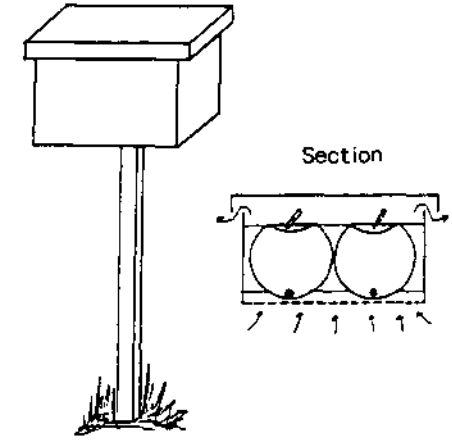
Lime Papers

Filter papers (Wattman nr. 1, diameter 12.5 cm) are impregnated for 1 min in a calcium hydroxide solution (400 g/20 l; continuously stirred) and dried at 105°C in fluoride-free air (1).

The lime papers were exposed at 1.50 m above ground level in well ventilated boxes and were protected from rain (Figure 1.)

Figure 1

Boxes for the Exposure of Lime Papers



Analysis

Six lime papers were exposed together for 7 or 28 days. After exposure, a sector of $^1/_6$ is taken from each paper, cut and mixed together (approx. 1 g). Another part of the lime paper is dried at 75°C for 2 hrs for determination of dry weight. The lime paper sample is ashed at 600°C for 4 hrs in a porcelain crucible which is covered by an aluminum sheet to avoid absorption of fluorides which can be released by the oven. After cooling, the ash is moistened with droplets of distilled water and left overnight. The fluorides are dissolved by carefully adding 2 ml 1/1 hydrogen chloride solution. After filtration and adding a buffer solution (TISAB – buffer with acetic acid, sodium chloride and sodium citrate) at pH 5 (by adding sodium hydroxide) the fluoride concentration is measured by means of a specific electrode.

The deposited amount of fluorides is calculated using the following formula:

$$Fd = \frac{(C_1 - C_0) \cdot W}{T \cdot S} = \mu g\ F \cdot dm^{-2} \cdot day^{-1}$$

Fd: deposited amount of fluorides on the lime papers (rate of deposition)
C_1: concentration of fluorides in the lime papers $\mu g\ F \cdot g^{-1}$ on dry weight basis
C_0: concentration of fluorides in non-exposed lime papers ($\mu g\ F \cdot g^{-1}$)
W: dry weight of 1 lime paper (g)
T: exposure time (days)
S: surface of 1 lime paper; both sides (dm^2)

Emission Measurements

The emissions were measured using the single filter method (impregnated with sodium formate) by Elfers and Decker (15), slightly modified by Verduyn et al. (16).

Results

The measured fluoride deposition rates in the neighborhood of a fertilizer plant given in Table 1 cover 5 years of experiments.

The integration period (exposure time) is normally 28 days. At some places lime papers with an integration period of 7 days were also used. Four weekly periods correspond with a 28 day period. The relationship between deposition rates of four successive exposure periods of 7 days and the deposition rate based on a corresponding 28 day exposure period is calculated by a multiple linear regression. The results are presented in Table 2 for all observations and

Table 1

Deposition Rate of Fluorides in the Neighborhood of a Phosphate Fertilizer Plant ($\mu g \cdot dm^{-2} \cdot day^{-1}$; 28 days integration).

situation opposite source	period	number of observations	mean rate of deposition F_dT	min/max
500 m ENE	April-October	35	11.3	2.8 -28.3
	November-March	24	10.6	2.2 -46.3
1000 m ENE	April-October	25	2.0	0.65-4.7
	November-March	18	2.8	0.79-10.1
5200 m NE	April-October	14	1.6	0.46-1.77
750 m W	April-October	21	2.8	0.60-9.3
1250 m W	April-October	14	1.95	0.74-3.72
1000 m SSW	April-October	21	2.8	0.65-14.4
Reference	April-October	35	0.27	0.14-0.70
Reference 2	April-October	14	0.20	0.11-0.33

Table 2

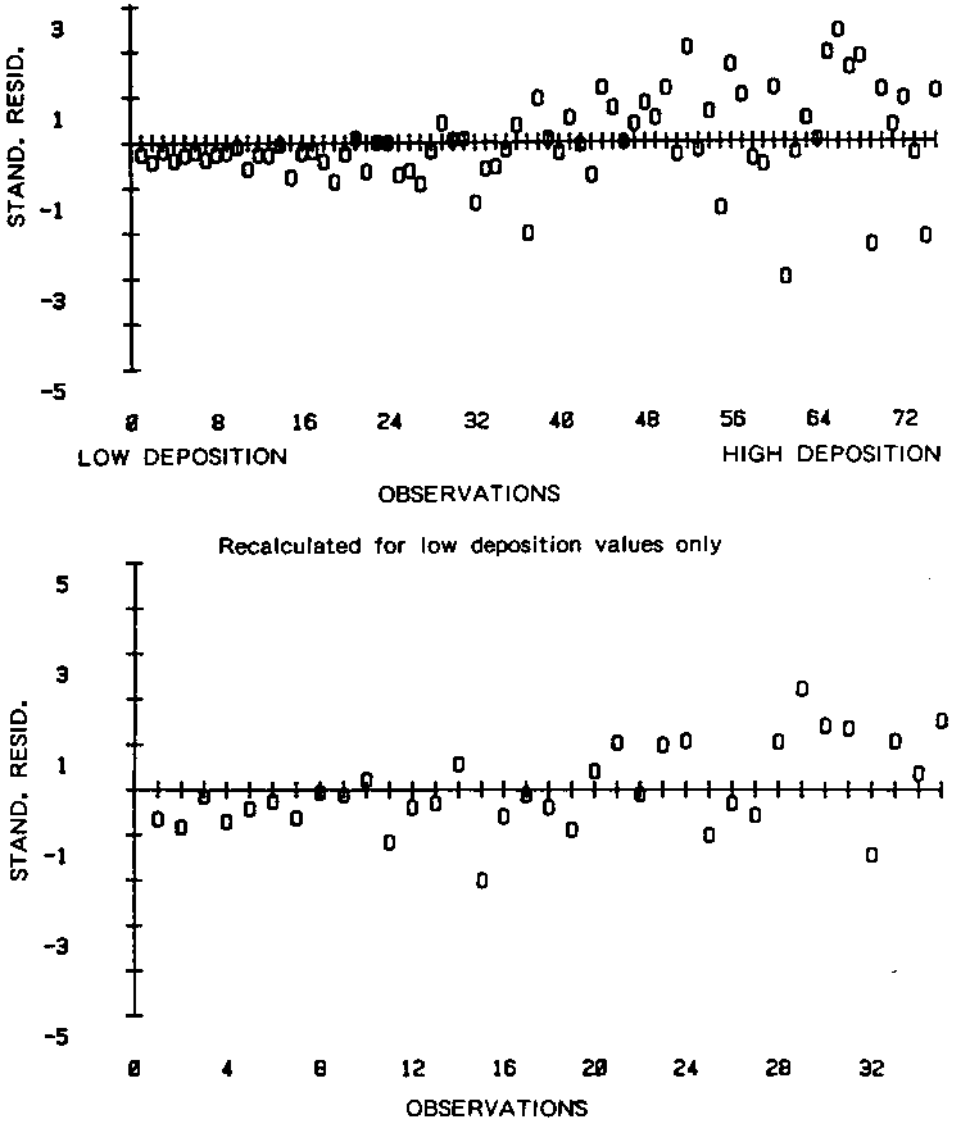
Relation Between Lime Papers Exposed During 4 Weeks and Lime Papers Exposed During 7 Days for 4 Corresponding Weeks

$y = ax_1 + bx_2 + cx_3 + dx_4 + e$	R^2	standard deviation
All observations (n = 75); equation 1 $F_dT = 0.12\ F_1 + 0.33\ F_2 + 0.15\ F_3 + 0.14\ F_4 + 0.09$	0.98	0.96
Only low values ($F_dT < 4$) (n = 35); equation 2 $F_dT' = 0.19\ F_1 + 0.19\ F_2 + 0.10\ F_3 + 0.035\ F_4 + 0.19$	0.71	0.26

recalculated separately for the lowest deposition rates (FdT < 4). The standard residuals (difference between calculated and observed values) are presented in Figure 2.

