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FLUORIDE: A TOXIC SUBSTANCE

Over 40 years ago, I started research on fluoride, predominantly its effect on the metabolism of organisms. The research has centered on enzymatic reactions in higher plants and on bones in animals.

Fluoride is inhibitory both *in vivo* and *in vitro* to enzymes in glycolysis, respiration, photosynthesis and in other pathways in higher plants. Among these enzymes or systems affected are glucose 6-phosphate dehydrogenase, enolase, succinic dehydrogenase, catalase, cytochrome oxidase, ATPase activity and transport, ATPase activity of the plasma membrane, the chloroplast coupling factor, and others. Some enzymes like enolase and plasma membrane ATPase are inhibited by fluoride at mMolar or lower concentrations. These are physiological levels as shown by the accumulation of the HF form of fluoride across membranes of organelles at different pH values.

Fluoride has been used effectively in humans in therapy, but there are nearly always toxic side effects due to the effect of fluoride on the metabolism. Even the use of fluoride in toothpaste must now be accompanied by a poison warning as directed by the US Food and Drug Administration. This warning is necessary because of laws that went into effect April, 1997. In this issue we report on a recent newspaper article on the subject by Don Oldenburg, a staff writer of the *Washington Post*.

The International Society for Fluoride Research has presented research for the past 30 years on fluoride toxicity that has too often been ignored by medical and dental groups.

Gene W Miller

XXIInd CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

The following dates and venue are at present planned

August 24-27, 1998

Lakeway Inn, Bellingham, Washington, USA

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FLUORIDE IN CALIFORNIA WINES AND RAISINS

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SUMMARY: Fluoride ion-selective electrode analyses of nineteen California wines revealed fluoride concentrations ranging from 0.23 to 2.80 ppm (mean 1.02 ppm, with seven samples above the international limit of 1 ppm). The water-extractable F content of five brands of California raisins varied from 0.83 to 5.20 ppm (mean 2.71 ppm). Elevated F levels in these wines and raisins appear to result from pesticide use of cryolite (Na_3AlF_6) in the vineyards. Potential toxic effects of F in conjunction with aluminum and sulfites in wine are discussed.

Key words: Aluminum; Analysis; California; Cryolite; Fluoride; Raisins; Sulfites; Wines.

INTRODUCTION

In a recent large-scale study, the fluoride (F) content of juices, juice-flavored drinks, and distilled-water-reconstituted frozen concentrates sold in the United States was found to range from 0.02 to 2.80 ppm (mg/L), with white grape juice having the highest mean F concentration of 1.45 ppm.¹ This high F level in white grape juice, which, along with that in other kinds of juices, may be contributing to increased dental fluorosis,¹ evidently results from the use of F-containing pesticides, since grape juice made from only the interior portion of grapes is reported to contain "no detectable amounts of fluoride."²

Because the legally-permitted use of natural cryolite (Na_3AlF_6) in vineyards to control leaf-eating insects is apparently fairly common, especially in the Central Valley of California,³ it would not be surprising to find that "traces of fluoride can sometimes remain in the finished wine products when this pesticide is used."³ By international agreement the F content of wine should not exceed 1 ppm,³ and although F levels in certain European and South American wines have been found to be well below this limit,⁴⁻⁶ the F content of present-day US California wines is not on record, at least in publications included in *Chemical Abstracts*. We therefore undertook such determinations, and we now report F analyses of nineteen California wines along with the amount of water-extractable F in five brands of California raisins.

MATERIALS AND METHODS

With the exception of an earlier package of Rainbow raisins, the various samples of California wines and raisins were purchased during January-April 1997.

Fluoride determinations were performed in duplicate or triplicate by use of an Orion Model 94-09 fluoride ion-selective electrode and a Corning Catalog No. 476350 calomel reference electrode connected to an Orion Model 407A Ion Analyzer. Calibrations were made with 10.0, 1.00, and 0.100 ppm F solutions prepared from reagent-grade sodium fluoride and distilled deionized water.

