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# FLUORIDE

## Quarterly Reports

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The Fifteenth Conference of the International Society for Fluoride Research will be held on the Utah State University Campus, July 30–August 2, 1986. Our host will be Professor G.W. Miller, Department of Biology, UMC 45, Utah State University, Logan, Utah 84322.

## OSTEOPOROTIC HIP FRACTURES AND FLUORIDATION

For over two decades now, both laboratory and epidemiological investigations have consistently failed to show that fluoridation of drinking water provides any improvement in bone quality or any significant help in preventing symptomatic bone thinning (osteoporosis), especially in the elderly (1-6). Particularly noteworthy among these studies is a U.S. National Health Interview Survey of hospitalization records for 1973-1977 which indicated that water fluoride levels used in fluoridation do not "prevent osteoporotic hip fractures" (5). As seen in Table 1, this work also clearly showed that both with and without fluoridation: "Among adults 45 years of age and over the rates [of hip fractures] for females are about twice as high as those for males."

Table 1

Annual Hip Fracture Hospitalization Rates per 1,000 Population (Whites only) Aged 45 and Over by Sex and Fluoride Exposure Status in the United States for the Period 1973-1977 (from ref. 5).

Fluoride Exposure*	Men		Women	
	Rate	(Number)	Rate	(Number)
Less than 20%	1.0	(25,997)	2.4	(30,473)
At least 80%	1.1	(18,034)	2.2	(21,810)

- \* According to per cent of the population in respondent's county of residence served with at least 0.7 ppm F in the water supplies.

During the past year, however, in a highly publicized report (7), O. Simonen and O. Laitinen of the Finnish National Board of Health and Kivela Hospital, Helsinki, have credited fluoridation in the town of Kuopio, Finland, with lower rates of hip fractures among men aged 50 and older and among women aged 70 and older in comparison to higher rates among men and women of the same age groups in the nonfluoridated town of Jyvaskyla.

Table 2 shows the results of their study for annual femoral neck (hip) fracture rates for the period 1967-1978 among persons aged 50 and older residing in these two central Finnish communities. The drinking water in Kuopio has been fluoridated at 1 ppm since 1959, and the F content of the water supplies in Jyvaskyla is  $\leq 0.1$  ppm. The hip fracture data were collected from the National Registry of hospital discharge records, and the age 50-and-over population and employment characteristics of the two towns were reported to be similar.

Although, as expected, the data in Table 2 reveal significantly higher rates of hip fractures among women than among men in all age categories in Kuopio, the same does not seem to be true in Jyvaskyla. There, surprisingly — and unaccountably — and in contrast to the findings in Kuopio as well as in the U.S. National Survey (5), the hip fracture rates are nearly the same for men and women in the first three age categories (50-59, 60-69, and 70-79),

Table 2

Annual Mean Incidence of Femoral Neck (Hip) Fractures per 1,000 Inhabitants by Sex and Age in Kuopio (fluoridated) and Jyvaskyla (nonfluoridated), Finland, During the Period 1967-1978 (from ref. 7).

Place	Incidence per Age Group*				Total+
	50-59	60-69	70-79	≥80	
Kuopio (F)					
Men	0.09(3)	0.34(8)	1.30(13)	3.20(7)	0.45(31)
Women	0.35(16)	0.80(31)	2.68(59)	7.62(42)	1.32(148)
Combined+	0.24(19)	0.63(39)	2.25(72)	6.36(49)	0.99(179)
Jyvaskyla (NF)					
Men	0.45(13)	0.67(14)	3.24(25)	5.75(8)	1.02(60)
Women	0.35(14)	0.70(23)	3.93(60)	13.00(49)	1.59(146)
Combined+	0.39(27)	0.69(37)	3.70(85)	11.05(57)	1.37(206)

\* Number of patients over 11-year period, 1967-1978, in parenthesis.

+ Calculated from data in ref. 7.

which strongly suggests that the data for Jyvaskyla may not be truly representative of a typical low-fluoride comparison, even if the overall incidence of hip fractures is not significantly different from that of other nonfluoridated communities in Finland, as claimed by Simonen and Laitinen.

In fact, this suspicion is greatly strengthened by the results of an independent contemporary study by I. Arnala in collaboration with E.M. Alhava and P. Kauranen at the University of Kuopio (6). Arnala compiled hospital records of upper femur (hip) fracture treatments of residents of various Finnish communities during the period 1972-1981 and found that the annual age-specific incidence rates "did not differ significantly with three different concentrations of fluoride in drinking water. Nor did the type of upper femoral fractures differ significantly from each other in the three areas examined."

The results of Arnala's study, which should be compared with the combined incidence figures of Simonen and Laitinen in Table 2, are given in Table 3. In Arnala's investigation, "all fractures due to severe trauma, such as falls from heights and traffic accidents, were excluded." Although it is not clear whether persons with such injuries were omitted from the Board of Health study (7), some of the discrepancies between the results of the two studies, at least for the low-fluoride areas, might be attributable to differences in recording criteria. Still, it is difficult to understand why even the rates for fluoridated Kuopio — covering nearly the same period of time — should differ as much as they do between the two studies.

Although Arnala's findings were available in print two years before the Board of Health report, the latter made no reference to them. On the other hand, Arnala not only cited the pertinent previous preliminary work by Simonen and Laitinen (8), but he also noted that the annual hip fracture rates in all three areas in his study were in close agreement with the annual rate (1.175

Table 3

Annual Mean Incidence of Upper Femur (Hip) Fractures per 1,000 Inhabitants by Age in Low-Fluoride, Fluoridated, and High-Fluoride Areas in Finland During the Period 1972-1981 (adapted from ref. 6).

Area	Incidence per Age Group*				Total
	50-59	60-69	70-79	≥80	
Low Fluoride+ (0.0-0.3 ppm)	0.12(5)	0.78(27)	1.64(39)	9.32(64)	1.24(135)
Fluoridated (Kuopio, 1 ppm)	0.19(13)	0.77(38)	1.63(50)	9.36(85)	1.19(186)
High fluoride‡ (1.5-5.4 ppm)	0.07(3)	0.80(28)	2.05(52)	7.40(57)	1.24(140)

\* Number of patients over 10-year period, 1972-1981, in parenthesis.

+ Centrally located communities of Kaavi, Keitele, Lepavirta, Suonenjoki, and Vierema.

‡ Southeast communities of Hamina, Pyhtaa, Ruotsinpyhtaa, Vehkalahti, and Virolahti.

per 1,000 inhabitants) determined earlier for a Finnish population of 1.1 million (9). He concluded, therefore, in contrast to Simonen and Laitinen, that "the incidence of hip fractures does not depend on fluoride intake" and that "low or high fluoride neither protects against nor increases the risk of hip fractures."

Arnala also supported these conclusions by extensive histomorphometric evaluation of iliac crest samples from contemporary cadavers and from hip fracture patients. Fluoridated water failed to protect against either osteoporosis or bone fragility, although it did produce substantial increases in bone fluoride levels, especially among persons with renal impairment and to a lesser extent among men than women. "Between hip fracture groups," Arnala noted, "there was no difference in histomorphometric parameters [volumetric density of trabecular bone and osteoid; osteoid-covered surface of trabecular bone; trabecular resorption surface] between the low-fluoride area and the fluoridated-water area."

Finally, Arnala found that osteomalacia (bone softening) as well as osteoporosis (bone thinning) were common among the hip fracture patients. "Abnormal biochemical changes, especially hypocalcemia, were frequent. The fluoride status in these patients from different areas supports the findings based on cadaver material. The volumetric density of trabecular bone did not change significantly in the three areas examined. . . . In these three regions with different contents of fluoride in the drinking water, the epidemiological study did not reveal any differences in incidence of fractures of the upper femur."

What must one logically conclude from all this? Clearly, despite Simonen and Laitinen's recent tentative suggestion to the contrary, the weight of both laboratory and epidemiological evidence continues to show that fluoridation of drinking water provides little or no protection against bone fragility and osteoporosis in the elderly.

A.W.B.

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# FLUORIDE CONTENT OF VARIOUS SODIUM CHLORIDE SAMPLES

by

Nobuhide Kokubu\* and Akira Yamasaki  
Tokyo, Japan

**SUMMARY:** Fluoride content was determined for various sodium chloride samples including reagent chemicals, cooking salts, and natural evaporites. After preconcentration on a Zirconium-loaded cation exchange resin column, fluoride was determined by a lanthanum fluoride ion selective electrode. The content is 0.045-0.125 ppm ( $\mu\text{gF/gNaCl}$ ) for reagent chemicals, and 0.15-7.4 ppm for commercial cooking salts; 20.6 ppm for one sample of crude marine salts, was the highest level found.

## Introduction

The determination of inorganic fluoride has been much improved by the introduction of fluoride-ion sensitive lanthanum fluoride electrodes (1). This method requires constant ion activity coefficients by addition of a large excess of spectator electrolytes. For this purpose, a Total Ionic Strength Adjusting Buffer (usually abbreviated as TISAB) has been devised and widely used (2). However, non-negligible blank values of fluoride have been found occasionally in TISAB whenever low-level fluoride is determined in rain or river water samples (3). The sodium chloride used for maintaining constant ionic strength (2) often contains traces of fluoride. Hence the determination of fluoride content in such sodium chloride samples is necessary, and it therefore seems important to estimate the limit of TISAB usage prepared with sodium chloride.

## Method

An apparatus was constructed with ordinary conditioned cation exchange resin [Dowex 50W-X8  $\phi$ 12mm, ca.10 ml vol.]<sup>†</sup> column (see Figure 1). After the zirconium loading and repeated fluoride filling (3) the aqueous sodium chloride solution (about 1 M) was prepared from the sodium chloride sample, placed in the beaker and poured into the resin column through a siphon tube. After all the solution was passed, the siphon tube was removed and the column was washed with deionized water. The adsorbed fluoride was eluted with 2 M sodium hydroxide. The eluate (100 ml, ten times the volume of the column) was collected and made into a definite volume. An aliquot was mixed with an equal volume of TISAB (3 M acetic acid and 1 M sodium acetate dissolved in 1 liter of deionized water), and the fluoride concentration was determined by the standard-addition technique with a micro-horizontal burette (for micro-

\* From Laboratory of Applied Chemistry, The University of Electro-Communications, Chofu, Tokyo 182, Japan.

† The freshly prepared column shows very poor yields because of the existence of non-elutable fluoride sites or "pits," in the column. Therefore, the pretreatment with 1% aqueous sodium fluoride solution following the sodium hydroxide elution seems to be necessary to accomplish the quantitative preconcentration. This process is called "pit-filling" (4).

diffusion analysis). The recovery of this column preconcentration and separation was  $95 \pm 5\%$  for sub-millimolar fluoride solutions (4).

### Results

Tables 1 and 2 present the observed fluoride content in various sodium chloride samples. The reagent grade sodium chloride contains 0.045 to 0.125  $\mu\text{gF/g}$  (ppm) of sodium chloride. The highest value of these samples corresponds to a background concentration of 0.41 micromol fluoride when the TISAB is prepared under the standard prescription of 1 M sodium chloride. In some natural water samples, including rainwater, the fluoride concentration often reaches micromolar levels.

Figure 1

Aparatus for fluoride preconcentration by Zr(IV) column.

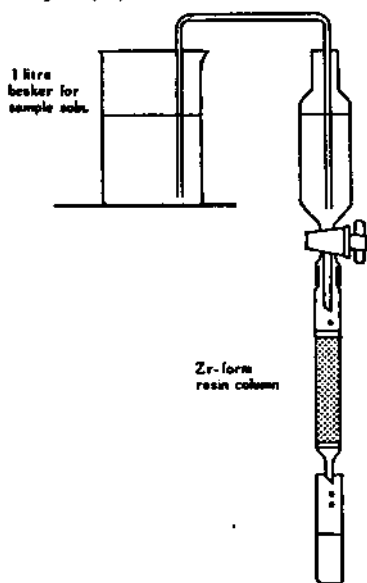


Table 1

Fluoride Content of NaCl Samples  
Reagent Chemicals

Brand	Lot #	$\text{F}^-$ ( $10^{-6}$ g/g NaCl)
Merck	5268091	0.071 $\pm$ 0.002
BDH Analar	1194754	0.069 $\pm$ 0.014
Matsunaga	0427	0.045 $\pm$ 0.002
Koso Chem.	S9A0912	0.059 $\pm$ 0.002
Nakarai	V8T5439	0.090 $\pm$ 0.002
Kokusan Chem	3A831532	0.120 $\pm$ 0.010
Kanto Chem.	312A5534	0.120 $\pm$ 0.020
Wako Chem.	0448	0.125 $\pm$ 0.015
Wako Chem.	AL1067	0.047 $\pm$ 0.001

Commercial Cooking Salts

Sample	$\text{F}^-$ ( $10^{-6}$ g/g sample)
Japan I (Table Salt)	0.255 $\pm$ 0.015
Japan II ("Enriched")	0.155 $\pm$ 0.015
Finland I	0.230 $\pm$ 0.020
Finland II	0.300 $\pm$ 0.020
Turkey I	5.8 $\pm$ 0.1
Turkey II	7.4 $\pm$ 0.1
U.S.A. (Hawaii)	0.34 $\pm$ 0.10
U.S.S.R.	3.85 $\pm$ 0.05

### Conclusion

Use of sodium chloride as the spectator electrolyte should be avoided in preparing TISAB whenever fluoride at micromolar levels is to be determined without preconcentration. It is therefore advisable to use other electrolytes, such as ammonium chloride or sodium perchlorate, which contain much less fluoride.

Table 2  
F<sup>-</sup> Content of NaCl Samples  
Crude Salts and Lake Evaporites

Origin (Locality)	F <sup>-</sup> (10 <sup>-6</sup> g/g sample)
Rock Salt (U.S.A.)	3.7 ±0.1
Rock Salt (China)	9.4 ±0.1
Rock Salt (Yeman)	7.6 ±0.1
Rock Salt (Spain)	6.4 ±0.2
Marine Salt (Egypt)	20.6 ±0.2
Marine Salt (Vietnam)	11.0 ±0.1
Marine Salt (Taiwan)	5.9 ±0.1
Japanese Marine Salts (Kyushu Area)	
Tsuyazaki, Fukuoka	13.3 ±0.1 (Heat Evaporation)
Obama, Nagasaki I	13.1 ±0.2 (Heat Evaporation)
Obama, Nagasaki II	9.2 ±0.1 (Heat Evaporation)
Obama, Nagasaki III	6.3 ±0.1 (Heat Evaporation)
Obama, Nagasaki IV	16.4 ±0.1 (Vacuum Evaporation)
Lake Evaporites from Turkey	
Lake Tuz Sample I	4.1 ±0.1
Lake Tuz Sample II	4.3 ±0.1

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EFFECT OF FLUORIDE ON SOME HEMATOLOGICAL PARAMETERS OF  
AN ESTUARINE MUDSKIPPER, BOLEOPHTHALMUS DUSSUMIERI

by

Y.A. Shaikh\* and P.K. Hiradhar  
Surat, India

SUMMARY: Estuarine mudskipper, Boleophthalmus dussumieri was exposed to sublethal concentrations of 40 and 80 ppm fluoride for 288 hr. The fish showed decreases in hemoglobin, white blood corpuscles (WBC), monocytes and neutrophils, and increases in red blood corpuscles (RBC) and lymphocytes.