Figure 2

Comparison between the calculated and observed 'Fdt'-values of the relation between 28 days exposure and four successive consecutive 7-day exposures (between -5 and +5 times the Standard Deviation).



From the regression equations it can be deduced that the quantity of fluorides deposited on lime papers during the full period of 28 days is lower than that deposited during four corresponding consecutive weeks. Indeed, at a constant deposition rate over the full period, the equations 1 and 2 can be transformed to:

$$F_dT = \frac{F_1 + F_2 + F_3 + F_4}{4}$$

or under a more convenient form (equation 3)

$$F_dT = 0.25\ F_1 + 0.25\ F_2 + 0.25\ F_3 + 0.25\ F_4 = 1\ F_x$$

Considering Fx the average deposition rate measured during an integration period of 7 days ($F_x = F_1 = F_2 = F_3 = F_4$; because there is no evidence that there is a different deposition rate in four consecutive periods if the averages are based on sufficient observations), the equations 1 and 2 can be transformed into (equations 4 and 5 respectively):

$$F_dT = 0.74\ F_x$$

$$F_dT' = 0.48\ F_x$$

As the coefficient of equations 4 and 5 differ from equation 3 it is clear that F_dT is much lower than F_x.

The lower deposition rate by lime papers exposed during 28 days must be due to a loss of reactivity, as a result of the reaction of calcium hydroxide with CO_2 to calcium carbonate. The longer the lime papers are exposed, the more carbonate is formed which is neutral and much less reactive to HF.

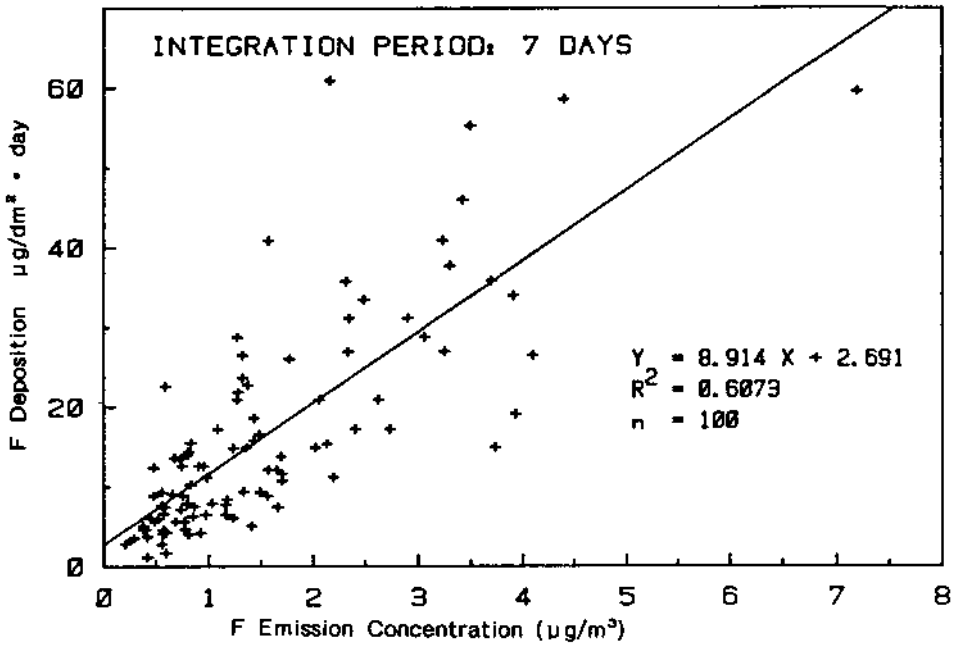
When all data are taken into account (equation 1) there is no clear evidence of a decreasing reactivity of the lime papers in function of time. The coefficients "a" to "d" are of the same order of magnitude. However, if only the lower levels are used (equation 2), a distinct decrease of the coefficients of the last week appears.

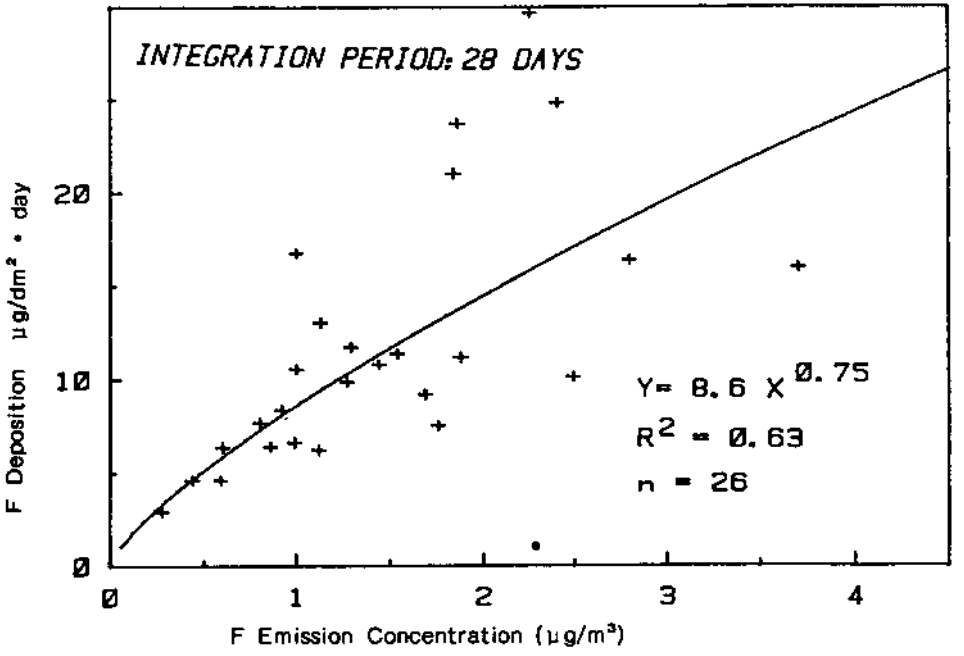
Indeed, the contribution of the last week to the total amount of deposited fluoride during the whole period of 28 days is negligable. The loss of reactivity can be deduced from the equations 4 and 5. As the coefficient for the whole set is 0.74 and 0.48 for the lowest deposition rates, the loss of effectness during the four weeks compared to four corresponding consecutive weekly exposures is about 26% for all data and 52% when only the lowest values ($F_dT < 4$) are considered. It is clear that exposure of fresh lime papers each week results in a higher fluoride deposition because (beginning with the second week) the amount of the more reactive calcium hydroxide is higher each time than in the lime papers exposed for four weeks.

Decreased reactivity is also shown by comparing ambient concentrations and depositions (Figure 3). Where a linear relationship has been found for weekly exposures, an exponential curve fits much better for 28 day exposures. Therefore, deposition measurements must be carried out over short integration periods.

Figure 3

Relation of Emission Concentration to Deposition





The most important parameter for deposition of fluorides on lime papers is ambient gaseous fluoride concentration as shown in Figure 3 where results of lime papers are compared with mean emission values in the respective periods. In spite of good correlation, big differences are possible due to other parameters. Indeed, at a higher wind speed, deposition of fluorides will be much higher because of a quicker diffusion of fluorides to the lime paper due to a lower boundary layer resistance at high wind speed. As this process is generally the same for plants, deposition measurements with lime papers are probably more closely related to processes of fluoride accumulation by plants than to emission data. The relation between ambient air concentration and deposition rate is also described by deposition velocity vd.

Loss of reactivity during long exposure periods is reflected in the deposition velocity coefficient which can be defined by

$$vd = \frac{\text{rate of deposition } (\mu g \cdot m^{-2} \cdot sec^{-1}}{\text{mean emission concentration } (\mu g \cdot m^{-3})} = ms^{-1}$$

For lime papers with 28 days exposure, the deposition velocity is 10.1 mms^{-1} (±3.7) whereas deposition velocity for 7 day exposure is higher namely 13.6 mms^{-1} (±6.4).