For each determination, a 5.0-mL portion of the sample solution was mixed with 5.0 mL of TISAB with CDTA (Total Ionic Strength Adjustment Buffer with 1,2-cyclohexylenedinitrilotetraacetic acid to liberate F from complexes of poly-

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valent cations). After the electrodes were immersed in the mixture of sample and buffer, constant readings were usually obtained in 5 to 8 min.

Wine samples were analyzed directly without prior treatment. For determination of the water-extractable F in raisins, 20.0-gram samples of each brand were suspended in 100.0 mL of distilled deionized water and stirred vigorously for 10 min in a Waring Vortex 7 blender. To facilitate filtration without introduction of F contamination from Filter Aid or Celite,⁷ the blended mixtures were heated at 90°C for 10 min. They were then partially suction filtered hot through 5.5-cm diameter Whatman No. 1 filter paper that had been freshly washed with 100 mL of hot distilled deionized water.

RESULTS AND DISCUSSION

As seen in Table 1, the F content of these California wines ranged from 0.23 to 2.80 ppm, with 7 of the 19 samples (37%) testing above 1 ppm F. Thus many of these wines appear to be contaminated by what are probably cryolite pesticide residues. If the samples are representative, then at least a third of California wines may have F levels above the international limit of 1 ppm.

TABLE 1. Fluoride content of California wines

Brand name	Vintage *	Type	(Color)	F ppm (mg/L)
Sutter Home	1990	Zinfandel	(red)	0.23
M. G. Vallejo	1994	Cabernet Sauvignon	(red)	0.38
Ernest and Julio Gallo	NS	White Zinfandel	(white)	0.41
Sutter Home	1993	Zinfandel	(red)	0.58
Sutter Home	1995	Cabernet Sauvignon	(red)	0.66
Sutter Home	1995	Chardonnay	(white)	0.68
Sutter Home	1994	Chardonnay Sauvignon Blanc	(white)	0.73
Blossom Hill	NS	White Zinfandel	(white)	0.82
Sutter Home	1995	Soleo	(red)	0.86
Ernest and Julio Gallo	1992	Chardonnay	(pink)	0.90
Vendange	NS	Cabernet Sauvignon	(red)	0.96
Sutter Home	1995	White Zinfandel	(white)	0.98
Vendange	NS	White Zinfandel Autumn Harvest	(white)	1.18
Glen Ellen	1995	Chardonnay	(pink)	1.28
Fairbanks	1995	White Port	(white)	1.35
Livingston Cellars	1996	Burgundy	(red)	1.41
Cook's	NS	Brut Imperial American Champagne	(white)	1.50
Ernest and Julio Gallo	1996	Ruby Cabernet	(red)	1.58
Inglenook	1993	Burgundy Red Table Wine	(red)	2.80
Mean				1.02
Median				0.90

NS = not specified

In contrast to these findings, European wines made from relatively uncontaminated grapes have been reported to contain only 0.2 to 0.38 ppm F.^{4,5} Likewise, at least in the past, Argentine wines were found to contain between 0.04 and 0.5 ppm F, with only 16 of 244 samples (6.6%) testing above 0.5 ppm.⁶ On the other hand, in Chile 17 samples of red wines contained 0.084 to 0.94 ppm F (mean 0.44 ppm), whereas 13 samples of white wines generally had higher F levels (like white grape juices, but unlike our California wines), ranging from 0.20 to 1.70 ppm (mean 0.63 ppm).⁸ Moreover, wines from vineyards located near F-polluting ceramic factories in Italy were found to have 0.5 to 9.5 ppm F.⁹

Table 2 records the concentrations of water-extractable F (and therefore less than the total F) in five brands of California raisins. As with the wine samples, the range of concentrations is fairly large, with the F level in Food Club raisins (5.20 ppm) being over six times that in a recently-purchased package of Rainbow raisins (0.83 ppm). That most of this F is on the outside of the raisins was demonstrated by the fact that simply soaking the raisins in distilled water for 1 to 2 hr released 70 to 90% of the amount of F found by the blending procedure. Thus these results, as with grape juices,^{1,2} point to varying levels of vineyard exposure to an F-containing pesticide such as cryolite.