KEY WORDS: Mudskipper; Hemoglobin; WBC; Monocytes; Neutrophils; RBC; Lymphocytes

Introduction

Fluoride toxicity to the following aquatic animals has been reported: crabs (1,2), bivalve molluscs (3,4) and fish (5,6). However, the majority of the above-mentioned reports are concerned with lethal concentrations, tissue accumulation or biochemical alterations in tissues due to fluoride toxicity. Studies on fluoride-induced changes in hematological parameters in aquatic animals are rare.

The present study concerns the effect of sublethal concentrations of fluoride on some hematological parameters (hemoglobin, monocytes, lymphocytes, neutrophils, red blood corpuscles (RBC), and white blood corpuscles (WBC) of an estuarine mudskipper, B. dussumieri (Cuvier and Valenciennes).

Materials and Methods

Mudskippers, B. dussumieri, collected from Dumas Coast of South Gujarat, were brought to the laboratory and acclimated to artificial sea water(7) for 7 days [photoperiod, 12 hr. D/N; temperature, 25° ±2° C; salinity, 27‰; pH 8.05 ±0.1]. Feeding with commercial fish food twice a day was continued up to 24 hr prior to experimentation. Twenty five mudskippers - 10.0 - 14.0 g in weight and 8.0 - 12.0 cm in length - were treated in artificial sea water containing 40.0 and 80.0 ppm fluoride in polypropylene containers. These concentrations were considered sublethal since the 96 hr LC 50 was 120 ppm fluoride. Fluoride concentrations were prepared by dissolving the appropriate amount of NaF in artificial sea water. The same number of mudskippers maintained in artificial sea water without fluoride served as control. The exposure media were changed daily. At the end of every 24 hrs, 3 fish were removed from each of the containers. They were anaesthetized with 0.5% solution of MS 222 and blood was drawn from the caudal fin. At the end of 96 hrs., the sampling interval was increased to 48 hrs. After 288 hrs. the experiment was terminated.

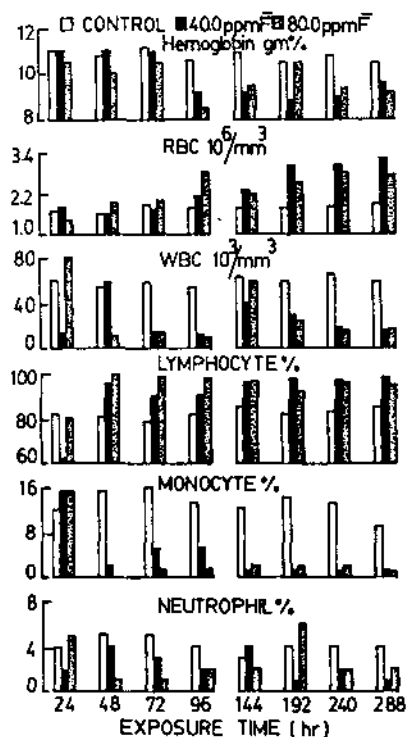
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From the Department of Biosciences, South Gujarat University, Surat -395 007, India.

RBC and WBC were counted using a hemocytometer with improved Neubauer ruling (Germany). Blood smears were stained with Leishman's stain (8) and differential counts were made. Hemoglobin was measured colorimetrically using the cyanomethemoglobin method.

Figure 1

Hb - quantity and blood cell populations in fluoride-exposed *B. dussumieri*.



fish, is sensitive to an abnormal fluoride level. Changes in blood glucose, glycogen and SDH activity in liver and muscles were observed in *B. dussumieri* when exposed to 40.0 and 80.0 ppm fluoride (13). Increases in RBC in the present study are probably due to the mobility of blood cells from the blood depot to the cardiovascular space as reported by Qayyum *et al* (14). Although the number of RBC was elevated, the hemoglobin content, surprisingly, was lower. Decreases in hemoglobin due to fluoride toxicity has been reported in mammals (15). Tom *et al* (16) reported inhibition of globin synthesis by fluoride. Changes in WBC, monocytes, neutrophils and lymphocytes observed in the present study are similar to what has been found in mammals (17). The mudskipper is sensitive to fluoride like other freshwater and marine fish.

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#### Results

Mudskippers exposed to 40 and 80 ppm fluoride showed considerable increase in RBC, compared to controls after 96 hrs. treatment (Figure 1). This increase was especially prevalent in those exposed to 40 ppm fluoride. Compared to the controls, WBC count declined in fluoride-exposed mudskippers, predominantly after 72 hrs. Monocytes and neutrophils declined, whereas lymphocytes increased in fluoride-exposed mudskippers. Monocytes decreased more than neutrophils. Changes in monocytes, neutrophils and lymphocytes were marked after 48 hr exposure to fluoride. Mudskippers exposed to fluoride showed a decrease in hemoglobin content after 96 hrs. exposure (Figure 1).

#### Discussion

Several studies have shown that fluoride affects various tissue metabolites in freshwater (9,10) and marine (11,12) fish; however, fluoride toxicity in estuarine fish has not been given attention. It has been shown that the estuarine sturdy fish mudskipper (*B. dussumieri*), like freshwater or marine

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# FLUORIDE INTOXICATION IN CATTLE DUE TO INDUSTRIAL POLLUTION CAUSED BY PROCESSING ROCK PHOSPHATE

by

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**SUMMARY:** Fluoride intoxication in cattle occurred as a consequence of atmospheric pollution by 4 phosphate processing factories located in the city of Rio Grande, Southern Brazil. Dental fluorosis was observed in 19 farms located in a range of 4.5 to 17.5 km from those factories. Severity of lesions was related to the distance between the farms and the factories as a linear function,  $y=2.13+(-0.12.X)$ ;  $r^2=0.77$  ( $P<0.001$ ). Lameness and hyperostosis were observed in a farm located 6 km distant from the factories. Histological bone lesions, in animals without hyperostosis, were characterized by osteoporosis, atrophy of osteoblasts and variation in shape and size of osteons with irregular distribution of osteocytes. Fluoride levels in bone varied between 1400 ppm and 5750 ppm.

**KEY WORDS:** Cattle; Environmental pollution; Fluoride; Fluorosis; Osteoporosis

## Introduction

In 1980, four rock phosphate processing factories for fertilizer production were located at the city of Rio Grande, State of Rio Grande do Sul, Southern Brazil. Atmospheric emissions of these industries are a well known source of fluoride contamination of pastures (1,2). Because many dairy farms are located near the city of Rio Grande, this work was carried out to study the occurrence of fluoride poisoning in cattle in this area.

## Material and Methods

Dental lesions in cattle were studied at 19 farms located between 4.5 and 17.5 km from 4 rock phosphate processing factories (Fig. 1). In each farm, incisor teeth of a variable number of cattle (Table 1) were observed and photographed, for black and white markings, the degree of lesion of each incisor tooth was classified according to the following, adapted from Suttie (3): 0] without lesions; 1] slight mottling of enamel; 2] definite mottling; 3] definite mottling, hypoplasia or hypocalcification and increased wear. The mean score of all permanent incisor teeth observed on each farm was designated mean degree of lesion. The degree of lesion in each farm, as a result of the distance from the factories was studied by regression analysis.

Lesions in molar and premolar teeth were observed in 3 slaughtered animals from farm A.

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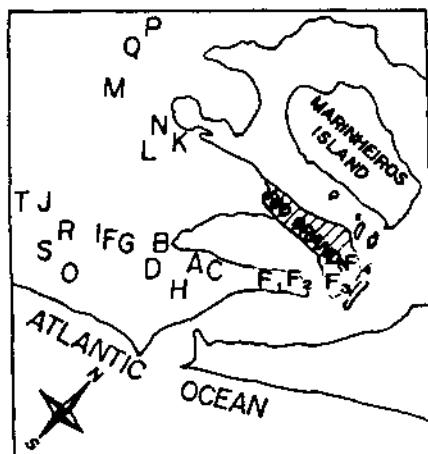
Incisor and mandible from 7 steers 3.5 to 5 years old from farms A and H and the proximal epiphysis-metaphysis of the right humerus of 3 of them, were fixed in 10% formalin, decalcified with formic acid and sodium citrate and embedded in parafin. Sections were stained with hematoxylin and eosin.

Fluoride levels in bone ash from the seven animals previously mentioned were determined at Cornell University by the fluoride ion electrode method quoted by Krook and Maylin (4).

### Results

Lesions of permanent incisor teeth were characterized by chalky white, yellow or brown discoloration, hypoplasia, pitting and loss of enamel, with exposure of dentine in some cases.

Figure 1  
Area studied.



Farms indicated by letters A to T; factories as F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, and F<sub>4</sub>, located in or close to city of Rio Grande. Wind direction from east 12% of time and from northeast 26% of time.

Table 1  
Farms studied with distance from factories

Farm	Distance (km)	No. of incisor teeth affected and degree of lesion					Mean degree	No. of cattle
		0	1	2	3	4		
P	17.5	86	2	0	0	0	0.023	15
J	14.5	91	3	5	4	0	0.24	15
R	13	32	5	3	0	0	0.27	14
S	14	18	1	1	1	0	0.28	16
O	13	121	24	17	3	0	0.41	23
T	15	24	2	3	2	0	0.45	6
Q	17	31	4	6	1	0	0.45	9
K	11	76	12	20	2	0	0.53	17
L	11	101	34	24	0	6	0.64	23
D	8	23	19	11	0	0	0.77	14
M	15	94	21	26	9	5	0.77	29
F	9.5	36	19	17	0	1	0.78	17
I	10	31	17	17	2	0	0.85	17
N	12	41	5	9	6	5	0.92	10
B	7.5	14	7	18	1	0	1.15	9
G	8.5	7	6	11	0	0	1.17	7
C	4.5	2	4	16	0	0	1.64	4
H	6	15	17	19	16	4	1.68	9
A	5.5	8	12	106	9	9	1.99	25



Table 2

F<sup>-</sup> (ppm) in bone ash of 3.5 to 5 year old steers from two farms

Farm	No. of animal	Humerus	Mandible
A	1	5750	1950
	2	2200	1400
B	1		3000
	2		2050
	3		3000
	4	2000	1750
	5		1650

Figure 2

Dental fluorosis in 4 year old steer, from farm A. Score 4 in both incisors. Gingival hyperplasia.

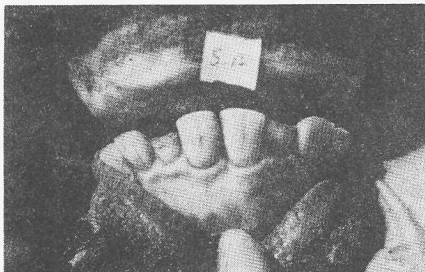
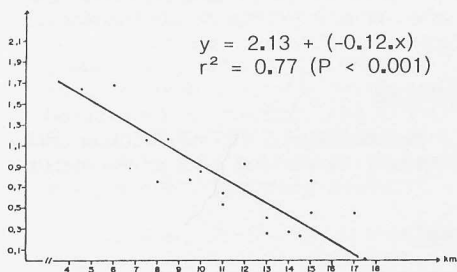


Figure 3

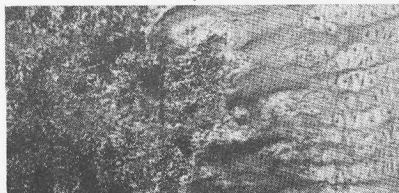
Fluoride Intoxication in Cattle due to industrial pollution caused by rock phosphate processing.



Mean degree of tooth lesion in each farm (y) as a function of distance from factories (x).  $y=2.13 + (-0.12 \cdot x)$ ;  $r^2=0.77$  ( $P<0.001$ ).

Figure 4

Proximal epiphyseal cartilage and primary spongiosa in humerus of 3 year old steer.



Osteoblasts scanty and atrophic, very little formation of primary spongiosa. Osteoporosis. (H.E. x100.)

Increased attrition was also observed (Figure 2). Many animals showed gingival hyperplasia. The number of cattle observed in each farm, the number of permanent incisor teeth within each degree of lesion, the mean degree of lesion and the distance from the factories are shown in Table 1. Regression analysis of the degree of lesion as a function of the distance from the factories is presented in Figure 3.

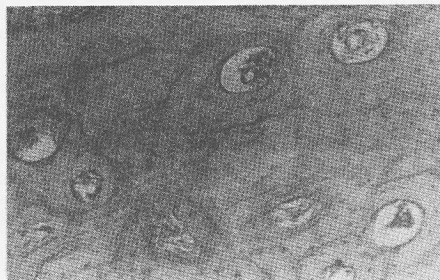
Molar and premolar teeth showed excessive and uneven attrition. On farm H, 2 cows showed lameness and 1 had hyperostosis of metacarpal bones.

Histological lesions of permanent incisors were hyperplasia of the cement and disturbed incremental lines of the dentine.

Bone lesions at proximal epiphysis-metaphysis of the humerus were characterized by osteoporosis, with very little formation of primary spongiosa and a small number of osteoblasts which appeared reduced in size and flattened (Figure 4). In compact mandibular bone, osteons were irregular in shape, size and distribution, with enlarged Haversian canals, irregular distribution of osteocytes and numerous cementing lines (Figure 5), interstitial lamellae were increased and irregular.

Figure 5

Mandibular bone of steer from farm A



Numerous cementing lines, irregular shapes and sizes of osteons with irregular distribution of osteocytes. Enlarged Haversian canals. (H.E. x100.)

Results of fluoride determinations in bone ash are shown in Table 2.

### Discussion

The occurrence of severe dental lesions, hyperostosis, lameness and toxic levels of fluoride in bones, confirmed the diagnosis of fluoride intoxication in cattle, as a consequence of atmospheric pollution caused by rock phosphate processing factories of Rio Grande. Following the criteria mentioned by Krook and Maylin (4), and Suttie (2) such intoxication seems to cause economic losses, at least in the farms situated close to the factories.

In the histological studies, which did not include animals with hyperostosis, bone lesions in compact bone were characterized by variation in shape and size of osteons with irregular distribution of osteocytes. Such alterations are the result of an imbalance between bone apposition and resorption (1). Osteofluorosis can result in different morphological lesions including hyperostosis, osteopetrosis and osteoporosis (1,4). In the animals studied in this survey, the main bone lesion was osteoporosis, a result of decreased bone apposition in consequence of osteoblastic insufficiency.

Considering the fact that the factories are located very close to or inside the city of Rio Grande, one must point to the potential risk of fluoride intoxication for humans, mainly workers in the factories and people residing in the environs of the factory.

Before this research was carried out, dental and osteofluorosis in cattle were undetected by farmers in the area. This fact provides evidence of the possibility of the occurrence of fluorosis in other areas of Brazil where aluminium reduction, phosphate processing, steel manufacturing or combustion of coal for various purposes are being performed.