Acknowledgement

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FLUORIDE ADMINISTRATION EFFECTS ON DENTAL CARIES DEVELOPMENT IN RATS FED A CARIOGENIC HEATED-SKIM-MILK-BASED DIET

by

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SUMMARY: Development of carious lesions in rats fed a cariogenic diet based on heated-skim-milk powder, with or without fluoride or lysine supplements was monitored. Lysine or fluoride supplementation was effective in reducing dental caries; the most effective cariostatic treatment was the combined use of these dietary factors.

KEY WORDS: Dental caries; Diet deficiency; Lysine deficiency

Introduction

Lysine is one of the most studied of the essential amino acids (1). Relative to other amino acids there is a deficit of lysine in several staple grains and the possibility of lysine fortification programs to improve diets of developing nations with a chronic suboptimal food supply has received considerable attention (2).

Sharpenak (3) has pointed out that the onset of dental caries may, at least in part, be affected systemically by nutritional agents including dietary protein, thiamine and lysine. Ingestion of diets based on heated-skim-milk powder by growing rats results in an increase in number and severity of dental caries when compared to controls (4-8). The cariogenicity of this diet may be attributed to a decrease in the diet's lysine content due to heating since lysine added to heated skim-milk powder-based diet restores the number and severity of various lesions to control levels (6-8).

It has been proposed, through animal and human studies, that addition of controlled amounts of fluoride (F) to drinking water exerts a cariostatic effect (9-11).

The purpose of this short report was to study the effect of F added to drinking water of rats ingesting a heated skim-milk-based diet (lysine poor, cariogenic) on development of smooth surface dental caries and to delineate any possible interrelationships between these two dietary factors. A preliminary report of these studies has appeared elsewhere (12).

Materials and Methods

Animals and Diets

Male, albino, inbred, Sprague-Dawley rats, very close in age viz. 22-25 days old, weighing 48 gm on average, were housed in individual stainless steel

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elevated cages at constant temperature (22°C) and a 12-hr light/dark cycle during a 7-week experimental period. Animals were weighed at day zero and once a week throughout the 7-week test period after which the animals were decapitated using a guillotine. Groups of 7-8 rats were randomly assigned to different diets (Table 1). They were fed, ad lib., either an unheated skim-milk-powder-based diet (control) or a caries producing skim-milk-powder-based diet. In the latter experimental diet, the skim milk portion was autoclaved at 17 psi, 12-15 min before inclusion in the total diet.

Some diets were supplemented with 2% lysine. The control diet, patterned on that described by McClure and Folk (4), contained cornstarch, 45%; glucose, 19%; commercial spray-dried, non-fat skim-milk powder, 35%; vitamin fortification mixture (Teklad Vitamin Fortification Mixture 40060. Teklad Test Diets, Madison, Wisc.), 1%. This diet differed from that of McClure and Folk (4) in that desicated liver and oral administration of vitamins A, D and E were replaced by the vitamin fortification mix. The mineral mix described by Anderson and Draper (13) comprised 1% of the diet. Heating of skim-milk powder decreases the lysine content of the diet about 49% with only minor decreases in other essential amino acids (8). For drinking water the animals were provided with either double-distilled water or double-distilled water containing 10 ppm F.

To minimize the effects of quantitatively differing food intakes, individual rats were pair fed unheated skim-milk-based diets in amounts that were identical with the amounts by their respective pair partners which received heated skim-milk-based diets.

Analytical Procedures: A modification of the McClure technique (14) was used for dental caries scoring. Only smooth surface lesions in the molars of the lower jaws were scored. All other analytical procedures have been described elsewhere (15).

Statistical Analyses: The data are expressed as means ± standard error (SE) of the mean; significance between groups was determined using Student's t test.

Results

Food intake and % weight gains of the rats receiving different diets are given in Table 1. Ingestion of heated skim-milk-based diet reduced food intake and % weight gain sharply: food intake dropped from 20.2 ±2.0 g/day (unheated diet, no F supplement) to 6.9 ±0.5 g/day (heated diet); % weight gain decreased from 619.8 ±24.3 to 13.2 ±5.5, respectively. It may be presumed from previous experience that the severely limited weight gain of the rats ingesting the heated-skim-milk diet is a consequence of the decreased food intake and/or the unavailability of lysine in their diet (8). To examine the former possibility, the weight gains of pair-fed groups of animals were compared. Although the pair-fed partners of the three heated-skim-milk-based diet groups consumed an identical amount of unheated skim-milk-based diet, their % weight gain was considerably higher e.g., 130.6 ±23.4 vs. 13.2 ±5.5 for the unheated skim-milk, pair-fed group vs. the heated skim-milk group, respectively. This was due no doubt to the presence of lysine in the unheated diet. Addition of a 2% lysine supplement to the heated diet restored both food intake and % weight gain to almost the levels achieved by rats ingesting unheated diets, but the dif-

Table 1

Food Intake and Weight Gains

Type of skim-milk-based diet	ppm F	Dietary lysine supplement (%)	Food intake (g/day)	Weight gain (%)
Unheated	0	0	20.2 ±2.0[2]	619.8 ±24.3[2]
Unheated	10	0	20.3 ±2.1[2]	667.9 ±19.8[2]
Heated	0	0	6.9 ±0.5[3]	13.2 ±5.5[3]
Unheated, pair-fed	0	0		130.6 ±23.4[4]
Heated	10	0	6.1 ±0.4[3]	19.9 ±5.6[3]
Unheated, pair-fed	10	0		71.8 ±7.0[4]
Heated	0	2	18.6 ±2.3[2]	549.7 ±33.0[2]
Unheated, pair-fed	0	2		573.5 ±24.3[2]
Heated	10	2	18.4 ±2.1[2]	532.5 ±18.7[2]
Unheated, pair-fed	10	2		553.7 ±14.7[2]

Data are expressed as means ± standard error of the mean. 7 or 8 rats were used in each dietary group. Values in the same column not sharing a common superscript number are significantly different, $p<0.05$.

ferences observed were not statistically significant. Inclusion of 10 ppm F in the drinking water of animals ingesting any of the dietaries was without significant effect on either daily food intake or weight gains during the course of the study.

The effect of the dietary treatments on the incidence and severity of dental caries is given in Table 2. Consumption of heated skim-milk-based diet resulted in an increase in the number of carious lesions, their severity and the number of carious teeth when compared to rats consuming the unheated diet. Pair feeding the unheated diet and/or supplementing the diets with 2% lysine reduced the incidence and severity of carious lesions. However, whereas lysine supplementation to heated diets restored daily food intake and % weight gains completely (Table 1) it did not decrease the number of carious lesions or the severity of the dental caries to the control, unheated diet level, although significant decreases were observed (e.g. total number of carious lesions: 6.8 ±0.7, unheated diet; 14.5 ±1.0, heated diet; 10.9 ±0.8 heated diet + lysine). Lysine supplementation of heated diet was without effect on the number of carious teeth. A dietary lysine supplement of 2% to the unheated, pair-fed group, no fluoride diet, caused an increase in dental caries parameters when compared to unheated controls. There is no basis to explain this observation. F exerted a beneficial cariostatic effect similar in pattern to that of lysine supplementation to heated diets, namely it reduced the number and

severity of carious lesions in rats consuming a heated-skim-milk based diet but not completely to the control, unheated diet level (e.g., severity: 8.0 ±0.7, unheated diet; 20.8 ±1.5, heated; 14.4 ±0.9, heated diet + F). F did not reduce the number of carious teeth (4.9 ±0.4, unheated diet; 5.9 ±0.1, heated diet; 5.9 ±0.1, heated diet + F).