TABLE 2. Water-extractable fluoride content of California raisins

Brand name	F ppm (mg/kg)
Rainbow	0.83
Rainbow (earlier, 1996 purchase)	1.85
Sweet Harvest	2.65
Champion	2.85
Sun-Maid	2.85
Food Club	5.20
Mean	2.71

Although chronic fluoride intoxication would seem unlikely from any but the highest F wines or raisins examined here, in the past habitual heavy users of wines containing 8 to 72 ppm F illegally added to retard fermentation have developed a peculiar type of alcohol-related skeletal fluorosis characterized by severe and painful exostoses and joint deformities.¹⁰ On the other hand, recent studies have shown that rats exposed to as little as 0.5 ppm AlF_3 in their drinking water for 45 to 52 weeks exhibited not only a general decline in body appearance and increased mortality but also significant microvasculature disturbances associated with abnormalities in and loss of neuronal brain cells.¹¹⁻¹³ Since ingested aluminum itself can be neurotoxic^{14,15} and is also able, at elevated levels of intake, to decrease F absorption,¹⁶ it is noteworthy that these and other studies with rats as well as studies with rabbits have shown that toxic cellular uptake of Al in the diet is actually increased by F.^{13,17,18} Consequently, with mean Al levels in US table wine and raisins officially reported to be 0.93 and 3.08 ppm, respectively,¹⁹ possible subtle, long-term synergistic toxic effects from the presence of both F and Al in these products should not be overlooked.

In view of the foregoing evidence of F enhancement of Al absorption and intoxication, the question naturally arises: might F also increase the potential health risks of other toxic substances in wine and raisins? In this light the age-old tradition of adding sulfites as preservatives in wine-making³ bears examination. In the United States sulfites are usually added at the crush stage to bring the SO₂ equivalent level in wine to 30-90 ppm.²⁰ Any such US-market wine bottled after January 1988 must carry a notice that it "contains sulfites,"²¹ and all the wines examined here were so labeled. Allergic-asthmatic reactions to sulfites in the diet are well documented²¹⁻²⁴ and allergic hypersensitivity to low-level F intake is equally well documented.²⁵

Other recent research has shown that chronic intake by rats of sodium metabisulfite (5 mg/day/kg body weight for up to 15 days) leads to significant disruption of lactate dehydrogenase activity and indications of cellular damage to the rat kidney.²⁶ Since kidney cell damage in rats from long-term ingestion of low-level F is on record^{27,28} and has now been confirmed and amplified,¹³ the potential for F to enhance or to combine with sulfite toxicity in wines should also be considered.

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SERUM 25-HYDROXY VITAMIN D₃ IN ENDEMIC GENU VALGUM AND FLUOROSIS

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SUMMARY: Vitamin D nutritional status was assessed by determining serum 25-hydroxy vitamin D₃ (25-OH-D₃) levels in subjects suffering from endemic fluorosis and endemic genu valgum. Appropriate age and sex matched controls from the endemic and non-endemic areas were also studied for comparison. No evidence of vitamin D deficiency was found in any of the groups studied. On the other hand, serum 25-OH-D₃ levels were significantly elevated in genu valgum subjects. Thus, the results indicate that vitamin D deficiency may not be one of the factors responsible for bone manifestations seen in endemic fluorosis and endemic genu valgum.

Key words: Bone; Genu valgum; Skeletal fluorosis; 25-hydroxy vitamin D₃ (25-OH-D₃).