### Acknowledgements

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# SPECIFIC PHYSICOCHEMICAL PROPERTIES OF FLUORIDATED CARBONATE APATITES

by

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**SUMMARY:** In addition to the synthesis of carbonate ( $\text{CO}_3^{2-}$ ) apatites with various carbonate contents and crystallinity, fluoridated  $\text{CO}_3^{2-}$  apatites were synthesized at  $80^\circ\text{C}$  and pH 7.4. The degree of increase in the solubility of  $\text{CO}_3^{2-}$  apatites with high crystallinity similar to that of tooth enamel changed greatly in the region of 0-3 wt% of carbonate content. Such sensitive susceptibility of  $\text{CO}_3^{2-}$  apatites to acid decreased dramatically with substitution of small amount of  $\text{F}^-$  ions into the apatite crystals. These solubility and dissolution behaviors of fluoridated  $\text{CO}_3^{2-}$  apatites did not parallel the crystallinity, which increased slightly, then decreased with the degree of fluoridation, and finally increased greatly when the fluoride content approached that of francolite.

**KEY WORDS:**  $\text{CO}_3^{2-}$  apatites; Fluoride; Crystallinity; Solubility

## Introduction

Incorporation of fluoride into the crystalline lattice of apatitic tooth mineral induces greater stability (1-3). On the other hand, the inorganic phases of tooth enamel are mainly carbonate-containing apatites and carbonate in the apatites may weaken the chemical bonds in the crystals (4-6). In most studies, however, both synthetic apatites and even tooth apatites examined were carbonate-free fluoridated hydroxyapatite (HAp), although a few investigators (7,8) have touched briefly upon the subject of fluoridated  $\text{CO}_3\text{Ap}$ . It is important to examine the interaction between the caries-preventive action of fluoride and the caries-promoting action of carbonate in apatite crystals.

At first, the caries susceptibility of fluoride-free  $\text{CO}_3\text{Ap}$ , then the interaction of fluoride and carbonate content affecting the physicochemical properties of synthetic fluoridated  $\text{CO}_3\text{Ap}$  with special attention to the relationship of apatite crystallinity to solubility behavior was examined.

## Materials and Methods

$\text{CO}_3\text{Ap}$  with various carbonate contents were prepared at 40, 60, and  $80^\circ\text{C}$ , and pH 7.3-8.0, by feeding 0.5 l of 50 mM  $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$  solution and 0.5 l of 30 mM  $\text{NH}_4\text{H}_2\text{PO}_4$  solution containing 0-0.3 M concentrations of  $(\text{NH}_4)_2\text{CO}_3$  into 1 l of a mechanically-stirred 1.3 M  $\text{CH}_3\text{COONH}_4$  solution. One series of fluoridated HAp and two series of fluoridated  $\text{CO}_3\text{Ap}$  with various fluoride contents were also prepared at  $80^\circ\text{C}$ , by feeding 0.5 l of 100 mM  $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$  solution and 0.5 l of 60 mM  $\text{NH}_4\text{H}_2\text{PO}_4$  solution containing 0-20 mM concentrations of HF and 0, 60, 120 mM concentrations of

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$(\text{NH}_4)_2\text{CO}_3$  into 1 l of mechanically stirred 1.3 M  $\text{CH}_3\text{COONH}_4$  solution. The pH was maintained at 7.4 by occasional addition of concentrated  $\text{NH}_4\text{OH}$  solution.

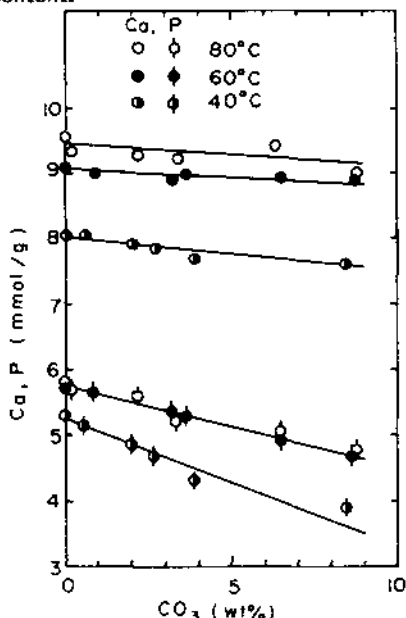
X-ray diffraction was employed to identify precipitates and estimate their lattice constants and crystallinity. The a- and c-axis dimensions were calculated from (300) and (002) reflections, respectively. To estimate the crystallinity of samples, the inverse of the half-value breadth was calculated for the (300) and (002) reflections as representations of the a- and c-axis.

Calcium concentrations were determined by atomic absorption spectrophotometry; total phosphate concentrations by the UV-spectrophotometric method; fluoride concentrations by an Orion Specific Meter; carbonate concentrations by the Conway method (9).

Solubility experiments were carried out at 0.5 M acetate buffer solution. After incubation at 37°C for 1 month, the calcium and phosphate concentrations in the solution were determined. The calcium concentration was adopted as the apparent solubility of the apatites.

Figure 1

Calcium and phosphate content of  $\text{CO}_3\text{Ap}$  as a function of carbonate content.



The X-ray diffraction patterns for all samples were characteristic of calcium apatites (Figure 2). The apatites, synthesized with low carbonate concentration at 80°C, were well crystallized similar to that of human enamel (Figure 1B), which crystallographic orientation is almost diminished in powdered

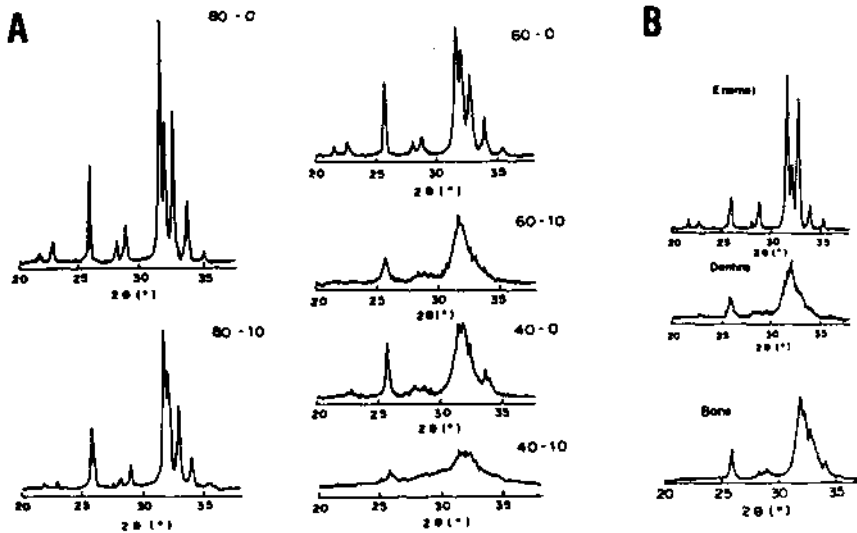
To examine the dissolution rate of fluoridated  $\text{CO}_3\text{Ap}$ , pellets with a surface area of 3.3  $\text{cm}^2$  were prepared by compressing each powder sample under 2 tons and then heating it at 500°C to prevent its disintegration in solution. The pellets were partially dissolved in a 0.5 M acetate buffer solution [pH 4.0, 25°C] by agitating at 500 rpm. The dissolved calcium concentration in the solution during the initial 1 hr period was adopted as the average dissolution rate of the fluoridated apatites, details of which have been reported previously (10-12).

## Results

$\text{CO}_3\text{Ap}$ : Figure 1 shows the chemical compositions of precipitates synthesized at 40, 60 and 80°C. Whereas the decrease in calcium content was slight, with an increase in carbonate content, the phosphate content decreased greatly.

Figure 2

Typical examples of x-ray diffraction patterns of  $\text{CO}_3\text{Ap}$  synthesized at 40, 60 and 80°C (A), with those of human enamel, dentine and bone (B).



Numbers to the right indicate temperature and  $\text{CO}_3/\text{P}$  feed molar ratio.

sample. The a-axis dimensions decreased with the increase in carbonate content (10), in the same way as the  $\text{CO}_3\text{Ap}$  synthesized in aqueous systems by LeGeros (13). When considered with the results of chemical and infrared absorption (10) analyses, these results

indicate that  $\text{CO}_3^{2-}$  ions taken into the apatite crystal may be substituted into  $\text{PO}_4^{3-}$  positions. The crystallinity of  $\text{CO}_3\text{Ap}$  decreased with carbonate content and increased with temperature. The crystals of  $\text{CO}_3\text{Ap}$  became smaller and more spheroidal with the increase in carbonate content, and with the decrease of temperature.

Figure 3

Apparent solubility of  $\text{CO}_3\text{Ap}$  shown as the dissolved calcium concentration.

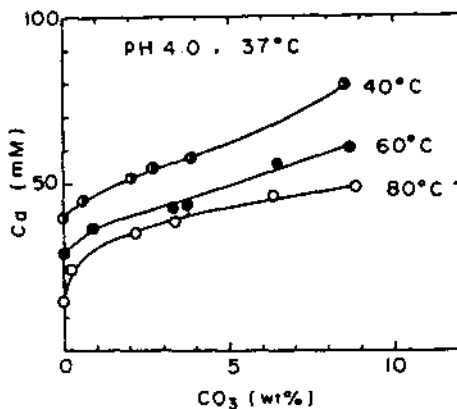
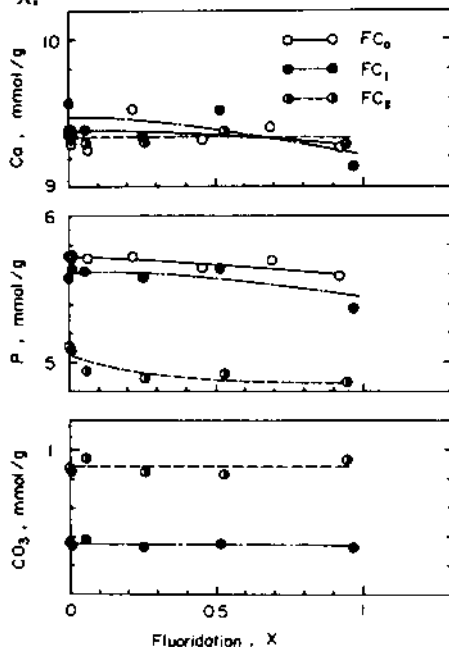


Figure 3 shows the dissolved calcium concentrations as the apparent solubility of  $\text{CO}_3\text{Ap}$  at pH 4.0 and 37°C. The solubility of  $\text{CO}_3\text{Ap}$  synthesized at each temperature increased with the increase in carbonate content. In particular, the solubility of  $\text{CO}_3\text{Ap}$  synthesized at 80°C tended to increase rapidly at low carbonate content.

**Fluoridated  $\text{CO}_3\text{Ap}$ :** The fluoride content of precipitates (Figure 4) increased with the fluoride concentration in the solution, whereas neither calcium nor phosphate content changed significantly with fluoride content. The carbonate content was not affected by fluoride content. X-ray diffraction patterns

**Figure 4**

Calcium, phosphate and carbonate contents of fluoridated HAp ( $\text{FC}_0$ ) and fluoridated  $\text{CO}_3\text{Ap}$  ( $\text{FC}_I$  and  $\text{FC}_{II}$ ) as function of degree of fluoridation,  $X$ .



fluoridated HAp corresponded approximately to the variations in crystallinity. Although the unexpected crystallinity phenomena of the fluoridated  $\text{CO}_3\text{Ap}$  could not be clearly observed under the electron microscope, perhaps because of the small and coagulated crystals, variations in the surface area of fluoridated  $\text{CO}_3\text{Ap}_{II}$  with increasing fluoride content roughly supported the unexpected crystallinity phenomena (12).

The dissolved calcium concentrations shown in Figure 7 indicate the apparent solubility of fluoridated HAp and fluoridated  $\text{CO}_3\text{Ap}$  at pH 4.0 and 37°C. The solubility for each series of samples decreased with the amount of fluoride. In particular, low fluoride content tended to greatly decrease solubility.

Figure 8 shows the average dissolution rate of fluoridated apatite pellets during the initial 1 hr in agitated acetate solution at pH 4.0 and 25°C.  $\gamma_{\text{Ca}}$  indicates the calcium concentration in the solution based on unit surface area of the pellet per unit time.

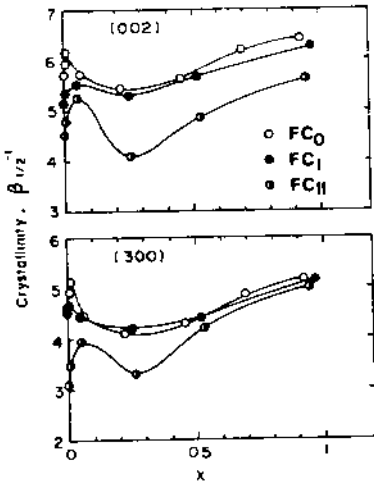
of all samples were characteristic of calcium apatites. A lower degree of crystallization was associated with increase in carbonate content. No extraneous peaks due to  $\text{CaF}_2$  were found, indicating that the fluoride content of samples did not exceed 2 mmol/g, which is equal to that of fluorapatite  $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$  and francolite [carbonate fluorapatite]. With increased fluoride,  $X$ , the a-axis dimensions decreased, whereas the c-axis dimensions scarcely changed (12).

Figure 5 shows the crystallinity of fluoridated apatites as the inverse of the half-value breadths of (300) and (002) reflections. The crystallinity of each fluoridated  $\text{CO}_3\text{Ap}$  initially increased slightly, then decreased with the amount of fluoride, and finally increased greatly when the fluoride content approached that of francolite, analogous to that of the fluoridated HAp.

Figure 6 shows the typical transmission electron micrographs of fluoridated HAp and fluoridated  $\text{CO}_3\text{Ap}$  in each series. In general, it may be said that the morphology of the apatite crystals reflects on their crystallinity (14). The observation on the morphology of the

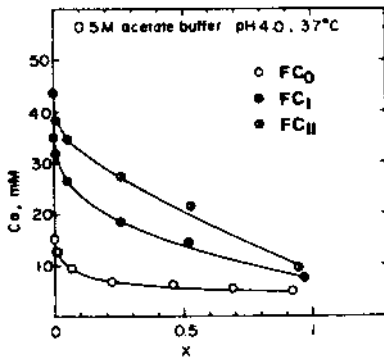
**Figure 5**

Crystallinity of fluoridated HAP ( $FC_0$ ) and fluoridated  $CO_3Ap$  ( $FC_I$  and  $FC_{II}$ ) shown as inverse of the half-value breadth of (300) and (002) reflections.



**Figure 7**

Apparent solubility of fluoridated HAP ( $FC_0$ ) and fluoridated  $CO_3Ap$  ( $FC_I$  and  $FC_{II}$ ) shown as dissolved calcium concentration.



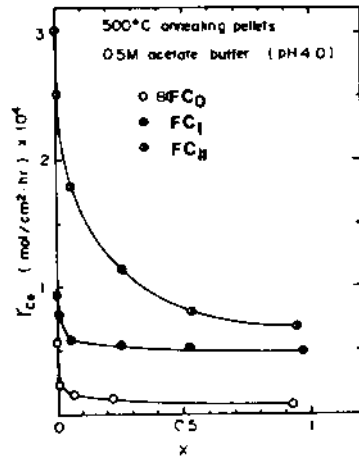
**Figure 6**

Transmission electron micrographs of fluoridated HAP ( $FC_0$ ) and fluoridated  $CO_3Ap$  ( $FC_I$  and  $FC_{II}$ ).