Table 2

Dental Caries Scoring

Type of skim-milk-based diet	ppm F	Dietary lysine supplement (%)	Total number of caries	Total severity	Number of teeth affected
Unheated	0	0	6.8 ±0.7[1]	8.0 ±0.7[1]	4.9 ±0.4[1]
Unheated	10	0	4.4 ±0.7[2]	4.9 ±0.9[2]	3.4 ±0.5[2]
Heated	0	0	14.5 ±1.0[5]	20.8 ±1.5[5]	5.9 ±0.1[3]
Unheated, pair-fed	0	0	9.1 ±0.6[3]	10.9 ±0.7[4]	4.6 ±0.4[1]
Heated	10	0	11.3 ±0.7[3]	14.4 ±0.9[3]	5.9 ±0.1[3]
Unheated, pair-fed	10	0	6.6 ±0.9[1]	8.0 ±1.0[1]	4.6 ±0.3[1,2]
Heated	0	2	10.9 ±0.8[3]	13.0 ±1.5[3]	5.6 ±0.2[3]
Unheated, pair-fed	0	2	11.9 ±0.8[3]	15.5 ±1.7[3]	5.8 ±0.2[3]
Heated	10	2	6.1 ±0.6[1,2]	7.4 ±1.0[1,2]	4.0 ±0.3[1,4]
Unheated, pair-fed	10	2	7.8 ±0.6[1]	10.3 ±1.0[4]	4.3 ±0.3[4]

Data are expressed as mean ± standard error of the mean. Values in the same column not sharing a common superscript are significantly different $p<0.05$.

Discussion

In rats on heated skim-milk-based diet, food intake and growth was reduced and the number and severity of carious lesions compared to controls increased. Supplementation of this diet with 2% lysine restored food intake and growth to control levels and resulted in a reduction of dental caries parameters measured. Addition of lysine to the unheated skim-milk-based diet (lysine sufficient) provided no added protection against dental caries. No differences in dental caries status of animals fed ad lib. versus controlled frequency paired feeding (Table 2) were observed. From these pair-feeding data it can be concluded that the cariogenic effect of lysine deficient diet is not related to depressed food intake. Administration of 10 ppm F in the drinking water was not totally effective in partial prevention of carious lesions induced by a lysine deficient diet. The most effective cariostatic treatment seemed to be the combined use of F in drinking water coupled with lysine supplemented to heated diet. Further studies, e.g., varying the age of the animal and length of test period, are warranted in order to fully support this conclu-

sion. The two dietary supplements, while operating in an additive form, may operate through quite different mechanisms (5,6,8,16-18). It has been argued that dentinal fluid may support the carious process (3,19-21). Once cariogenic bacteria have penetrated dentin they, theoretically, could receive some of their mutrients from pulpal fluids via dentinal fluids as well as oral fluids. Several reports, employing in vitro techniques, have suggested that pulpal or dentinal fluid may provide invading bacteria with growth limiting nutrients, such as lysine (3,19).

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BIOMONITORING OF ATMOSPHERIC FLUORIDE POLLUTION BY CHANGES IN PHYSIOLOGICAL ION MOBILIZATION IN PLANTS

by

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SUMMARY: Topaz, the most fluoride-sensitive variety of gladioli used in this experiment, was found to have a higher regression coefficient (r = 0.87) of foliar calcium vs fluoride content where atmospheric fluoride was lower.

In the Kambara district where a lower atmospheric fluoride is the sole pollutant, foliar sodium and chloride, respectively, were not associated with the fluoride content of gladioli. On the other hand, increases in foliar sodium and chloride were associated with the fluoride content in azalea and Myrica rubra in the Asaba district where both fluoride and chloride pollute the atmosphere.

KEY WORDS: Fluoride, Chloride, Pollution, Plant indicator, Physiological ion mobilization

Introduction

The white-flowered gladiolus (cv. Snow Princess) is a favorite biologic indicator for fluoride according to Hitchcock et al. (1) who summarized 10 years of study with fluoride on gladiolus. Whereas certain plant cultivars of gladiolus, apricot, prune, corn and grape are most sensitive to fluoride injury, celery, alfalfa, tomato, tobacco and some other species are resistant to fluoride but susceptible to sulfur dioxide.

Hendrix et al. (2) related certain leaf characteristics and flower color to atmospheric fluoride-sensitivity in gladiolus which were examined in 110 gladiolus varieties. Numerous studies concerning field observations of the visible effects of fluoride upon various types of vegetation have been reported. Previously (3), we reported that foliar fluoride accumulation was associated with increases in foliar sodium and calcium, and with decreases in foliar potassium and magnesium in mandarin, Japan-cedar and gladiolus. Thus, changes in the mobilization of physiological ions such as sodium, potassium, calcium, magnesium and chloride in plants by atmospheric fluoride were examined using the ornamental plants, gladiolus and azalea, and the fruit tree Myrica rubra. Moreover, to determine differences in gladioli varietal sensitivity to atmospheric fluoride, we examined the relationship to changes in sodium, potassium, calcium, magnesium and chloride in plant leaves to atmospheric fluoride-sensitivity.

Materials and Methods

Five varieties of gladiolus (Deep Purple, Red Beauty, Topaz, Traverer and

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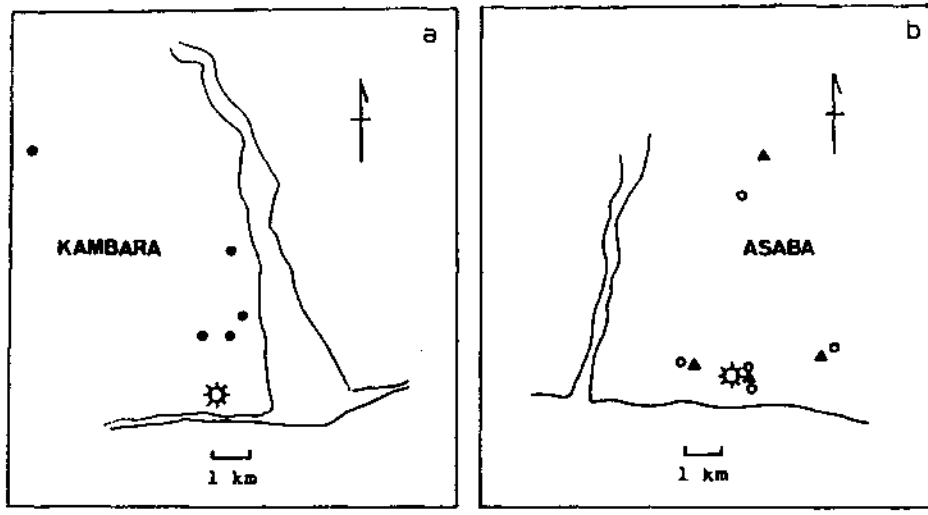
White Friendship) were used as phytometers in the Kambara district (Figure 1a). Field grown azalea and Myrica rubras were used as plant indicators in the Asabata district (Figure 1b); an aluminum refinery is located in the district of Kambara; an aluminum reproduction factory in the Asabata district.

Figures 1a and 1b

Location of Monitoring Stations in Kambara (a) and Asaba (b)



Monitoring stations: ● = with gladiolus, o = with azalea, ▲ = with Myrica rubra.

The tips (0-15 cm) of gladioli were collected for analysis during early August. Azalea and Myrica rubra were collected during March and September. Whole leaves from these plants were used for analysis. Plant leaf samples were washed with distilled water, and dried at 105°C for 24 hr prior to analysis for fluoride content. Dried samples were ground in Willey mill, ashed with calcium oxide at 600°C for 2 hr in a Muffles's ashing chamber after which they were fused with granular sodium hydroxide at the same temperature for 10 min.

A modification of the standard Willard and Winter (4) steam distillation procedure for fused samples was used to separate fluoride from interfering substances (5). Fluoride ions in the distillate were determined by using a fluoride ion-selective electrode (98-09-00, Orion Research, U.S.A.). Concentrations of sodium, potassium, magnesium and calcium were determined with an atomic absorption spectrophotometer (Hitachi Model 518) (6).

Atmospheric fluoride was collected with alkaline-treated filter paper, whereas atmospheric chloride was collected with a dust jar. Determinations of fluoride, chloride, sodium, potassium, calcium and magnesium were prepared using the same methods outlined for plants.