INTRODUCTION

Endemic skeletal fluorosis, a disease characterised by osteosclerosis, osteophytosis and calcification of ligaments and tendons, poses a severe public health problem in some parts of India. In these fluorosis prone areas, the drinking water contains excessive quantities of naturally occurring fluoride.^{1,2} Endemic genu valgum, which has been described as a manifestation of chronic fluoride toxicity in some fluorosis areas in India, is associated with osteoporosis of long bones, in addition to osteosclerosis of the spine.³ Predilection of this syndrome for males was another interesting feature. This fact, and the pre-adolescent nature of onset of the syndrome, suggest that hormones may be involved in its pathogenesis.³

Several studies have revealed the metabolic distinctiveness of endemic genu valgum in contrast to the chronic osteosclerotic type of skeletal fluorosis. Although the aetiopathogenesis of genu valgum subjects is not yet completely understood, it has been shown that in endemic genu valgum the circulating parathyroid hormone (PTH) and human growth hormone (HGH) levels increased significantly while that of calcitonin (CT) was found to decrease.^{4,5} Also, it was shown that the total ⁴⁷Ca turnover and accretion of calcium in bone are higher in endemic genu valgum.⁶ Based on analysis of food samples grown in the endemic genu valgum areas, it was suggested that a possible alteration in the molybdenum-copper ratio in the dietaries of genu valgum subjects may have a role in the aetiology of endemic genu valgum.⁷

Clinically there is no evidence of nutritional rickets in subjects with endemic genu valgum. However, the possibility of a defect in vitamin D₃ metabolism in these subjects cannot be ruled out. Also, there have been so far no studies on the metabolism of vitamin D in these subjects. Further, since the residents of endemic areas are adequately exposed to sunshine and temperature (28-42°C) throughout the year, it is not known whether the metabolic conversion of the available vitamin D₃ is defective in these endemic genu valgum subjects, leading

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to conditioned vitamin D deficiency. Vitamin D as such is inert and must undergo two sequential hydroxylations: first in the liver to form 25-OH-D₃ and subsequently hydroxylation occurs in the kidney to form 1,25 dihydroxy vitamin D₃ (1,25-(OH)₂D₃), the most active form of vitamin D⁸. It is well known that 25-OH-D₃ is the major circulating metabolite in the plasma of mammals which is known to reflect the vitamin D status of an individual.

In the present study the 25-OH-D₃ levels were studied in subjects suffering from endemic fluorosis with and without genu valgum.

MATERIALS AND METHODS

Chemicals: Crystalline 25-OH-D₃ was kindly donated by Hoffman-La-Roche, NJ USA 25-OH [23,24(n)³H] vitamin D₃ (spec. act. 107 Ci/mmol) was purchased from the radiochemical centre, Amersham, UK. All other chemicals were of analytical grade procured locally.

Subjects: The study was conducted in a total of 67 subjects belonging to an endemic (n = 50) or non-endemic (n = 17) fluorosis area. The fluoride content of drinking water was found to be 8-12 mg/L in the endemic area, and 1.2-1.4 mg/L in the non-endemic region.

Among the 50 subjects residing in the endemic fluorosis village, 30 were suffering from skeletal fluorosis, of whom 12 were adults suffering from the osteosclerotic type, and the other 18 had endemic genu valgum. The remaining 20 subjects (8 adults and 12 adolescents) selected from the same endemic area did not show any obvious clinical deformities.

Seventeen subjects residing in a non-endemic area served as controls (11 of them were age and sex matched to the skeletal fluorosis group, and 6 to the endemic genu valgum group).

Diagnosis of the endemic skeletal fluorosis and genu valgum subjects was based on clinical and radiological criteria. Procedures were in accord with guidelines of the Central Ethical Committee, Indian Council of Medical Research.

Serum parameters: Fasting blood samples were collected in the field and transported to the laboratory on ice. Serum was separated and used for determination of calcium, phosphorus, alkaline phosphatase and 25-OH-D₃ levels.

Serum calcium was determined in the presence of 0.1% LaCl₃ in an atomic absorption spectrophotometer (Model No. 1000, varian techtron).⁹ Serum phosphorus was estimated by the method of Chen *et al*¹⁰ and serum alkaline phosphatase by Bodansky's method.¹¹ Serum 25-OH-D₃ was estimated by the competitive protein binding assay using normal rat serum as a binding protein.¹² The recovery of extraction of added radiolabelled 25-OH-D₃ to serum was around 98%. The minimum detectable limit by our assay was 50 pg.