**Figure 8**

Dissolution rate of fluoridated HAP pellets ( $FC_0$ ) and fluoridated  $CO_3Ap$  pellets ( $FC_I$  and  $FC_{II}$ ).



**Discussion**

The  $CO_3Ap$  solubility was related approximately to crystallinity. However, the degree of increase in  $CO_3Ap$  solubility with high crystallinity changed greatly in the region of 0-3 wt% of carbonate content. This means that  $CO_3Ap$

solubility with high crystallinity in this region is greatly affected by small changes in carbonate content. This range of carbonate content, and possibly also crystallinity, correspond to those of enamel apatites. Thus, carbonate-containing apatites with high crystallinity are very sensitive to acid at low carbonate content.

It is interesting that these sensitive susceptibilities of  $\text{CO}_3\text{Ap}$  to acid decreased dramatically with substitution of a small amount of  $\text{F}^-$  ions into the apatite crystals. In addition, this chemical stability of fluoridated apatites in solution, as reflected in solubility and dissolution rate, cannot be explained in terms of crystallinity. It must be considered a specific action of fluoride in the crystallinity of fluoridated apatites.  $\text{F}^-$  ions may exist as an impurity (15) at relatively low fluoride content, in which case they would be expected to produce disorder within the crystal column. This disorder and/or random distribution of  $\text{F}^-$  ions in the crystals may possibly inhibit the crystal growth at relatively low fluoride content.

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# EFFECTS OF INHALED HF ON CHOLESTEROL METABOLISM IN GUINEA PIGS

by

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**SUMMARY:** Exposure to 5 mg HF/m<sup>3</sup> causes a significant increase in the plasma cholesterol levels in the guinea pig. Modifications of the cholesterol metabolism are due to the specific action of fluoride. Effects of HF on cholesterolemia are reversible however, and during a second exposure to HF, plasma cholesterol increases as in the first exposure. Cholesterol biosynthesis was studied. Acetate incorporation in intestinal tissue and lung was higher in intoxicated animals than in controls but mevalonate incorporation was comparable in the two groups. The enzyme catalyzing mevalonate synthesis,  $\beta$ -methyl- $\beta$ -hydroxyglutaryl CoA reductase, could be activated by HF.

**KEY WORDS:** Cholesterol; Guinea pigs; HF; Intestinal tissue; Lung.

## Introduction

Hydrogen fluoride inhalation for 84 hours (10 mg HF/m<sup>3</sup>) induces a significant increase in plasma cholesterol levels (1) in guinea pigs. The present study was undertaken to investigate which of two mechanisms might be involved in the development of hypercholesterolemia: a specific effects of hydrogen fluoride and/or an irritating action. The effects of controlled levels of hydrogen fluoride on plasma cholesterol were noted at various sampling times, as well as the reversibility of these effects.

## Method

**Animals and experimental design:** The male and female albino guinea pigs which were used weighed 350 g at the beginning of exposure. They were fed with commercially available pellets (purchased from Usine d'Alimentation Rationnelle "UAR", France) containing 20 ppm fluoride and with a fresh supply of carrots and vitamins. To study the effects of graded levels of hydrogen fluoride on plasma cholesterol, the animals were divided into five groups: A control group of guinea pigs was housed in a cylindrical plexiglass cage previously described (1) without gaseous HF. Four groups were exposed to 1.5, 3, 5 and 10 mg HF/m<sup>3</sup> respectively in the plexiglass cage for 84 hours. (Gaseous HF was produced by sending an aqueous solution of HF, by peristaltic pump, into a vaporization oven at 150°C. The desired level of HF in the atmosphere (1.5, 3, 5, and 10 mg HF/m<sup>3</sup>) was obtained by varying the concentration and volume of the solution and by modifying the amount of purified air used to dilute the HF vapor. The level of fluoride was checked every 3 hrs for 84 hrs with an automatic captor by trapping the HF in a known volume

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of air on a dry caustic soda-impregnated filter and assaying with a specific electrode (2). During the study the average room temperature was 20°C and the humidity 72%.

For comparison of the effect of hydrogen fluoride and of hydrogen chloride, a group of animals was exposed to 5 mg HCl/m<sup>3</sup> for 84 hrs. The effect of hydrogen fluoride on animals presenting hypercholesterolemia was investigated on a group of male albino guinea pigs receiving the same diet with 1% (w/w) additional cholesterol for seven days. All animals had free access to water and food during exposure and were fasted overnight before the blood samples were taken. On the other hand, the variations of plasma cholesterol concentration were studied for females as well as for males during prolonged exposure in the fluoride atmosphere (HF: 5 mg/m<sup>3</sup>).

Plasma cholesterol of three groups of guinea pigs of both sexes (randomly selected and healthy) was evaluated on D : 0 (control). The animals were then exposed to a constant fluoride atmosphere at 5 mg HF/m<sup>3</sup>. Each day a random selection of 5 animals was made and the value of plasma cholesterol was determined. Care was taken to insure that each animal was investigated every third day. For females, exposure was interrupted on the 15th day and re-exposure began on the 34th day and lasted until the 37th. The males were no longer exposed after day 14.

Technique: a) Cholesterol and fluoride determinations - Total cholesterol was estimated by the technique of Röschlau et al. (3). Plasma fluoride was determined by the method of Hall et al. (4). b) Statistical analysis: The dose factor is considered qualitatively (each day the animals receive a supplementary dose of HF). The homogeneity of the averages is tested (analysis of variances with repetition). If the averages differ significantly, the monotony of the response (amount of cholesterol) according to the dose of HF inhaled is studied. The response is supposedly linear. The linearity of the curve and its slope are tested.

Cholesterol metabolism: Materials - For incubation, tissues were immediately placed in cold saline. The ileum was opened longitudinally, flushed with cold saline, rinsed again and cut into 0.5 cm portions. Liver and lung were sliced. Ileum or liver or lung (200 mg) was placed in a flask with 2 ml of Krebs-Ringer buffer pH 7.5. Five experiments were prepared for each sample. After a 10 minute preincubation period at 37°C, in a metabolic shaker, the precursor was added: 0.25 µCi/ml of [1-<sup>14</sup>C]-acetate or 0.25 µCi/ml of [1-<sup>14</sup>C]-mevalonate following which the flasks were gassed 95% O<sub>2</sub> - 5% CO<sub>2</sub>, capped and incubated for 90 minutes according to Turley et al. procedure (5) modified by Sablé and Sicart (6).

Extraction and separation of lipids: At the end of the incubation period, the tissues were rapidly removed, placed in a flask containing a chloroform-methanol mixture (2:1 v/v) and homogenized. Total lipids were extracted by Folch's method, then fractionated into cholesterol and cholesterol esters by thin layer chromatography on silica gel G coated plates (0.25 mm thickness, Merck, Darmstadt, West Germany) in acetic acid-diethyl ether - light petroleum ether (1:20:80, v/v). After development of the chromatogram, the silica gel plate areas corresponding to the labeled lipids were scraped off and put into a vial containing 10 ml liquid scintillation mixture. The radioactivity was measured in a Packard Tricarb 3320 liquid scintillation spectrometer.

## RESULTS

The specific effect of hydrogen fluoride in the development of hypercholesterolemia is shown by plasma cholesterol determinations in two groups of guinea pigs. One was exposed to HF (5 mg/m<sup>3</sup>) the other to HCl (5 mg/m<sup>3</sup>), a gas the irritant action of which is similar to HF. Guinea pigs exposed to hydrogen fluoride presented a plasma fluoride concentration of 2,000 µg/liter whereas, in animals exposed to HCl, levels were 200 µg/liter. Cholesterol levels were significantly increased ( $p < 0.001$ ) in the fluoride group compared with levels in the HCl-exposed group. Mean values were 1.58 mmole/liter in the fluoride group, 0.68 mmole/liter in the chloride group. Therefore, increase of cholesterol is due to a specific effect of hydrogen fluoride.

To study the effects of different concentrations of hydrogen fluoride on cholesterol levels, the animals were divided into four groups and exposed to 1.5, 3, 5 and 10 mg HF/m<sup>3</sup> for 84 hours. Only when guinea pigs were exposed to 5 and 10 mg HF/m<sup>3</sup> was plasma fluoride concentration increased compared with controls (Table 1). Modifications in plasma cholesterol concentration were also observed (Table 2). Cholesterol levels were significantly increased in groups exposed to 5 and 10 mg HF/m<sup>3</sup>, but no cholesterol increase was registered in guinea pigs exposed to 1.5 or 3 mg HF/m<sup>3</sup> for four days or more.

Table 1  
Plasma Fluoride Levels of Guinea Pigs  
Exposed to Fluoride Graded Levels

HF in Atmosphere (mg/m <sup>3</sup> )	Plasma F (mg/l)
1.5	1.2 ±0.19
3	1.4 ±0.21
5	2 ±0.23
10	2.5 ±0.25
0 (control)	0.2 ±0.15

Table 2  
Plasma Cholesterol Levels of Guinea Pigs Before and After  
Exposure to Fluoride Graded Levels (Total Cholesterol: mmol/l)

Groups	n	Before Exposure		mg HF/m <sup>3</sup>	After Exposure	
		m	S.D.		m	S.D.
1	6	0.740	0.123	1.5	0.683	0.089
2	6	0.725	0.092	3	0.713	0.107
3	15	0.870	0.200	5	1.580	0.430
4	46	0.850	0.490	10	1.530	0.630

m = mean; S.D. = standard deviation; n = number of animals

Figure 1

Plasma Cholesterol Level (mmole/l) of Male Guinea Pigs during HF Exposure ( $5 \text{ mg/m}^3$ ). 1: Start of Exposure; 2: End of Exposure.

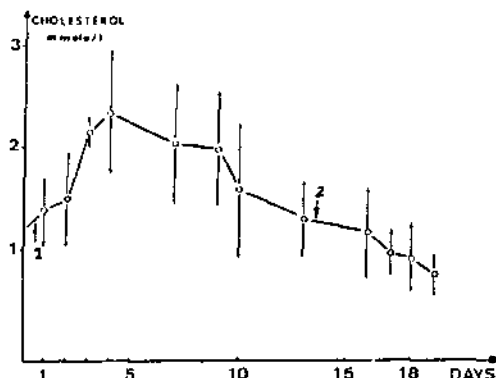
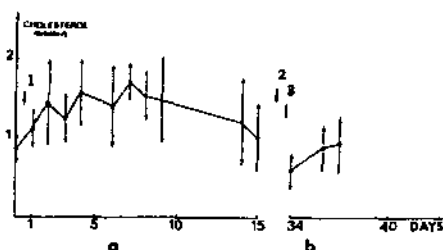


Figure 2

Plasma Cholesterol Level (mmole/l) of Female Guinea Pigs during First HF Exposure [a] and during a Second HF Exposure [b] after Two Weeks without Intoxication. 1: Start of First Exposure; 2: End of First Exposure; 3: Start of Second Exposure.



Exposure time to HF ( $5 \text{ mg/m}^3$ ) in relation to plasma cholesterol levels was studied in males and females (Figs. 1 and 2). For female guinea pigs, a test of homogeneity of the means (D : 0 and D : 9) shows a significant difference ( $F_{4,8}^9 = 4$ ) at 0.001. Analysis of the variance shows that between D : 0 and D : 9, the curve crossing through the set of points does not significantly deviate from linearity ( $F_{4,8}^1 = 1.15$  N.S.) and furthermore that the slope is very significant ( $F_{4,8}^1 = 24$  at 0.001). After withdrawing the animals from the fluoride atmosphere for a period of 10 days, and again exposing them plasma cholesterol increased but the new increase was smaller (Tables 3, 4, 5). For male guinea pigs, a test of homogeneity of the means (D : 0 and D : 4) shows a significant difference. Analysis of variance shows that between D : 0 and D : 4, the curve crossing through the set of points does not significantly deviate from linearity ( $F_{2,2}^3 = 0.7$  N.S.) and furthermore that the slope is very significant ( $F_{2,2}^1 = 22.7$  at 0.001). However in male guinea pigs, the increase

Table 3

Cholesterol Levels of  
Female Guinea Pigs Before HF Exposure  
( $5 \text{ mg/m}^3$ ) (Total Cholesterol: mmol/l)

Groups	n	m	s <sup>2</sup>
A	5	1.066	0.027
B	5	0.902	0.020
C	5	0.876	0.042
A+B+C	15	0.948	0.182

m = mean; S.D. = standard deviation;  
n = number of animals

**Table 4**  
Cholesterol Levels of Female Guinea Pigs at Different Sample Times During HF Exposure (5 mg/m<sup>3</sup>) (Total cholesterol: mmol/l)

Days	0	1	2	3	4	6	7	8	9
Groups	A+B+C	A	B	C	A	B	C	A	B
n	15	5	5	5	5	5	5	5	5
m	0.948	1.134	1.440	1.262	1.584	1.420	1.738	1.547	1.510
S.D.	0.182	0.243	0.545	0.317	0.424	0.519	0.229	0.313	0.529

m = mean; S.D. = standard deviation; n = number of animals

**Table 5**  
Cholesterol Levels of Female Guinea Pigs at Different Sample Times During a Second HF Exposure (Total cholesterol: mmol/l)

Days	34	36	38
n	12	5	6
m	0.678	0.907	1.050
S.D.	0.228	0.280	0.372

m = mean; S.D. = standard deviation;  
n = number of animals

**Table 6**  
Cholesterol Levels of Male Guinea Pigs at Different Sample Times During HF Exposure (5 mg/m<sup>3</sup>) (Total cholesterol: mmol/l)

Days	0	1	2	3	4
Groups	A+B+C	A	B	C	A
n	15	5	5	5	5
m	1.190	1.384	1.472	2.140	2.350
S.D.	0.15	0.601	0.491	0.190	0.641

m = mean; S.D. = standard deviation; n = number of animals

of plasma cholesterol is faster and the maximum value of cholesterol is reached more quickly than in females (Table 6).

These variations led to the study of the effect of HF on the rate of cholesterol biosynthesis by means of [ $1-^{14}\text{C}$ ]-acetate and [ $1-^{14}\text{C}$ ]-mevalonate incorporation in controls and intoxicated animals. In the HF group, the experiment was performed when serum cholesterol had reached its maximum value. Acetate incorporation in intestinal tissue and lung was higher in intoxicated animals than in controls (Table 7) but mevalonate incorporation was comparable in the two groups (Table 8). In contrast, when the same experiment was performed

Table 7

1- <sup>14</sup> C -acetate incorporation in Cholesterol of Ileum, Lung and Liver of Controls and Intoxicated Animals		
	Controls	Exposed
Ileum:		
m	1907*	8730
S.D.	1165	628
n	4	4
difference statistically significant at P<0.001		
Lung:		
m	1910	7663
S.D.	1233	3874
n	4	4
note dispersion of levels in exposed guinea pigs.		
Liver:		
m	2250	2863
S.D.	860	705
n	4	4

m = mean; S.D. = standard deviation;  
n = number of animals

\* dpm/gram tissue/hour.