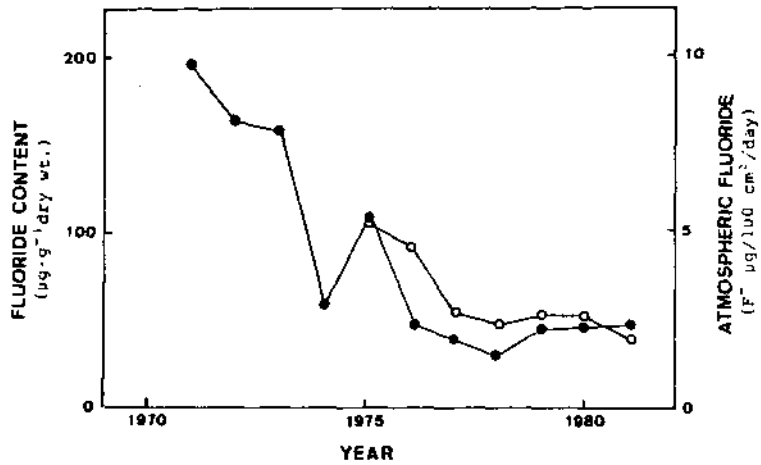
Results and Discussion

Varietal sensitivity to fluoride injury in gladioli: In the Kambara district where

gladiolus, taro and mandarin have been used as biomonitors of atmospheric fluoride pollution since 1969, fluoride content in gladiolus leaves (Purple Supreme and Deep Purple) gradually decreased from year to year since 1972 (Figure 2). To determine varietal sensitivity to atmospheric fluoride among gladioli, tentative examination of length of burned tissue versus fluoride accumulation, five varieties of gladiolus, namely, Deep Purple, Red Beauty, Topaz, Traverer and White Friendship (Figure 3), showed Topaz was most sensitive. On the other hand, length of burned tissue was related to distance from fluoride-emitting source (Table 1). Foliar injuries in stations A, B and C differed significantly from those in the control station.

Figure 2

Response of Foliar Fluoride content in Gladiolus to Atmospheric Fluoride



o : Atmospheric Fluoride, ● : Foliar Fluoride

Table 1

Visible Fluoride Injury (Length of Foliar Burned Tissue)

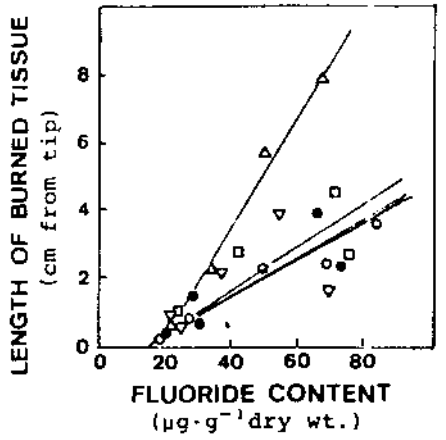
Station	Distance and direction from source (km)	Length of Foliar Burned Tissue (cm)				
		Deep purple	Traverer	Topaz	Red beauty	White friendship
A	1.5 NNE	3.93 ±0.36 (9.83)*	3.63 ±0.72 (18.2)*	5.71 ±1.01 (17.8)*	4.53 ±0.60 (6.47)*	3.93 ±0.36 (7.56)*
B	1.9 NNE	2.37 ±0.38 (5.93)	2.48 ±0.54 (12.4)	7.89 ±0.91 (24.7)	3.13 ±0.40 (4.47)	1.67 ±0.36 (3.21)
C	1.4 NNW	1.46 ±0.14 (3.65)	2.26 ±0.35 (11.3)	2.17 ±0.35 (6.78)	2.70 ±0.43 (3.86)	2.22 ±0.45 (4.27)
D	3.2 N	0.66 ±0.14 (1.65)	0.83 ±0.20 (4.15)	0.66 ±0.18 (2.06)	1.01 ±0.13 (1.44)	0.80 ±0.19 (1.54)
Control	8.8 NW	0.40 ±0.12 (1)	0.20 ±0.54 (1)	0.32 ±0.10 (1)	0.70 ±0.15 (1)	0.52 ±0.28 (1)

*Numbers in parenthesis are relative values for control.

Mobilization of sodium and potassium related to fluoride-sensitivity in gladiolus: In previous research (3), elevation of foliar sodium was associated with fluoride accumulation in gladiolus (Purple Supreme and Deep Purple). To determine differences in sensitivity to atmospheric fluoride, we examined relationship of changes in sodium and potassium in the five varieties of gladioli to atmospheric fluoride-sensitivity in these plants (Figure 4). Changes in foliar sodium content were not associated with a range (0-100 µg/g dry wt) of fluoride accumulation in the five varieties of gladiolus used in this experiment. These results could be due to decrease in atmospheric fluoride concentration (Figure 2). On the other hand, decrease in foliar potassium content responded to increases in foliar fluoride in Topaz and White Friendship gladiolus in 1981 as shown in Figures 4h and 4j.

Figure 3

Foliar Burning Related to F^- Content in Gladiolus Leaves.



Δ = Topaz, ▽ = White Friendship, □ = Red Beauty, ○ = Traverer, ● = Deep Purple

Topaz: $Y = -0.296X + 27.07$ ($r = -0.69$, $n = 5$)

White Friendship: $Y = -0.279X + 33.29$ ($r = -0.95$, $n = 5$)

(X: foliar fluoride content, Y: foliar potassium content)

Mobilization in calcium and magnesium related to fluoride-sensitivity in gladiolus: Fluoride accumulation in the tip of fir needles was associated with calcium translocation to the tip as shown by Garrec et al (7). We also observed that foliar calcium concentration increased with foliar fluoride accumulation in one gladiolus variety namely Purple Supreme. In this experiment, only one of the five varieties of gladiolus had a higher regression coefficient of foliar fluoride versus calcium content in 1981 as shown in Figure 5c.

Topaz: $Y = 0.0737X + 1.74$ ($r = 0.87$, $n = 5$)

White Friendship: $Y = 0.0645X + 3.81$ ($r = 0.23$, $n = 5$)

(X: foliar fluoride content, Y: foliar calcium content)

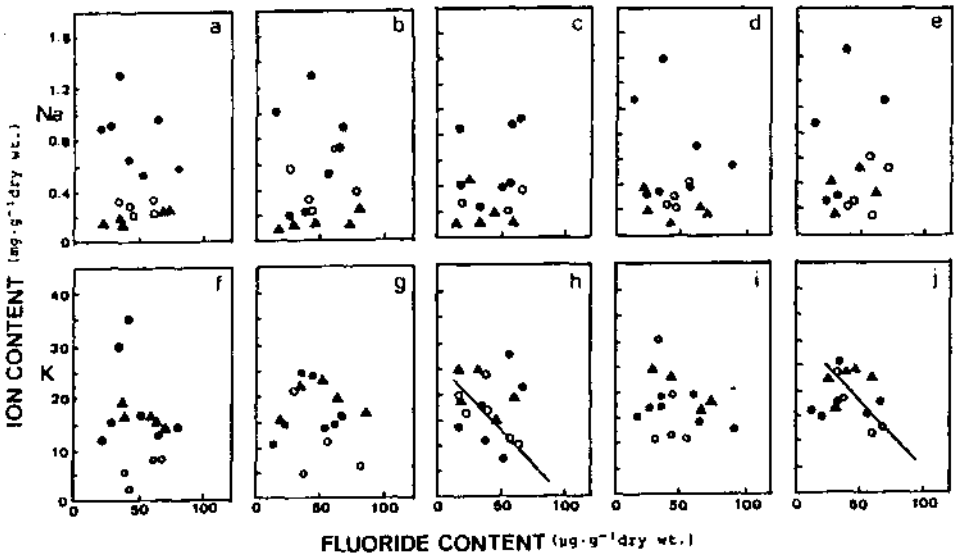
Foliar magnesium content had a negative regression coefficient as the fluoride accumulation in mandarin ($r = -0.48$, $n = 12$) (3). Foliar magnesium content in Deep purple increased markedly with the rise in fluoride content (Figure 5f).

Deep Purple: $Y = -0.0547X + 6.134$ ($r = -0.80$, $n = 5$, in Aug., 1981)

(X: foliar fluoride content, Y: foliar magnesium content)

Figure 4

Response of foliar sodium and potassium to the F^- content in gladiolus leaves.



a,f: Deep Purple, b,g: Traverer, c,h: Topaz, d,i: Red Beauty, e,j: White Friendship.

Gladiolus Leaves: o = in 1981, ● = in 1982, ▲ = in 1983.

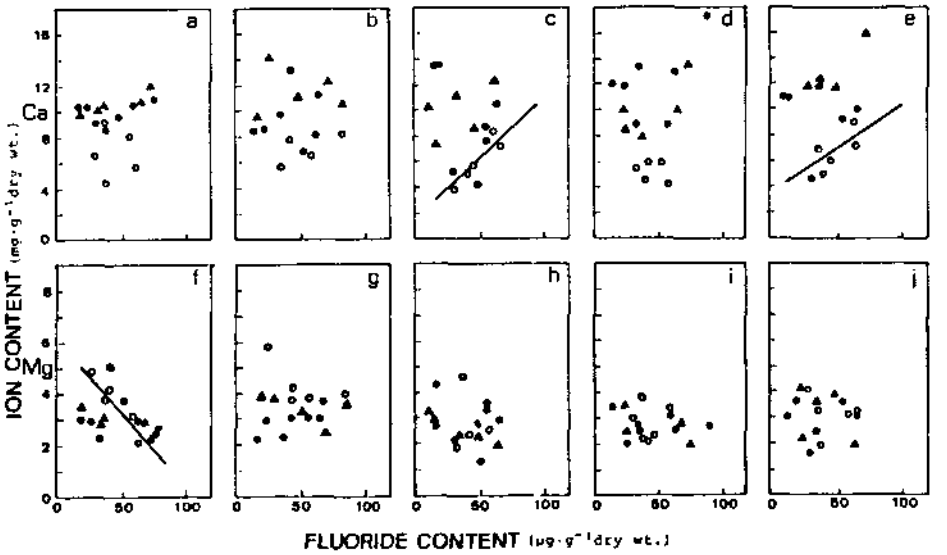
Changes in ion content in gladioli related to distance from fluoride-emitting source: To determine the accumulation of fluoride, sodium and calcium in gladiolus leaves from sea salt, the chloride, potassium and magnesium content in leaves from a fluoride-emitting source was plotted (Figure 6a-f).

Foliar content of fluoride, sodium and calcium decreased in relation to the distance from the fluoride-emitting source (Figures 6a, c and e). Their patterns suggested the possibility that it was caused by sea salt. Moreover, chloride content in gladiolus leaves was determined to learn the influence of sea-salt on fluoride content in plant leaves. The chloride content was not associated with fluoride accumulation and sodium content in leaves. Thus, the contribution from sea salt to foliar fluoride accumulation in gladiolus was small in this district. Moreover, the decrease in foliar magnesium and potassium content was not dependent on sea salt, but was due to atmospheric fluoride-emission.

To monitor fluoride and chloride, azalea and Myrica rubra were used as plant indicators: In Asaba district, some ornamental plants and fruit trees such as azalea and Myrica rubra were damaged by concomitant pollution of the atmosphere by fluoride and chloride. Seasonal variations in atmospheric fluoride and chloride near an aluminum reproduction factory are shown in Figures 7a and b. Increases in foliar sodium and chloride versus foliar fluoride in azalea were found to have higher regression coefficients, respectively (Figure 8c and d).

Figure 5

Response of foliar calcium and magnesium to F^- in gladiolus leaves.



a,f: Deep Purple; b,g: Traverer; c,h: Topaz; d,i: Red Beauty; e,j: White Friendship.

Gladiolus Leaves: o = in 1981, ● = in 1982, ▲= in 1983.

Azalea (foliar sodium vs foliar fluoride):

Y = 0.047X + 2.116 (r = 0.63, n = 5, in March, 1982)

Y = 0.387X - 5.904 (r = 0.99, n = 5, in Sept., 1982)

Y = 0.091X - 1.625 (r = 0.90, n = 5, in Sept., 1983)

(X: foliar fluoride content, Y: foliar sodium content)

Azalea (foliar chloride vs foliar fluoride):

Y = 0.079X - 1.262 (r = 1.03, n = 5, in March, 1982)

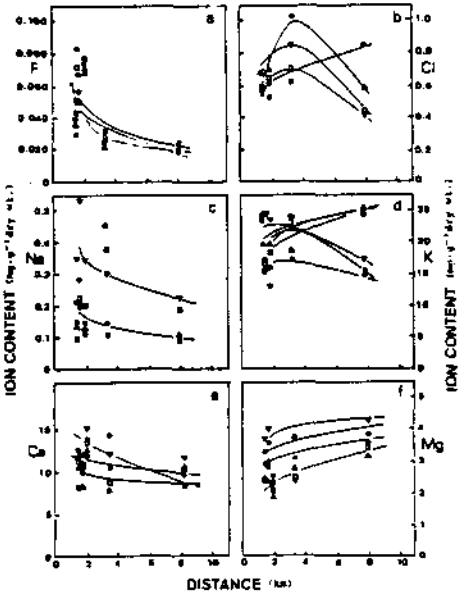
Y = 0.319X - 1.951 (r = 0.92, n = 5, in Sept., 1982)

Y = 0.035X - 0.236 (r = 0.88, n = 5, in Sept., 1983)

X: foliar fluoride content, Y: foliar chloride content)

Figure 6

Distance from the F^--emitting source Related to foliar ion in gladiolus.

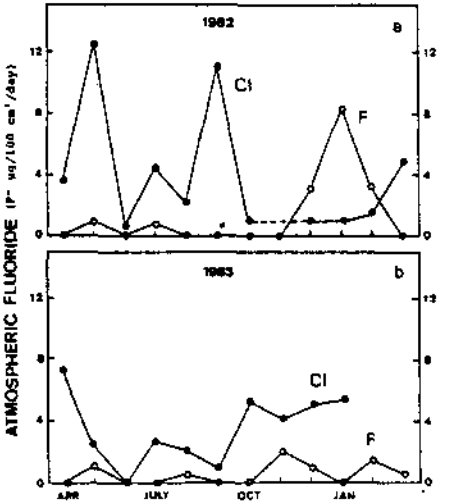


Foliar content:
a = fluoride; b = chloride; c = sodium; d = potassium; e = calcium; f = magnesium

Δ = Topaz; ∇ = White Friendship; □ = Red Beauty; O = Traverer; ● = Deep Purple

Figure 7

Seasonal Variations of Atmospheri Fluoride and Chloride in Asaba.



a = Atmospheric F^- and Cl^- in 198
b = Atmospheric F^- and Cl^- in 1983.

O = F^-; ● = Cl^-

Moreover, regression coefficient for foliar potassium, calcium and magnesium versus foliar fluoride was low with the exception of calcium in azalea (Figure 8b).

Azalea (foliar calcium vs foliar fluoride):

$$Y = -0.154X + 18.09 \quad (r = -0.76,\ n = 5,\ \text{in March, 1982})$$

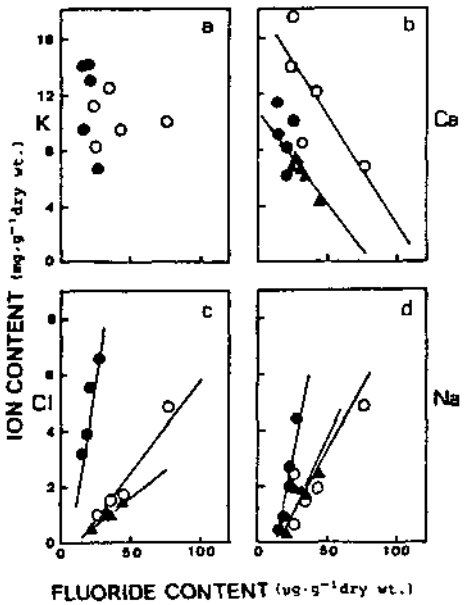
$$Y = -0.136X + 11.37 \quad (r = -0.80,\ n = 5,\ \text{in Sept., 1983})$$

(X: foliar fluoride content, Y: foliar calcium content)

On the other hand, regression coefficients for foliar sodium and chloride showed higher values in Myrica rubra, whereas the coefficients for foliar potassium, magnesium and calcium were lower (Figure 9).

Figure 8

Response of Foliar Physiological Ions to F^- in Azalea.