Table 8

1- <sup>14</sup> C -mevalonate incorporation of Ileum and Lung of Controls and Intoxicated Guinea Pigs		
	Controls	Exposed
Ileum:		
m	6849*	4219
S.D.	1767	1329
n	4	4
difference not statistically significant		
Lung:		
m	10764	11276
S.D.	4381	4116
n	4	4
difference not statistically significant		

m = mean; S.D. = standard deviation;  
n = number of animals  
\* dpm/gram tissue/hour.

during the serum cholesterol decrease in intestinal tissue of intoxicated animals, acetate incorporation was low (2203 ± 546 dpm/g. ileum/h.). The rate of acetate incorporation in the liver was much lower than that in ileum and lung. The rates of sterol synthesis are similar in control and exposed groups (2250 ± 860 compared to 2863 ± 705 dpm/g. liver/h.).

### Discussion

Fluoride inhalation produces a more constant impregnation of fluoride than oral ingestion. The percentage of fluoride metabolized by the latter is only about 45%. Therefore, in this study, fluoride inhalation was chosen. Guinea pigs received 0.75, 1.5, 2 and 4 mg of gaseous HF/day plus about 1.5 mg of fluoride in the diet per day. Control guinea pigs received only 1.5 of dietary fluoride.

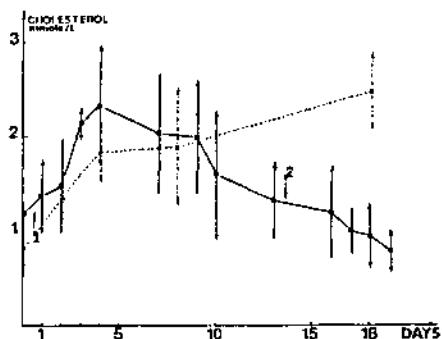
The specific action of HF on cholesterol metabolism is shown by comparing the effects of HF and HCl. Acetate and mevalonate incorporation suggests that β-OH, βmethylglutaryl CoA reductase (E.C.1.1.134), the enzyme catalyzing mevalonate synthesis, could be activated by HF. The enzyme might then be inhibited by the high quantity of cholesterol produced in the first phase of intoxication as previously demonstrated for the regulation of cholesterol biosynthesis in different mammalian species (7,8). The regulation of cholesterol biosynthesis in the guinea pig, however, seems to be different. Some authors have pointed out that guinea pig and rabbit cholesterolemia, in contrast to that in the rat, progressively increases with a cholesterol-rich diet slowly reaching a maximum value (6). Thus it seems that exogenous cholesterol does not produce a negative feed-back control in this species.

To assess this hypothesis, plasma cholesterol and |1-<sup>14</sup>C|-acetate incorporation were studied in cholesterol-fed guinea pigs. In contrast to results observed

in intoxicated groups, plasma cholesterol and  $[1-^{14}C]$ -acetate incorporation slowly increased and no diminution of the parameters was observed when the diet was continued (Figure 3.) Thus endogenous cholesterol seems to be able to produce a negative retro-control on its own biosynthesis but exogenous sterol, contained in a cholesterol-enriched diet, should not cause such inhibition. Furthermore, effects of exogenous and endogenous cholesterol are cumulative. When effects of HF inhalation on the plasma cholesterol level and  $[1-^{14}C]$ -acetate incorporation were studied in cholesterol-fed guinea pigs, acetate incorporation in ileum was higher in intoxicated animals than in controls, both groups receiving a cholesterol rich diet ( $10480 \pm 5147$  compared to  $5290 \pm 2823$  dpm/g. ileum/h.). The effects of HF on cholesterolemia are reversible when exposure to HF is discontinued. During a second exposure, however, cholesterol biosynthesis increases again (Figure 2).

**Figure 3**

Comparative Effects of Endogenous (●—●) and Exogenous (\*—\*) Cholesterol on Guinea Pig Cholesterolemia during Exposure to HF and Cholesterol-Rich Diet Respectively.



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# EFFECT OF FLUORIDE AND MERCURY UPON THE ACTIVITY OF AMINOTRANSFERASES.

by

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**SUMMARY:** The authors carried out experiments, using blood serum of Wistar rats, to study the synergistic effect of fluoride and mercury upon the activity of aspartate aminotransferase and alanine aminotransferase. When sodium fluoride and mercury chloride were combined enzymatic activity apparently increased in comparison to controls (AspAT -  $p < 0.01$  and AlAT -  $p < 0.01$ ). A more conspicuous increase in activities of the studied enzymes was obtained compared to controls, following administration of mercury chloride and sodium fluoride separately ( $HgCl_2$  - AspAT -  $p < 0.001$  and AlAT -  $p < 0.005$ , NaF - AspAT -  $p < 0.001$  and AlAT -  $p < 0.001$ ).

**KEY WORDS:** Aminotransferases; Fluoride; Mercury; Serum

## Introduction

Fluoride and mercury ions can participate in metabolic changes within the organism. Above a threshold, plants, animals and man display symptoms of disease. Inspired by the increasing levels of fluoride and mercury in the natural environment, the authors studied the aminotransferase activity, which is an index of liver and kidney damage.

## Materials and Methods

The animals were divided into 4 groups, each of which consisted of 8 males and 8 females; daily subcutaneous injections of the following reagents were administered: 1.] 0.9% NaCl in the amount of 0.5 ml/rat treatment was continued for the subsequent seven days; enzymatic activity was estimated after last injection (control group). 2.] Mercury chloride in the dose of 0.8 mg/l kg of body weight, which corresponds to 0.6 mg of pure mercury per 1 kg of body weight in the amount of 0.5 ml/rat; treatment was continued for the subsequent seven days, and enzymatic activity was estimated after last injection (experimental group). 3.] Sodium fluoride in the dose of 60 mg/l kg of body weight, which corresponds to 27 mg of fluoride per 1 kg of body weight in the amount of 0.5 ml/rat; treatment was continued for the subsequent seven days, and enzymatic activity was estimated after last injection (experimental group). 4.] A mixture of mercury chloride and sodium fluoride in the same doses as in groups 2 and 3; treatment was continued for the subsequent seven days, and enzymatic activity was estimated after last injection (experimental group). The possible synergistic effect of both fluoride and mercury upon the activity of aspartate aminotransferase (aspAT) and alanine

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aminotransferase (AlAT) in blood serum of Wistar rats (male and female) was investigated according to the method developed by Reitman and Frankel (1).

### Discussion and Results

The experiments yielded the following results: enzymatic activity in I.U. (international units) 1 ml serum - control group: AspAT = 38.0 ( $\pm 1.2$  S.D.), AlAT = 42.0 ( $\pm 1.8$  S.D.); the animals administered mercury chloride: AspAT = 51.0 ( $\pm 2.4$  S.D.), AlAT = 49.0 ( $\pm 1.6$ ); the animals administered sodium fluoride: AspAT = 52.0 ( $\pm 2.2$  S.D.), AlAT = 56.0 ( $\pm 2.4$ ); the animals receiving a mixture of mercury chloride and sodium fluoride: AspAT = 42.0 ( $\pm 1.2$  S.D.), AlAT = 46.0 ( $\pm 1.2$  S.D.).

Table 1

The Activity of Aspartate Aminotransferase (AspAT) and Alanine Aminotransferase (AlAT) in Blood Serum of Rats after Injection: HgCl<sub>2</sub>; NaF; HgCl<sub>2</sub> + NaF and Control Group.

Groups of Animals Values	Control		Experimental					
	AspAT	AlAT	HgCl <sub>2</sub>		NaF		HgCl <sub>2</sub> + NaF	
			AspAT	AlAT	AspAT	AlAT	AspAT	AlAT
Enzyme Activity (I.U./1 ml serum)	38.0	42.0	51.0	49.0	52.0	56.0	42.0	46.0
Standard Deviation ( $\pm$ S.D.)	$\pm 1.2$	$\pm 1.8$	$\pm 2.4$	$\pm 1.6$	$\pm 2.2$	$\pm 2.4$	$\pm 1.2$	$\pm 1.2$
Level of Significance	---	---	p < 0.001	p < 0.005	p < 0.001	p < 0.001	p < 0.01	p < 0.01

In group 4, in which both fluoride and mercury ions were administered, the activity of investigated enzymes was much stronger than in controls but at the same time much weaker than in serum of animals which were treated with both sodium fluoride and mercury chloride.

### Conclusion

It is concluded that toxicity is greater when the organism is exposed to the action of the two investigated elements separately. Obviously, the "synergistic effect" is also toxic for the organism, but the character of the effect appears to be of a different nature. A highly unstable compound HgF<sub>2</sub> may be formed that may cause a longer latent period of toxicity.

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# ROENTGEN DIAGNOSIS OF INDUSTRIAL SKELETAL FLUOROSIS (A REPORT OF 100 CASES)

by

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**SUMMARY:** This paper presents X-ray manifestations of industrial fluorosis in 100 cases. It is recognized that increasing density, bony structure changes and periosteal hyperplasia with calcification or ossification, especially the process of hyperplastic calcification of the posterior margin of tibia and interosseous membrane of radius and ulna, constitute the main criteria for the diagnosis of this clinical entity. Often all the above-mentioned processes make their appearance in the early stage of the disease but they may be delayed until the later stages. Preliminary analysis of the causes of this manifestation and classification of the stages of this disease with its various types are herein discussed.

**KEYWORDS:** Industrial fluorosis; Roentgenological characteristics; Classification stage.

## Introduction

Osteosclerosis and periosteal hyperplastic calcification or ossification in industrial skeletal fluorosis have been reported in the literature (1,2). The morphological characteristics of the periosteal tissues, the susceptible sites, and the etiological causes of this disease, however, are rarely mentioned. Often changes due to periosteal hyperplastic calcification have been considered a main diagnostic criteria in the late stage of this disease (3). Recently, in fluoride workers, changes due to periosteal hyperplastic calcification demonstrable by X-ray have been observed in the early stage of this disease which is of great diagnostic value to the clinician. This paper presents an analysis of 100 cases of industrial skeletal fluorosis with respect to its roentgenological characteristics and susceptible sites. To explore the causes of its formation as well as its diagnostic value, the findings were compared with 65 cases of normal workers.

## Material and Methods

One hundred patients (99 males and 1 female), average age 42.6 years (28-60), had worked in contact with fluorides an average 15.9 years (9-28). Included were electrolysis workers, anode workers, aluminum tapping workers, furnace workers and raw material packaging workers. Twenty-six of them were diagnosed as Stage 0-I (cases reserved for further observation), 70 as Stage I and 4 as Stage II. Fifty-eight males and 7 females, average age 39.0 years

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(28-60), and average standing 16.3 years (3-29), whose work included administrative staff, fitters, lathe operators and forgers, served as controls.

The air fluoride concentration in their work place ranged, during the past few years, from 0.03 to 19.6 mg F/M<sup>3</sup>. The drinking water contained less than 1 ppm fluoride. The main clinical symptoms presented by the 100 patients with industrial fluorosis were neurasthenic syndrome (52%), lumbar pain (72%), arthralgia of the limbs (58%), chronic infection of upper respiratory tract (47%) and restriction of motion of the joints. Among patients, urinary fluoride averaged 2.98 (0.4-11.6) mg/l; among controls, 1.02 (0.3-3.4) mg/l. The difference between the two groups ( $p < 0.01$ ) was statistically significant.

Changes in skeletal structure, observed by X-ray in the industrial fluorosis group, were chiefly manifested through changes of skeletal trabeculi. They were particularly significant in the lower lateral aspects of the sacroiliac joint and in the body of the ilium as well as in the vicinity of the symphysis pubis and were seen even in the wings of the ilium and in the vicinity of tibial tuberosities. The trabeculi were thickened, increased in density, tortuous, uneven in degree of thickness and arranged in disorder.

Among patients, in 13.1% of the cases (Fig. 1) the trabeculi were significantly thickened with clear edge and markedly decreased in number. The net holes were increased in size, simulating coarse thin gunny cloths. In 50.5% of the cases, they were obscure in demarcation and slightly thickened; the net holes resembled thin dense gauze, obscure in contour. In 30%, tiny condensed mottling could be seen in the form of structureless sand grains (Fig. 2). The remaining 6.1% of the cases showed no change of any kind.

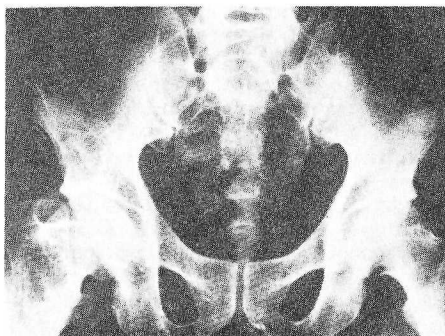
Skeletal changes in industrial fluorosis are generally much less intense than those in endemic fluorosis. The main bone changes increase in density even though the bony matrix structure was markedly sparse in some cases. Yet the trabeculi of the bones were comparatively thick with high density. In 6.1% of the patients bone density was markedly increased; in 35.4%, it was slightly increased; in 4.0%, bone density decreased (3).

Soft tissue changes in industrial fluorosis, were more marked. The formation of calcification and ossification in interosseous membrane, tendons or ligaments was seen frequently in the upper middle section of tibia, the interosseous ridge of the radius and the obturator foramen periosteum. Next in frequency the following occurred: calcification and ossification of periosteum, ulna and fibula, iliolumbar ligament, the sites of attachment of the sacrospinous ligament, sacrotuberous ligament and the paravertebral ligament.

Among patients, periosteal hyperplastic ossifications were mainly situated at the posterior margin of the sites of attachment of the tendons on the upper middle section of the tibia with a considerably wide range and polymorphous in nature. The periosteum of the hyperplastic ossifications in some cases was shaped like a tape parallel to the cortex of the bone; the medial margin was less dense than that of the bone cortex, thus forming the so-called "double frame" shape; some of them were shaped like small bony spicules, some were undulatory; the remainder were saw-toothed. In severe cases, they appeared in the form of multiple disseminations with their bases attached to the cortex of the bone and their tips opposite the proximal joints in the form of "candle tears" (Fig. 3).

Figure 1

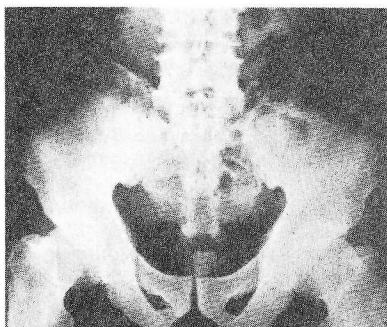
Male, aged 54, electrolysis worker.



Trabeculi significantly thickened, net-holes were increased in size, simulating coarse, thin gunny sack cloth.

Figure 2

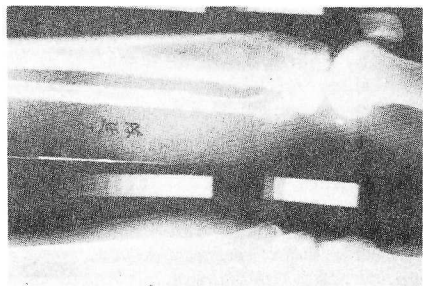
Male, aged 39, electrolysis worker.



Trabeculi slightly thickened, form structureless, simulating sand grains.

Figure 3

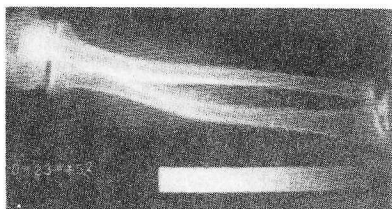
Male, aged 55, electrolysis worker.