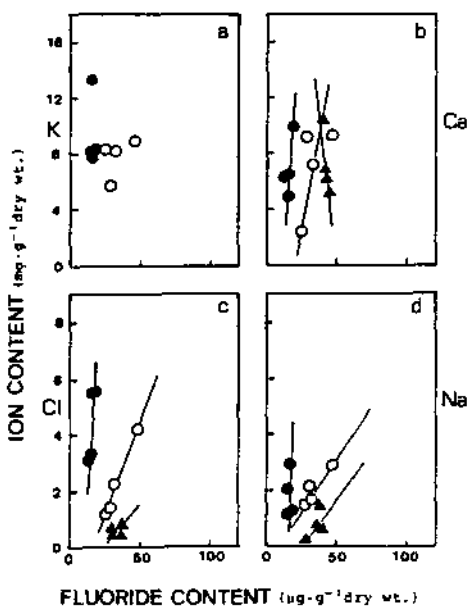


Foliar content:
a = potassium; b = calcium;
c = chloride; d = sodium.

Foliar ion content:
O = March, 1982; ● = September, 1982
▲ = September, 1983

Figure 9

Response of Foliar Physiological Ions to F^- in Myrica rubra.



Foliar content:
a = potassium; b = calcium;
c = chloride; d = sodium.

Foliar ion content:
O = March, 1982; ● = September, 1982
▲ = September, 1983

Acknowledgement

We express thanks to Messrs K. Sano (K) and Y. Furukawa (A) of the Public Offices of Kambara (K) and Asaba (A), for their support.

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

by

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Morioka, Japan

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

To estimate, in vitro, the fluoride concentration in deciduous teeth from areas with different fluoride concentrations in drinking water, fluoride concentrations in surface enamel of exfoliated deciduous molar teeth were measured from school children who had resided continuously, since birth for 6-10 years, 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 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). These results confirm previous findings that fluoride accumulates preferentially in the outer region of deciduous enamel.

The distribution of fluoride within a depth of about 50 μM from the enamel surface emphasized the marked decrease in fluoride concentration from the enamel surface to the interior. The difference between inner and outer enamel is smaller in the area with less than 0.1 ppm fluoride.

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

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CHANGES IN THE CARIES PREVALENCE OF 11-12-YEAR-OLD SCHOOLCHILDREN IN THE NORTHWEST OF ENGLAND FROM 1968 to 1981

by

V. Clereburgh, A.S. Blinkhor, M.C. Downer, H.C. Hodge, A.J. Rugg-Gunn, G.M. Mitropoulos, and H.V. Worthington
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(Abstracted from Community Dent. & Oral Epidemiology, 11:367-70, 1983)

The aim of this study was to compare the dental caries prevalence of 11-12 year old children attending schools in the northwest of England during

1968-78 with that of 11-12-year olds attending the same schools in 1980-1981. Five examiners revisited some of the schools which participated in the original trials. Each examiner looked at the teeth of 196 to 296 children. The time intervals between the original and repeat examinations were 12, 10, 8, 5, and 3 years. Percent caries reduction (PCRs) ranged from 19 to 33 for DMFT and from 24 to 35 for CMFs. The PCRs were greater on free smoth and approximate surfaces than on fissure surfaces and for the anterior teeth than for the mouth as a whole, suggesting that fluoride may have played a role in the reduction.

Caries prevalence was reduced in all five districts, with PCRs ranging from 19 to 33 DMFT and from 24 to 35 DMFS. However, the magnitude of the PCRs did not seem to relate to the intervals of time between examinations. For example, over the longest interval of 12 yrs., in district 1, mean DMFS were reduced by 24% from 9.0 to 6.9 (including radiographic data) yet, over the shortest interval of 3 yrs., in district 5, a 30% reduction occured, from 5.6 to 3.9 DMFS.

The DFS for fissure surfaces was reduced in all five districts, with PCRs ranging from 9 to 24. Free smooth surfaces showed greater PCRs than fissure surfaces in the most recent comparisons, over 8, 5 and 3 yrs. However, since DFS prevalences for free smooth surfaces are small, even a large PCR means that only a small fraction of a surface has been saved, e.g. in district 4, the DFS was reduced by 50% from 0.50 to 0.25; only a quarter of a surface was saved.

It is well documented that fluoride is more effective in reducing caries on free smoth and approximal surfaces than on fissure surfaces, and that anterior teeth benefit more than posteriors. Thus, the findings of this study are consistent with the hypothesis that fluoride may have played a role in the reductions noted. It would, however, be inappropriate to suggest that fluoride, whether in dentifrices, tablets or mouthrinces, was solely responsible. Other possible influences on the decline in caries prevalence include dental health education and changes in the pattern of sugar consumption. The varying reductions in caries prevalence, recorded in the districts of this study, may have resulted from the different geographical locations.

KEY WORDS: Caries prevalence decline; Dental caries; Fluoride; Northwest England, caries in.

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EFFECT OF FLUORIDE ON BONE IN FINLAND

by

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(Abstracted from Acta Orthop. Scand., 56:161-166, 1985)

The fluoride content and histomorphometry of iliac crest trabecular bone, taken from cadavers in several hospitals in three areas of Finland were studied: 1] low fluoride (0.0-0.3 ppm); 2] Kuopio (fluoridated since 1959 at 0.9-1.2 ppm); 3] a high F area >1.5 ppm in southeastern Finland where some wells contain eight times as much F as fluoridated Kuopio water. Sixty-five subjects (40 men and 25 women) in the low F group constituted controls; 43 (26 men and 17 women) in the second group, from autopsies at fluoridated Kuopio University Central Hospital, had lived at least ten of their last years in fluoridated Kuopio; 57 (40 males, 17 females) were selected for the high F group.

The fluoride content of trabecular bone differed in fluoridated and low-fluoride areas ($p<0.001$). In the area with fluoridated drinking water (1.2 ppm) linear regression analysis revealed correlations ($p<0.05$) between fluoride content in bone and osteoid volume ($r = 0.486$) and between fluoride content and osteoid-covered trabecular bone surface ($r = 0.541$) in women. In males, the highest fluoride content in bone was 2750 ppm with no histological changes. In females, the highest fluoride concentration in bone (3890 ppm) was found in a subject with impaired renal function; she had increased osteoid volume ($V = 5.5$). All subjects with slightly impaired renal function had a higher content of fluoride in bone (2090 ±1010 ppm; mean ±SD) than did those with normal creatinine level.

In the high fluoride area bone F was high, and osteoid values were increased in both sexes. Differences between the high and low fluoride areas were significant for the fluoride content of trabecular bone, the volumetric density of osteoid, and osteoid-covered trabecular bone surface in both sexes. Osteoid volume was increased in the high-fluoride area. Osteoid seam width was correlated with bone fluoride in both women ($r = 0.462$, $p<0.05$) and men ($r = 0.503$, $p<0.001$). The highest fluoride content measured in trabecular bone was 10,890 ppm in a 66-year-old female; in males 7090 ppm.

The fluoride content of drinking water was not correlated with volume density of trabecular bone, nor was there correlation between ages of patients and the fluoride content of bone.

The main histological change induced by fluoride, namely increased osteoid volume, has been shown in studies where osteoporosis was treated with fluoride preparations. Elevated concentrations of fluoride in drinking water increased osteoid surface and volume abnormally and may also increase resorption. This increase in osteoid parameters was already observed in the present study at fluoride concentrations above 1.5 ppm.

As evaluated by histomorphometry, fluoridation does not seem to protect against bone loss in old age. To establish a safe fluoride concentration in drinking water is difficult because individual susceptibility to fluoride varies.

KEY WORDS: Bone, histomorphometry; Finland; Fluoride water.

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INFLUENCE OF FLUORINE COMPOUNDS ON PLANTS SOWN IN POTS

by

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Poznan, Poland

(Abstracted from Metabolizm Fluoru, 1982, p. 195)

Pot experiments were initiated in a vegetation hall on May 15 and June 20, 1976. Oats, white mustard and perko were sown in pots containing 7 kg soil with identical basic fertilization supplemented with NaF in amounts of 5.0 g, 7.5 g, 10.0 g and 12.5 NaF per pot. Hence the fluoride concentrations ranged from 323 to 807 ppm, which corresponded to F^- concentrations found in soil in the environs of an aluminum works. Each experiment had 5 combinations and 6 replicates. Control pots were not supplemented with NaF.