Marked periosteal hyperplastic ossifications of upper middle section of femur, posterior margin.

Figure 4

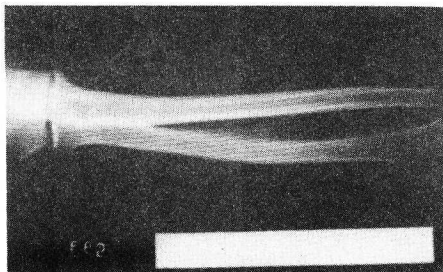
Male, aged 53, electrolysis worker.



Marked radial ridge increase in width, uneven density.

Figure 5

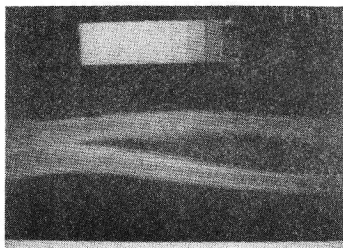
Male, aged 41  
phosphoric fertilizer worker.



The radial ridge increased in width, margin of the horny radial ridge simulating fish fins.

Figure 6

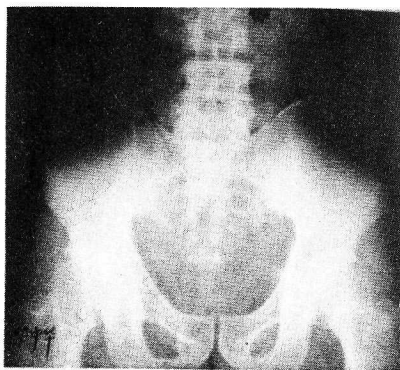
Male, aged 44,  
phosphoric fertilizer worker.



Marked ossification of periosteum of ulna.

Figure 7

Male, aged 39,  
phosphoric fertilizer worker.



Marked calcification of membrane of obturator foramen.

In the control group, although light and localized periosteum shadows were seen, most of them were localized on the lateral side of the upper section of the tibia. They presented themselves as hilly processes with smooth margins.

In the patient group, the periosteal hyperplastic ossification was situated on the upper middle part of the interosseous ridge of the radius; the radius ridge was increased in width; the measurement of its thickness averaged 8.38 (5.7-13.6) mm; thus the corresponding interosseous space was narrowed. Primarily, the margin of the hyperplastic interosseous membrane was irregular with increased density forming a hairy calcified zone after which it developed into unevenness of the density of the radial ridge in the form of cloudy flakes; at the medial margin of the corresponding cortex of the bone there was also the presence of irregular hyperplasia and, in severe cases, the external margin of the radial ridge presented horny processes simulating fish fins, all of which protruded toward the distal side (Fig. 4,5). The periosteum of the corresponding ulna, likewise, showed hyperplastic changes, but they were generally less extensive (Fig. 6).

In the control group, although the ridge of the radius became widened, the density was even and homogeneous with smooth margins; no marginal horny processes were seen. The thickness of the radial ridge measured 7.22 (5-9) mm, a statistically significant difference ( $p < 0.001$ ) from the patients.

Among patients, calcification or ossification of the membrane of the obturator foramen appeared mostly in the inner margin. It could also appear in the outer as well as the upper and lower margins, presenting horny or hairy processes with irregular margins (Fig. 7). Such changes occurred in 56 cases (56.6%) of this group, in 42.6% of which the lengths of the processes were over 0.5 cm.

In the control group, 6 cases (92%) had hyperplasia of the membrane of the obturator foramen; in only 1 case was the length over 0.5 cm. Most of them manifested the presence of bilaterally symmetrical processes with triangular shape and smooth margins. Regarding joint changes, only slight hyperplastic sclerosis of the bone was seen namely, the intercondylar protuberance became sharp, protrusion of the margin of the iliac fossa was lip-shaped. The articular surface of the symphysis pubis was slightly sclerotic but the articular space was not narrowed.

To summarize the above manifestations, industrial fluorosis might produce such changes as osteosclerosis, structural bone changes and periosteal hyperplasia, calcification and ossification. All these changes might occur in different cases with varied intensities. The 100 cases have been classified into the following three types: bone matrix, periosteal and mixed, based on whether the main changes were in bone substance, periosteal, or both. There were 25 (25%) cases in the bone matrix type, 33 (33%) in the periosteal, and 42 (42%) cases in the mixed type.

### Discussion

The skeleton and teeth are the main target organs most frequently affected by fluoride. More than 90% of fluoride in the human body is in the bones. Based on analysis of 100 cases presented in this paper, the main X-ray changes of the skeleton were increased bone density, thickening and increased concentration of the trabeculi, and structural disorder with the formation of network, all of which took place mainly in the body of the ilium and in the outer lower aspect of the iliosacral joint. With thickening of the trabeculi, their number was also decreased and the net squares were enlarged; some were compact and obscure; nevertheless the bone was denser. Better nutrition among fluoride workers, sufficient calcium intake as well as dose and duration of contact with fluoride may explain why osteoporosis and osteomalacia were rarely seen, an observation compatible with data reported by Dominok (4).

Why were such changes as periosteal hyperplastic calcification in our patients restricted chiefly to the upper middle section of the posterior margin of the tibia, radius and ulna? The periosteum and interosseous ridge of the radius are the site of attachment of many muscles of the forearm while the proximal end is finer in the radius, thus building mainly the wrist joint; and its distal end finer and proximal end of the ulna is thicker forming mainly the elbow joint. Such a specific structure pattern causes the line of stress to pass through the interosseous ridge of the radius. When the hand is straining,

the strength will be transmitted mainly through the wrist joint to the radius, whence it passes through the interosseous membrane of the radial ridge to be again transmitted to the ulna and elbow joint. In this way the interosseous membrane of the radius ridge serves as the conduction medium to the force. Similarly, the upper middle section of the posterior margin of the tibia serves as the site of attachment to the various muscles of the middle and deep layers causing thickening of the periosteum. With flexion movement of the leg, the periosteum and interosseous membrane are often brought into traction causing the above-mentioned sites to move and be subjected to the effect of strength, thus promoting local circulation and metabolism. Therefore, under the effect of fluoride, osteogenetic and osteoclastic activities are markedly activated. Hyperplasia of both inner and outer periosteum as well as thickening and roughening of the bony surface which result lead finally to ossification and formation of external osteophyte of various forms, thickening of the bone cortex and narrowing of the bone marrow cavity. These changes of crucial importance in the diagnosis of industrial fluorosis might be the main reasons for the appearance of early hyperplastic calcification and ossification in the above-mentioned sites.

Based on our criteria, industrial fluorosis has been classified into the following four stages according to skeletal roentgenological changes: Stage 0-I (including candidates reserved for observation), Stages I, II and III. This classification is chiefly based on the extent of osteosclerosis, bony structures, and periosteal hyperplastic calcification. The main manifestations in stage 0-I consist of bone density within the normal range, slight thickening of the trabeculi and slight changes in the periosteum; in stage I, bone density is slightly increased with bone structure presenting changes simulating the shape of "gauze" and slight periosteal calcification; in stage II, bone density is markedly increased; trabeculi are markedly thickened, presenting a form of "gunny cloth" appearance with marked and extensive periosteal changes; in stage III, all the above-mentioned changes are more marked and prominent. They were not seen in the 100 cases herewith presented.

The following points constitute the differences in our classification and that of Rohlm and Grinberg (3). According to these authors, periosteal changes are included in stages II and III. In such cases, however, we found periosteal hyperplastic calcification of different intensities. This change appeared early at a higher positive rate with identical sites of appearance, easily recognizable under X-ray and did not necessarily appear after the trabeculi changes. More and more studies of the preskeletal phase of this disease have been appearing in recent years (5). We believe that, due to progress in science and medical technology, it will be possible to find an early non-osteophytic diagnostic criterion.

#### Conclusion

Increase in density of bone, thickening and increased density of trabeculi, disorder in the arrangement of the structure and periosteal hyperplastic calcification and ossification are the main skeletal changes in industrial fluorosis, shown by X-ray. Calcification and ossification at the sites of muscular attachment, on the interosseous membrane of the middle section of the radius and the upper middle part of the posterior margin of the tibia, have important diagnostic value for industrial fluorosis.

### Acknowledgement

We are grateful for the cooperation of the Medial-South Area Group for Fluoride Protection for submitting patients and to Dr. Li Yumin (Hunan Metallurgical Industrial Hygiene Institute) for valuable assistance in reviewing this manuscript.

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### BINDING OF FLUORIDE ION TO EGG ALBUMIN STUDIED WITH THE FLUORIDE ION SELECTIVE ELECTRODE

by

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SUMMARY: The binding of fluoride ion to egg albumin at pH 5.75 was studied with a fluoride ion selective electrode. Significant binding was found with this new technique. When the data were plotted according to the Klotz equation, a value of 1600 was obtained for the product  $nK$ , where  $n$  is the number of binding sites and  $K$  is the binding constant which is the same for all sites.

KEY WORDS: Egg albumin; Fluoride ion; Fluoride ion selective electrode

### Introduction

It has been known for a long time that many enzymes are inhibited by fluoride ion. Usually the binding of the fluoride ion to the enzyme has been studied by kinetic methods. Only a limited number of enzymes have been

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studied in enough detail to obtain an inhibition constant (1). Even when an inhibition constant can be obtained from kinetic studies, it is questionable whether this gives the thermodynamic binding constant (2).

In a limited number of cases the binding of fluoride ion to enzymes has been studied by other methods. Only a few of these studies gave thermodynamic binding constants (3-7).

The binding of fluoride ion to proteins that are not enzymes is not as easy to study because of the shortage of analytical methods (8). Equilibrium dialysis and UV-visible spectrophotometry have been used to study binding of fluoride ion to human serum albumin and bovine serum albumin. Significant binding was found (9-11). The presence of calcium ion greatly increases the binding of fluoride ion to bovine serum albumin, apparently forming a ternary complex (12). A detailed study of the binding of fluoride ion to a denatured bovine serum albumin using an ion exchange potential method found  $K_1 = 3600$  for the first binding site,  $K_2 = 150$  for the next 8 sites and  $K_3 = 5$  for the next 18 sites. Binding increased as pH increased in the range 5.20 to 6.35 (13). Another detailed study of bovine serum albumin in 0.05 M acetate buffer used a gel filtration technique. Two classes of binding sites were found with  $K_1 = 280$  for 8 binding sites and  $K_2 = 10$  for the next 22 sites. Less binding of fluoride ion was found at pH 4.9 than at 3.9 where the detailed study was done (14).

No studies of the binding of fluoride ion to egg albumin have been reported.

The invention of the fluoride ion selection electrode has made it possible to measure fluoride ion activity conveniently down to  $10^{-6}$  M (15). Accurate measurements can now be made on volumes of a microliter or less (16). A number of people have used the fluoride ion selective electrode to measure fluoride ion concentration in biological fluids (17-19).

This study uses the fluoride ion selective electrode to measure the binding of fluoride ion to egg albumin. This new technique has the advantages that thermodynamic binding constants can be obtained, the protein does not need to be an enzyme or have a strong UV-visible absorbance, and small sample volumes can be used.

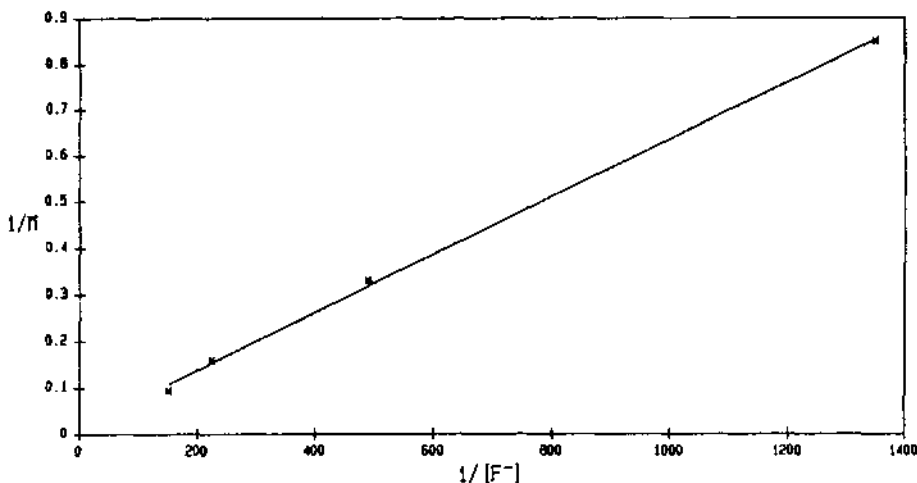
#### Materials and Methods

Sigma Chemical Co. egg albumin was dissolved in acetate buffers of pH 5.75 and an ionic strength of 0.10. The Orion fluoride ion selective electrode was calibrated with known concentrations of sodium fluoride solutions with ionic strength of 0.10 using a Leeds & Northrup expanded scale pH meter. The egg albumin was about 0.2 mM and the fluoride ion varied from 0.4 to 8 mM during the titration.

#### Results and Discussion

The data did not fit a model of one or two binding sites. Therefore, the results were plotted using the Klotz equation:  $1/\bar{n} = (1/nK) (1/[F^-]) + 1/n$  where  $\bar{n}$  is the number of fluoride ions bound to the protein at the particular molar concentration of fluoride ion,  $n$  is the maximum number of fluoride ions bound, and  $K$  is the binding constant which is the same for all  $n$  sites (20).

Figure 1.  
Klotz Plot for Titration of Egg Albumin with Fluoride Ion.



Klotz, I.M., Walker, F.M. and Pivan, R.B.: The Binding of Organic Ions by Proteins, *J. Am. Chem. Soc.*, 68:1486-1490, 1946.

The slope in Fig. 1 is 0.00063 which gives 1600 for  $nK$ . The intercept at  $1/[F^-]$  equals zero is  $1/n$ . While the data in Fig. 1 give a reliable value for  $nK$  and indicate significant binding of fluoride ion to the protein, the value of the intercept is sufficiently uncertain that the individual values of  $n$  and  $K$  must be considered uncertain.

This study has shown that the fluoride ion selective electrode can be used to study the binding of fluoride ion to proteins, even if they are not enzymes.

#### Acknowledgement

The authors thank the undergraduate research students who did preliminary work and Prof. F.D. Williams and Prof. T.L. Warrington for helpful discussions.

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DURATION AND COURSE OF PREGNANCY OF WOMEN  
LIVING NEAR THE POLICE CHEMICAL PLANT -  
A CLINICAL STUDY

by

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(Abstracted from *Medycyna Pracy*, 36:51-54, 1985)

In an investigation of potential detrimental effects of industrial fluorine compounds in the environment, the pregnancies of 312 women residing near a fluoride-polluting plant in Police, Poland, were compared during 1980-81 with the pregnancies of 137 women living in the non-polluted community of Dabie-Szczecin. The two groups of women were similar with respect to the distribution of their occupations and types of delivery of their babies. However, the proportion of women over age 30 was greater in Police (81/312=26%) than in Dabie-Szczecin (19/137=13.9%).

Although the incidence of miscarriages (9.6% vs. 11.7%) and late premature births (6.1% vs. 4.4%) was not significantly different between Police and Dabie-Szczecin, the difference in the number of early premature (immature) deliveries (14=4.5% vs. 1=0.7%) was significant at the  $0.1 > p > 0.05$  level ( $\chi^2 = 3.08$ ). The women of Police also had 4 stillbirths (1.3%) and 1 defective birth (0.3%) compared to none in either category in Dabie-Szczecin.

The authors suggest that a larger data base is desirable to determine and verify characteristic differences.

Abstracted by A.W. Burgstahler

KEY WORDS: Immature births; Pregnancy; Police, Poland

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THE USE AND POTENTIAL MISUSE OF FLUORIDE

by

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and Howard R. Creamer, Ph.D.

(Abstracted from *Journal of Oregon Dental Association*, 54:17-18, 1985)

Subgingival irrigation with fluoride solution to control periodontal disease represents a significant change from past use and presents a number of potential hazards not encountered when these solutions were used solely as oral topical agents.

Irrigation with relatively concentrated fluoride solutions assures that high doses of this anion come into contact with living unprotected cells. Concentrations of fluoride considerably less than those used in oral rinses, gels, or tooth-pastes, are cytotoxic to a variety of cells. Of particular importance are the effects of fluoride on leukocytes and, more specifically, on the principal host-defense cell, the neutrophil or PMN. The number of neutrophils in crevicular fluid and the severity of periodontal disease are closely correlated. Neutrophils mainly protect a person against his own periodontal pathogens.

Among questions which the authors asked themselves were: Can fluoride alter neutrophil function and thereby compromise host defense? Does fluoride alter directed cellular migration chemotaxis? Does fluoride alter neutrophil degranulation?

Neutrophils show no evidence of loss of viability or membrane integrity when stored up to 2 hours in solutions containing up to 40 mM fluoride (759 ppm  $F^-$  or 0.31%  $SnF_2$ ). They can be maintained overnight in 3 mM fluoride without evidence of cellular damage. Whereas PMN chemotaxis is not inhibited when the fluoride concentration is 3 mM or less, at 10 mM fluoride it is inhibited. Preincubation of neutrophils for 2 hours in 0.5 mM fluoride inhibits chemotactic tripeptide induced  $O_2$  generation by approximately 25%; similar incubation in 5.0 mM fluoride completely abolishes production of this toxic oxygen radical.

Degranulation — the process whereby hydrolytic and peroxidative enzymes contained in membrane-bound cytoplasmic granules are emptied into phagocytic vacuoles or externally — is inhibited by fluoride in a dose-dependent fashion, starting at 1 mM concentration, with complete inhibition at 10 mM anion. Since PMN functions can be inhibited by fluoride, and since PMN are our first line of defense against pathogens in periodontal pockets, it is not difficult to imagine that use of fluoride in this manner could compromise our local defenses, permitting pathogens to enter the body, possibly causing systemic disease.

Because of the attendant unknowns, it would seem prudent to avoid repeated application of fluoride subgingivally until such a time as its safety and efficacy have been established.

**KEY WORDS:** Chemotaxis; Neutrophil degranulation; Subgingival fluoride; Super-oxide generation

**REPRINTS:** Department of Biochemistry, OHSU School of Dentistry, Portland, Oregon

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## FLUORIDE IN TEA

A Preliminary Study to Estimate the Quantity of  
Fluoride Intake through Tea Drinking

by

Ibnu Effendi and Djajadi Wibowo

(Abstracted from *Odonto-Stomatologie Tropicale* 7:163-167, 1984)

To estimate fluoride ion concentrations of 49 different brands of tea, a beverage widely consumed in Indonesia, various test infusions were made using standard procedures with an Orion pH meter (407 A) and fluoride electrodes (type 96-09). Calibrations were conducted using distilled water.

Infusion time using 2, 4, 6, 8, and 10 gms of tea was, in some, for 6 minutes; in others, up to 24 hours. The fluoride ion concentration in the water used for all tests was 0.02 ppm. Fluoride concentrations from different samples of tea varied widely suggesting that samples from the same package could contain different proportions of "young" and "old" tea leaves, the latter being higher in fluoride.

Traditionally, tea is prepared in Indonesia by using 2 gm of tea leaves infused in 100 ml of boiling water for 6 minutes which produces a mean fluoride ion concentration of 1.7 ppm. Increasing the amount of tea used from 2 gm to 10 gm, or increasing the infusion time to 4 or more hours, produces an apparent maximal concentration of 9.0 ppm. Use of glass or plastic containers for making the tea did not make any difference but the quality of the tea may be a significant factor. "Low quality cheap" tea is likely to contain more "old leaves." The latter which contain more fluoride, are a potential source of fluoride intake.

Lack of quality control in preparation of "cheap" tea makes it difficult to predict the fluoride-ion concentration of a single cup of tea but, overall, "cheap" tea is likely to supply higher amounts of fluoride than "expensive" tea.

KEY WORDS: Tea, F<sup>-</sup> in; Tea infusions

REPRINTS: Directorate Dental Health, Ministry of Health, Indonesia.

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## EFFECT OF FLUORIDE IONS ON CARIOGENIC BACTERIA

by

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Warsaw, Poland(Abstracted from Czas. Stomat., 37:633-638, 1984)  
[Article in Polish]

This short survey reviews the influence of the fluoride ion on the dental surface bacteria [in Polish: Bacterial Blona Nazębna, abbreviated here as "bbn"]. The fluoride ion concentration in this "bbn" was found to depend directly on the concentration of the fluoride ion in water, used for daily drinking, according to studies in three African communities.

In "bbn" only 5 to 7% of the total fluoride exists in the active, ionized form, the rest being coupled with proteins and bacteria; 90% of the fluorine in cytoplasm exists in ionic form. A decrease of pH in the "bbn" leads to a very significant increase in the activity of the fluoride ion, and to an immediate increase in fluorine concentration in bacterial cells.

The presence of fluoride ion leads to a decrease in the rate of bacterial growth, especially of Streptococci mutans which are present in "bbn." This decrease in growth of bacteria is probably due to the alteration of the exchange rates of anions and cations - which are important for bacterial survival - as well as to the decrease in the rate of sugar metabolism, especially the process of glycolysis. It is known that lowering of pH is significantly smaller in bacteria when a mixture of sugar with fluoride is present in comparison with bacteria without fluoride, mostly due to the decrease in activity of the enzyme called "enolase."

By decreasing bacterial growth, the presence of fluoride in the "bbn" might lead to prevention of cariogenic decay of teeth. It is also established that, due to the presence of fluoride, "bbn" is not formed on the surface of teeth, thereby contributing to prevention of cariogenic activity of bacteria. However, usage of fluorine compounds on a regular basis may lead to insensitivity of the bacteria toward fluoride. Some bacteria, which are no longer sensitive to the presence of fluoride, exhibited much lower cariogenic activity than similar bacteria which were still sensitive to fluoride.

Abstracted by Bogdan Matuszewski, West Point, Pa.

KEY WORDS: Bacterial metabolism; Cariogenic bacteria; Fluoride ion.

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THE FLUORIDE CONTENT IN CULTIVATED SOIL  
UNDER DIFFERENT GEOGRAPHICAL CONDITIONS IN CHINA  
AND ITS RELATION TO ENDEMIC FLUOROSIS

by

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(Abstracted from Geographical Research, 4:30, 1985)

The geographical distribution of fluoride in cultivated soil and the relationship between its distribution and endemic fluorosis were studied. Total fluoride in cultivated soil is mainly contributed by bed-rock and the original material which formed the cultivated soil. Therefore total fluoride in cultivated soil is high in those areas where rock and minerals are rich in fluoride.

The law of zonality is obvious in the geographical distribution of dissoluble water-borne fluoride in cultivated soil. It is low in a torrid and semi-torrid zone, mostly less than 1 ppm, high in an arid and semi-arid region of temperate and semi-temperate zones, mostly more than 2 ppm. Total and dissoluble fluoride in cultivated soil contribute little to the fluoride content in crops, but dissoluble fluoride in cultivated soil and fluoride in shallow ground water are closely related. Thus through shallow ground water used for drinking, dissoluble fluoride in cultivated soil affects the human body and results in fluorosis.

KEY WORDS: Bed-rock; China; Crops; Cultivated soil fluoride; Dissoluble fluoride; Endemic fluorosis.

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THE FLUORIDE CONTENT OF SOME TEAS AVAILABLE IN AUSTRALIA

by

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(Abstracted from Australian Dent. J. 30:25-28, 1985)

The fluoride (F) content of teas commercially available in Brisbane, Queensland, was measured. A variety of blends in a wide price range, and at least one Australian grown tea were included for comparison.

Estimates of the F content of tea infusions made from a variety of brands of tea available in Queensland, between 1977 and 1983, based on different infusion times ranged from  $1.75 \pm 0.16$  to  $10.21 \pm 2.08$  ppm after one minute, from  $3.72 \pm 0.29$  to  $11.02 \pm 0.24$  ppm after 5 minutes; from  $4.22 \pm 0.43$  to  $12.65 \pm 1.25$  ppm after 10 minutes and  $3.93 \pm 0.53$  to  $13.51 \pm 1.59$  ppm after 30 minutes.



This study confirms the results of previous investigations in that the great majority of fluoride that will diffuse into a brew will have been released after five minutes. In the teas examined, the level of fluoride found in the 5 min. infusions ranged from  $4 \times 10^{-3}$  to  $11 \times 10^{-3}$  M F (Mean = 1.34 ppm.). Variations in teas of one brand will produce greater changes in fluoride levels than will be affected by the shift in a decimal place with sophisticated methodology.

Regarding tea intake by children, according to one survey, about one half of the six to eight-year-old children from three different places in Queensland drink tea. Their mean daily intake was between one and two cups. Some of these children, however, drank up to five cups per day, which could contribute between 0.8 and 1.6 mg of fluoride intake per day per child.

Since, in some cases, tea could be making significant contributions to the daily fluoride intake, dentists should be aware of the levels of fluoride in different brands of tea on the Australian market and of their potential contribution, particularly when fluoride supplements, for example tablets, are recommended

KEY WORDS: Australia, Queensland; Tea, fluoride in.

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EFFECT OF SODIUM FLUORIDE AND FLUOROACETAMIDE ON  
SISTER CHROMATID EXCHANGES AND CHROMOSOMAL ABERRATIONS  
IN CULTURED RED MUNTJAC (MUNTJACUS MUNTJAK) CELLS

by

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(Abstracted from Huanjian Kexue Xuebao 3:94-100, 1983)  
[in Chinese]

The mutagenicity of fluoride has been confirmed once more. Chromosomal aberrations, sister chromatid exchanges (SCEs) and cell cycle kinetics in cultured cells of Red Muntjac in vitro were used as indices in studying the mutagenic effects of NaF and fluoroacetamid (640-19-7). Both NaF and fluoroacetamid can cause chromosomal breakage, increase SCE frequency and lag cell cycle.

KEY WORDS: Chromosome aberrations; Fluoride effects on chromosomes; Fluoroacetamid and chromosomes; Red Muntjac cell cultures; Sister chromatid exchanges

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FLUORIDE

## THE EFFECTS OF FLUORIDE ON THE GASTRIC MUCOSA OF THE RAT

by

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(Abstracted from Journal of Oral Pathology 13:535-545, 1984)

To determine (1) the minimum fluoride concentration required to produce histologically evident gastric damage, (2) the minimum time required to produce significant mucosal damage, and (3) whether fluoride increased mucosal permeability to both small and large molecules, solutions of 0, 1, or 10 mM NaF in 0.1 N HCl were placed in rat stomachs in vivo for up to one hour. Histologic and SEM examinations revealed dose- and time-dependent damage to the surface mucous cells. The 10 mM, but not the 1 mM, NaF solution increased gastric mucosal permeability to small, but not to large molecules.

Topical fluoride gels are applied in plastic trays to an entire arch or by cotton-tipped applicators to quadrants of the mouth. Eventually swallowed, they make direct contact with gastric mucosa, a tissue capable of absorbing many solutes including fluoride. These agents are known to increase gastric mucosal permeability to small molecules. As little as 5 mM NaF caused marked inhibition of histamine stimulated acid secretion in the cat. Apparently, somehow, fluoride damages a protective "barrier" in the stomach. Since amounts of fluoride, retained on oral soft and hard tissues by patients, may range from 11-35 mg., fluoride itself probably contributes to gastric distress.

Histologically, use of 10 mM sodium fluoride in 0.1 N HCl consistently produced changes in the gastric mucosa. On gross inspection, the mucosa had a mildly hyperemic appearance. Microscopically, the entire surface layer of mucous cells was either missing or severely disrupted after 1 hr. Similar, but less dramatic, results were obtained at 10 and 30 min. Scanning electron microscopy confirmed the disruption of the surface mucous cell layer. Physiologically there was a highly significant increase in stomach volume after 10 mM NaF was placed in the stomach.

Stomach contents received at 10 and 30 min., often contained as much sodium, potassium and albumin as 60 min. samples. Following fluoride ingestion, the highest HF concentration in the body is found in the stomach. Ten millimolar NaF caused appreciable mucosal damage in as little as 10 min. After one hour, almost complete desquamation of all surface cells plus some disruption of gastric gland architecture which occurred tended to cause mucosal hyperemia in addition to disruption of gastric glands.

Although research data obtained in this report were from anesthetized rats, the results warrant further investigation.

KEY WORDS: Gastric mucosa; Rat

TOOTH WEAR, SOLUBILITY AND FLUORIDE CONCENTRATION OF  
MOLAR-TOOTH SURFACES IN RATS MAINTAINED ON SIMULTANEOUS OR  
SEPARATE INTAKE OF FOOD AND FLUORIDATED DRINKING WATER

by

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(Abstracted from Archives of Oral Biology 30:167-169, 1985)

The aim of this study was to examine tooth wear in rats maintained on simultaneous or separate intake of food and water, in the absence or presence of appreciable levels of  $F^-$ . Eighty male white rats of the Hebrew University strain Sabra, 20 days old and weighing about 72 g, were fed a standard animal-house diet containing up to 5 ppm F and drinking water containing up to 0.3 ppm  $F^-$  ad libitum prior to the start of the experiment. The rats were divided into four groups of 20 rats each. Group 1 received the standard diet in pellet form and deionized water ad libitum for 3 hr daily. Group 2 received daily the same diet for 3 hr followed by the deionized drinking water for 3 hr in order to provide further insight into the relationship between the physical character of the diet and tooth integrity. Groups 3 and 4 were subjected to the same mode of feeding as groups 1 and 2 respectively except that the drinking water contained 25 ppm  $F^-$ . The upper right maxillary jaw segments of each group of rats were chosen for evaluation of tooth wear of the first molars. The remaining three quadrants were used for examination of solubility and  $F^-$  concentration of molar occlusal surfaces. Entire crowns were immersed in order to secure enough enamel and cusp dentine for solubility and F examinations.