The plants developed normally only in the control pots. Even plants supplemented with 5.0 g NaF sprouted unequally, developed more slowly than those in control pots, were pale and thin. Higher fluoride rations were not investigated because it was found that the 323 ppm F^- concentration was toxic for experimental plants. The experiment was liquidated and plant material taken for further investigations.

On July 6, 1976, a new experiment with oats was initiated. In addition to basic fertilization NaF was added in amounts of 2.0, 3.0, 4.05, and 5.0 g which corresponded to concentrations of 132 to 323 ppm F^-. There was also a control group. The straw crop was already 2.0 g NaF, 10.7% lower than in the control group. With increasing F^- rations, it decreased by 28.5%, 64.8%, and 76.5% respectively. The yield of grain decreased more markedly, namely by 34.1%, 32.1%, 98.5%, and 99.9%

KEY WORDS: Plants; Fluoride; Soil

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ENAMEL MOTTLING AT DIFFERENT LEVELS OF FLUORIDE IN DRINKING WATER: IN AN ENDEMIC AREA

by

V.V. Subbareddy* and A. Tewari

(Abstracted from Journal of the Indian Dental Association, 57:205-212, 1985.)

To study the prevalence and severity of enamel mottling 1759 school children, aged 12-17 years with continuous residence, were selected in six specific rural areas where the fluoride level in drinking water was 0.30, 1.10, 2.00, 3.40, 5.40, and 10.40 ppm. All six areas, except one (i.e. 0.30 ppm — Chandigarh Admn.), were from the endemic fluoride area of Dist. Bhatinda, Punjab.

Environmenal factors such as eating habits, occupational and nutritional status, mean annual temperatures and living conditions, were similar in all six groups. Whereas at 0.30 ppm F, none of the children had enamel mottling, at 1.10 ppm F 88.08 percent exhibited some degree; at 2 ppm F and above the severity of enamel mottling increased proportionately.

In the human body, fluoride is the main bone seeking element. It accumulates in every tissue showing physiological or pathological calcification. Fluoride affects the ameloblasts in formative and maturative stages.

A variety of factors such as climatic conditions, water hardness, eating habits during tooth development, nutritional status, altitude, affect the severity of dental fluorosis.

Enamel Mottling Prevalence Among Children Aged 12-17 Related to Fluoride in Drinking Water

F^- in drinking water	No. of children examined	Percent with mottling	Severity of Mottling*					
			1	2	3	4	5	6
0.30	310	0	0	0	0	0	0	0
1.10	307	85.02	24.76	31.59	22.48	6.19	0	0
2.00	307	98.05	4.89	29.97	31.27	25.41	6.51	0
3.40	224	100.00	0	5.38	38.84	47.77	8.03	0
5.40	307	100.00	0	2.28	37.13	45.28	15.31	0

* According to original scale of Dean: 1 = questionable, 2 = very mild, 3 = mild, 4 = moderate, 5 = moderately severe, 6 = servere.

KEY WORDS: Enamel mottling; Endemic fluoride area; India, rural dental health.

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****

INFLUENCE OF NaF ON THE HISTOLOGICAL AND HISTOCHEMICAL CHANGES IN ORGANS OF WHITE RATS

by

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(Abstracted from Metabolizm Fluoru, 1982, p. 124)

Sodium fluoride (NaF) was administered with standard diet during 6 months to male Wistar rats in 10 or 20 mg/kg dose. Another group of animals was treated with 10 mg/kg NaF and, in addition, with calcium carbonate. In the NaF group multiplication of periosteal cells in iliac bone and fibrinoblastic process was noted. In the other group, treated additionally with $CaCO_3$, periosteal reactions were not visible. Fatty degeneration of hepatocytes in the group receiving 10 or 20 mg/kg of NaF was observed as well as histological changes in kidneys. The histochemical reations under the influence of NaF were altered.

KEY WORDS: Fluoride; Bone; Enzymes; Liver; Kidney

REPRINTS: Institute of Pharmacology and Toxicology, Pomeranian Medical Academy, 70-111 Szczecin, Poland.

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FLUORIDE CONTENT OF SELECTED HUMAN FOOD, PET FOOD AND RELATED MATERIALS

by

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(Abstracted from Z. Ernahrungswiss. 24:54-66, 1985)

This paper represents the results of a limited survey of the fluoride content of human and animal food, and related products.

In human food-items, which included ground beef, a variety of sausages, fish and fish meals as well as 5 different teas, fluoride ranged from 1.11 mg/kg or ppm dry weight (Big Mac) to 63.1 (canned sardines). Of 24 health foods, 10 varieties of table salt for human consumption and 3 dental impression materials, the F range for each was 1.4 to 848 ppm; 0.66 to 6.8 and 11,500 to 14,500 ppm respectively. Tea brewed an average of 3 minutes releases 91% of the fluoride obtained after 5 minutes, a period not normally exceeded.

Among health foods and preparations for self-medication, numerous products are concerned with bone formation and calcium supply besides general roborant effects. Bones and petrous raw material were used in their prepara-

tion. A short list of them — when analyzed for fluoride — revealed, for several products, unexpectedly high levels.

No declaration of high fluoride levels was given for any product, although intake of the recommended doses of products with 100 mgF/kg would result in fluoride uptake of the order of 1-3 mg/day. In sea salt up to 7 mgF/kg was found.

The analytical figures in this paper represent, if the source of fluoride is eaten or fed, intakes but not bioavailabilities. According to the data presented more attention should be paid, in certain areas of human life, to the possible occurrence of fluoride in the environment of man.

KEY WORDS: Fluoride, Human food, Health Food, Pet food.

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DENTAL HEALTH STATUS AND ATTITUDES TO DENTAL CARE IN FAMILIES PARTICIPATING IN A DANISH FLUORIDE TABLET PROGRAM

by

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(Abstracted from Community Dentistry & Oral Epidemiology, 12:303-7, 1984)

The caries experience and dental fluorosis of 84 Danish children, whose average age at the time of examination was 6.8 years, were compared with those of a group matching in sex, age, place of residence and socio-economic status. They had used fluoride tablets 1-4 years during 1976-80. Mothers' attitudes toward dental care and candy, their knowledge of tooth-brushing, and the number of teeth in maxilla showed no difference between the fluoride tablet group and the non-users group. Moreover, there was no significant difference between the two groups with respect to dental caries. Mother in the fluoride tablet group apparently were more restrictive in candy consuming habits; in fact 30 children of this group had a fixed weekly day for candy to 17 in the non-users group.

On clinical examination, in the fluoride tablet group, 13 (12 + 1) out of 54 children examined showed slight degrees of dental fluorosis in the permanent first molar. In the non-users group all surface examined had a normal appearance. The two groups examined did not differ regarding dental fluorosis of the permanent central and lateral incisors. Since few enamel changes occured in the primary and secondary molars it was not possible to detect any difference between the fluoride tablet group and the non-users group. Nor has there been any significant difference in average caries experience in the two groups.

The similiarity between the two groups in terms of current caries activities is clearly demonstrated by the fact that they did not differ in number of active and inactive lesions. The same picture was obtained with regard to the primary second molar.

No difference in the prevalence of dental plaque and gingivitis has been observed between the two groups. Significant difference between the groups, either in terms of classical measures of caries experience or in current activities of caries was demonstrated. Increasing awareness of the importance of dental hygiene and dietary patterns is likely to play a role in reduced caries progression.

KEY WORDS: Attitude; Dental caries; Dental enamel; Dental health services; Dental plaque; Dental prophylaxis; Dentifrices; Family; Fluorides; Gingivitis

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GONADO- AND EMBROTOXICITY OF FLUORINE

by

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[in Russian]

Administration of 5 and 30 mg/kg F (as NaF solution) to male and female rats for 1 month showed a pronounced gonadotoxic effect, namely inhibition of spermatozoid function, loss of fertility in females, as well as disruption in the reproductive function of males. These doses, administered during 1-20 days of pregnancy, caused a dose-dependent embryotoxic effect — a decrease in fetal length and weight and a reduction in fertility and survivability.

KEY WORDS: Embryotoxicity; Gonadotoxicity; Sodium fluoride

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