Rats given, daily, food and water simultaneously for 3 hr only (group 1 and 3) gained more weight than rats provided with food for 3 hr followed by drinking water for 3 hr. Wear of the first maxillary molars, solubility and  $F^-$  concentration of the molar surfaces, respectively, were highly significantly increased ( $p < 0.001$ ) in the rats provided with fluoridated drinking water. Separating the intake of food and water failed to affect wear significantly, whereas solubility of mandibular and maxillary molars and  $F^-$  concentration of mandibular molar surfaces respectively increased significantly. As the  $F^-$  concentration in the molar surfaces increased, so did solubility of the molar surfaces, and vice versa. Wear of rat molar tooth surfaces, by removing the outer part of enamel, exposes areas of underlying dentine. Occasionally, wear may have been severe enough to expose the more soluble underlying F-rich dentine in groups 2 and 4 provided separately with food and drinking water. Wear of molar surfaces was significantly greater in groups whose drinking water contained 25 ppm.

Although our findings do not explain the reason for increased wear in the fluoridated drinking water groups, they confirm the finding that the rate of acid dissolution of worn surfaces is greater than that of intact enamel.

KEY WORDS: Molar surface fluoride; Tooth wear and  $F^-$  in rats

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FLUORIDE

SHORT-TERM EFFECTS OF FLUORIDE ON BIOSYNTHESIS OF ENAMEL  
MATRIX PROTEINS AND DENTINE COLLAGENS AND ON MINERALIZATION  
DURING HAMSTER TOOTH-GERM DEVELOPMENT IN ORGAN CULTURE

by

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(Abstracted from Archives of Oral Biology 30:181-191, 1985)

The effect of various concentrations of fluoride ( $F^-$ ) on cell proliferation, matrix formation and mineralization was examined in hamster molar tooth germs in premineralizing and mineralizing stages. The exposure lasted 16 h (mineralizing stages) and 24 h (premineralizing stages) and the  $F^-$  levels ranged from 2.63  $\mu M$  to 2.63 mM; [ $^3H$ ]-thymidine, [ $^3H$ ]-proline,  $^{45}Ca$  and  $^{32}PO_4$  were used as markers for cell proliferation, matrix formation and mineralization, respectively. The proline-labelled amelogenins were isolated by sequential extraction with water and formic acid and their nature examined by SDS-urea-polyacrylamide electrophoresis. Digestion by collagenase was used to assess the amount of proline incorporated into collagens.  $F^-$  in concentrations up to 1.31 mM inhibited neither biosynthesis or DNA and amelogenins, nor synthesis of collagens and their hydroxylation. Amelogenins extracted from  $F^-$  induced, non-mineralizing enamel matrix had the same electrophoretic mobility and the same degree of phosphorylation as amelogenins from normal, mineralizing enamel. However,  $F^-$  increased the uptake of  $^{45}Ca$  and TCA-soluble  $^{32}P$  dose-dependently, starting with 52  $\mu M$ . Thus, interference with secretion of enamel matrix by  $F^-$  takes place at much lower concentrations than required to inhibit biosynthesis of enamel matrix.

KEY WORDS: Cell formation, mineralization; F effect; Hamster; Molar teeth.

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INFLUENCE OF SOCIAL CLASS AND FLUORIDATION  
ON CHILD DENTAL HEALTH

by

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(Abstracted from Community Dent. Oral Epidemiol., 13:37-41, 1985)

Analysis of official statistics for Auckland, New Zealand, showed a significant correlation between social class and child dental health. Treatment levels which have continued to decline in both fluoridated and non-fluoridated areas are more related to social class factors than to the presence or absence of fluoridation. All children in unfluoridated areas, but only selected children in

fluoridated areas, had received regular topical treatment but use of toothpaste and oral hygiene had been encouraged in both areas.

In fluoridated Auckland, 15 years after initiation of fluoridation, dental treatment requirements of children and their social class were still significantly correlated. This applies to all teeth, including permanent teeth, despite the fact that total treatment requirements have dramatically declined by approximately the same percentages in most social rank areas.

A steady but less steep decline has also occurred in the unfluoridated part of Auckland, with permanent teeth in 1981 requiring much less treatment than in the same social class fluoridated area. Other social factors, such as "ethnicity," which includes racial differences, appear to be the same in both areas.

The situation is almost unique in New Zealand: a School Dental Service has provided, for the studied period, regular 6-monthly treatment of primary and permanent teeth for 98% of schoolchildren to a uniform standard, closely checked and supervised. At the time the information for this study was gathered, diagnostic criteria within the district under study were uniform. The numbers of children involved in this study are larger than those of many DMFT sample studies. In fact, insofar as 98% of schoolchildren attend these clinics, they are child populations, not samples, of the particular areas.

According to Table 4, here reproduced, caries-free percentages for 12-13 year-old children — both average income and from all school dental clinics in the three Health Districts of greater Auckland, fluoridated and unfluoridated — did not differ appreciably from each other.

Table 4

Fluoridation status	Health District	Average income (1981)	No. of Caries-free children		%
			No.	%	
Fluoridated	Auckland (above average)	\$8299	1414	230	16.3
	North	\$7545	4442	462	10.4
	South	\$7107	4627	459	9.9
	Auckland (below average)	\$6272	2297	194	8.4
	Greater Auckland	\$7272	12780	1345	10.5
Unfluoridated	South	\$7449	588	71	12.1
	North	\$6504	837	89	10.6
	Auckland	\$5625	179	16	8.9
	Greater Auckland	\$6584	1604	176	11.0

The results of this study suggest that child dental health is no better in fluoridated areas of the same socioeconomic level. It could be concluded, therefore, that topical fluorides (if they are the cause of the decline in dental decay) are just as effective as water fluoridation, if not more so, for all socioeconomic groups.

This author suspects that nutrition is an important factor for the relationship between social class and dental health, influenced by minerals and trace elements in soils and water. It is clear, from this study that, in Auckland, New

Zealand, levels of child dental health are related more to socioeconomic factors than to water fluoridation.

KEY WORDS: Child dental health; Epidemiology, oral; Fluoridation; Social class.

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## FATAL SYSTEMIC FLUOROSIS DUE TO HYDROFLUORIC ACID BURNS

by

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(Abstracted from *Annals of Emergency Medicine* 14:149-153, 1985)

A 23-year old relatively healthy white male, having sustained burns on his lower extremities from a 70% solution of hydrofluoric acid, died as a result of complications following systemic dissemination of fluoride ion. The second and third degree burns over both anterior thighs, which involved 9 to 10% of his body surface, resulted in hypocalcemia and intractable ventricular arrhythmias. Death occurred 17 hours after initial exposure in spite of heroic measures performed at Boston Massachusetts General Hospital. This death constitutes the second documented case of hypocalcemia from hydrofluoric acid burns and the first case to document myocardial injury and systemic fluorosis from a skin burn.

At the time of this accident, the patient was working in a well-ventilated room. A plastic face mask which he was wearing precluded any significant exposure through his respiratory system. Reportedly, immediately following the patient's contact with the hydrofluoric acid, his fellow workers removed his clothing and washed him in a shower. On admission to the hospital, his vital signs were stable and bronchospasm cleared with medication. However, approximately four and a half hours after the toxic exposure, he became hypotensive and cyanotic. He developed ventricular fibrillation, which responded to electric defibrillation and lidocaine. Due to major supportive measures, he was kept alive for approximately 12 more hours. Although he received large amounts of injectable calcium, his serum calcium ranged between 3.3 mg/100ml and 4.5 mg/100ml (normal 8.5 to 10.5 mg/100ml) apparently due to formation of insoluble calcium fluoride salts in the body. Another important laboratory finding was a low serum magnesium.†

Postmortem examination revealed pathological heart changes not previously described in patients suffering from systemic fluorosis due to ingestion, inhala-

† Low serum magnesium had been associated with intractable cardiac arrhythmias. Whether or not it would have made a difference had magnesium been administered with the calcium is difficult to say.

tion or burns with fluoride compounds. Fluoride levels in body tissues and fluid were extremely high; the serum level was 4.17 mcg/ml (normal 0.01 to 0.04 mcg/ml). At the burn site the fluoride level was 303 mcg/g dry tissue. The heart and lung contained approximately 15 mcg/g dry tissue, showing that the fluoride concentration was higher in soft tissues than in serum because of fluoride's binding to organic compounds in tissues.

This case history serves to emphasize the extremely severe potential toxicity of fluoride.

Abstracted by M.B. Schachter

KEY WORDS: Hydrofluoric acid burns; Fluorosis, fatal.

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#### THE EFFECTS OF CHRONIC HIGH FLUORIDE LEVELS ON FORMING ENAMEL IN THE RAT

by

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(Abstracted from Archives of Oral Biology 29:675-679, 1984)

To investigate the effects of chronic high levels of fluoride at secretory, maturation and postmaturation, stages of enamel formation, Sprague-Dawley rats (60-65 g) were randomly divided into two groups. The control group was provided with deionized water; the experimental group received either 75, 100 or 150 ppm fluoride in drinking water, ad libitum. Six control and seven experimental animals were used in each of four studies. After five weeks, fluoride, phosphorus and protein content of enamel in control and experimental animals were compared at three stages of enamel development.

The mineral content was reduced in pigmented enamel from animals given 75 ppm or more fluoride in drinking water. In all stages of fluorosed enamel development, fluoride content was elevated. At 75 ppm, the lowest fluoride level, a larger proline content was found in proteins of maturing, fluorosed enamel but protein content did not increase. In animals given 100 ppm fluoride in drinking water, proline content of protein was greater than in controls. Thus with increasing levels of fluoride in drinking water, an initial delay in loss of amelogenin proteins was followed by a decreased removal of total protein from enamel. Fluoride interfered with the normal post-secretory, pre-eruptive development of enamel.

Enamel obtained at three stages of development namely secretory, maturing and pigmented, was removed with a scalpel except the pigmented enamel from controls and those animals receiving 75 ppm fluoride in drinking water. Animals given 150 ppm fluoride in drinking water gained significantly less weight than

controls. Serum-fluoride concentration was directly related to concentration of fluoride in drinking water. In animals given chronic high levels of fluoride, this pigmented layer was progressively more disturbed. The erupted enamel of the experimental rats receiving 75 ppm fluoride in drinking water showed a series of transverse striations of alternating brown and white bands. With 100 ppm water-fluoride the bands became wider and less defined and the enamel was prone to fracture. Weight of secretory enamel that could be dissected from incisors of rats given 100 ppm fluoride was significantly less ( $P < 0.001$ ) than the weight of enamel dissected from controls.

Fluoride in the enamel at all stages was significantly higher in experimental animals than in their respective controls. Whereas density in control enamel increased with each successive stage of enamel development, in fluorosed enamel, increase in density between maturing and pigmented enamel was not significant. In animals given 100 ppm fluoride in drinking water, total protein content was greater in both maturing and pigmented enamel than in control enamel. Thus compositional changes in fluorotic enamel was related to both fluoride concentration in drinking water and serum-fluoride. However, protein content was greater in fluorosed pigmented enamel. The results also indicated that fluoride interfered with mineral deposition. Although phosphorus was lower in fluorosed, pigmented enamel, it was not reduced in maturing, fluorosed enamel.

Enamel maturation has been characterized by progressive deposition of mineral and withdrawal of organic matrix and water. It is evident that high chronic levels of fluoride interfere with this process.

**KEY WORDS:** Enamel formation in rats; Dental fluorosis in rats

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WEEKLY RINSING WITH A FLUORIDE MOUTHRINSE  
IN AN UNFLUORIDATED COMMUNITY:  
RESULTS AFTER SEVEN YEARS

by

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(Abstracted from J. of Public Health Dent., 45:95-100, 1985)

A decline in prevalence of caries in primary teeth among kindergartners prior to rinsing suggests that factors in addition to the mouthrinse program may have contributed to the caries decline. The data suggests that the greatest benefits of fluoride-rinsing do not go to those children who were most diligent in carrying out the prescribed procedures. Although frequency of rinsing was approximately the same in all grades, that the older children clearly



derived the greatest net declines in caries prevalence, tends to substantiate conclusions of other investigations namely those with the greatest propensity for disease will benefit most from a preventive program.

Of the three cohorts that rinsed for seven years — the current seventh-, eighth-, and ninth-grade children — eighth graders, who rinsed in grades one through seven, and ninth graders, who rinsed in grades two through eight, derived a much greater benefit from their seven years of rinsing than did seventh graders who rinsed from kindergarten through sixth grade. Thus the greatest benefits from the mouthrinsing procedure seemed to occur during the seventh and eighth grades. Because a large number of teeth were recently erupted, the number of new surfaces at risk has substantially increased.

A 43% decline in dfs prevalence in primary teeth of kindergartners over the seven years of the study could not be attributed to the rinsing program but to the secular decline in caries prevalence being experienced widely throughout the United States and other developed nations of the world.

The data suggest that seven years of rinsing in a supervised situation produces a benefit which approaches that of lifetime residence in a fluoridated community.

**KEYWORDS:** Sodium fluoride; Mouthrinsing; Dental caries; Permanent teeth; Primary teeth; Unfluoridated community

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#### TOXICITY OF FLUORIDE-CONTAINING DENTAL PREPARATIONS: A REVIEW

by

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(Abstracted from Sci. Total Environ., 43:41-61, 1985)

The potential for chronic fluoride ( $F^-$ ) toxicity is evaluated. The optimal  $F^-$  intake is considered to be 0.05-0.07 mg/kg body weight daily. As little as 0.08 mg/kg daily may be capable of inducing chronic poisoning. A 10-kg child living in a fluoridated area could be expected to ingest 0.3 mg  $F^-$  daily from water and at least 0.4 mg from food. If fluoridated toothpaste and mouthwash are used, an additional 0.7 mg  $F^-$  could be retained. 1 mg  $F^-$  taken at one time as in a tablet or drops, can acutely raise plasma  $F^-$  in a 10-kg child to levels known to inhibit a variety of enzymes. Infant formula made with fluoridated water may contain 100 times as much  $F^-$  as human milk. Excessive intake of  $F^-$  is suspected of causing birth defects, mutations, and cancer.  $F^-$

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toxicity in cattle results in recession of alveolar bone and gingiva. The uncontrolled use of fluoride-containing products could pose a hazard, especially where water is fluoridated.

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KEY WORDS:  $F^-$  toxicity; Fluoride preparations and supplements; Total  $F^-$  intake

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METABOLISM OF THE INHALED ANAESTHETICS:  
IMPLICATIONS OF ENZYME INDUCTION

by

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(Abstracted from British Journal of Anesthesia, 56:275-415, 1984)

Treatment with drugs and exposure to many environmental chemicals results in enzyme induction. However, the clinical significance of increased (or altered) metabolism of the inhaled anesthetics appears to be trivial. Enzyme induction does not affect the conduct of inhalation anesthesia. Thus, only delayed organ toxicity is at issue. Since methoxyflurane has fallen into disuse, nephrotoxicity secondary to its administration is no longer a problem. Nephrotoxicity as a result of enhanced defluorination of enflurane or isoflurane is also unlikely: enflurane biotransformation, in most circumstances, is uninducible; isoflurane is metabolized to such a small extent that any increase in its metabolism would be clinically inconsequential. Whether induction of halothane biotransformation and the production of reactive intermediates may lead to hepatotoxicity is not yet settled. It is quite clear that induction, in the presence of hypoxia, leads to hepatic necrosis in rats. However, a similar relationship has not been established in surgical patients.

KEY WORDS: Anaesthetics; Enflurane; Hepatotoxicity; Isoflurane.

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Author's Abstract

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