Statistical analysis: The significance of the differences was determined by the analysis of variance (ANOVA).

RESULTS

The mean ages of the skeletal fluorosis subjects and their sex matched controls were around 40 and 50 years, and the mean body weights of these two groups were about 42 and 44 kg respectively. The mean age of endemic genu

valgum subjects and their sex matched controls were 16 and 21 years and the mean body weights were around 36 and 38 kg respectively.

Serum calcium, phosphorus, alkaline phosphatase and 25-OH-D₃ levels in endemic genu valgum and fluorosis: There were no significant differences in the serum calcium and phosphorus levels between any of the groups studied. Some differences in alkaline phosphatase activity were observed but were within the normal range (Tables 1 and 2). The alkaline phosphatase activities tended to be in the higher range in fluorosis and endemic genu valgum subjects as compared to their respective controls. Even in the non-genu valgum subjects, the values were higher in subjects drawn from the fluorosis area than in their age matched controls drawn from the non-fluorosis area (Tables 1 and 2). However, no differences were observed in the adult subjects drawn either from the fluorosis or the non-fluorosis areas (Table 1 and 2).

TABLE 1. Serum parameters in endemic fluorosis

Subjects	Phosphorus (mg/dL)	Calcium (mg/dL)	Alkaline phosphatase B.U.	25-OH-D ₃ (ng/dL)
Endemic skeletal fluorosis (12)	5.6 ± 0.42	9.6 ± 0.39	7.1 ± 1.38	38.3 ± 8.86
Age matched residents without bone deformity in endemic area (8)	6.2 ± 1.11	10.8 ± 0.41	4.4 ± 0.85	47.9 ± 24.56
Age matched controls from non-endemic area (11)	4.8 ± 0.48	10.6 ± 0.38	5.5 ± 0.74	73.9 ± 20.67

Values are mean ± SE

Number of subjects in parentheses

TABLE 2. Serum parameters in endemic genu valgum

Subjects	Phosphorus (mg/dL)	Calcium (mg/dL)	Alkaline phosphatase B.U.	25-OH-D ₃ (ng/dL)
Endemic genu valgum (18)	5.9 ± 0.36	9.6 ± 0.27	11.1 ± 1.96	82.0 ± 11.87*
Age matched residents of endemic genu valgum area (12)	6.6 ± 0.30	9.3 ± 0.25	6.2 ± 0.90	36.9 ± 7.39
Age matched controls from non-genu valgum area (6)	6.3 ± 0.65	9.8 ± 0.65	3.2 ± 0.76	20.5 ± 5.84

Values are mean ± SE.

Number of subjects in parentheses.

* Difference from control; P < 0.05

Serum 25-OH-D₃ levels showed a wide variation within each of the groups studied. The values of serum 25-OH-D₃ levels were not different between subjects with clinically evident skeletal fluorosis and age matched subjects residing in the same area who had no obvious clinical signs of skeletal involvement (Table 1). The values for 25-OH-D₃ obtained in control subjects

from non-fluorosis areas were not statistically different from values observed in fluorosis. However, it was interesting to observe that the 25-OH-D₃ levels were significantly higher ($P < 0.05$) in genu valgum subjects as compared to non-genu valgum controls drawn either from the same area or from the non-endemic area (Table 2). Values obtained in subjects residing in the fluorosis area but who are not suffering from endemic genu valgum and skeletal fluorosis indicated that the levels were higher in genu valgum than in skeletal fluorosis after correcting for the age effect.

DISCUSSION

The presence of excessive quantities of fluorine in drinking water is accompanied by a characteristic sequence of changes in bone, teeth and peri-articular tissues. These changes lead to a variable degree of locomotor disability, ranging from simple mechanical back pain to severe, crippling, combined locomotor and neurological impairment, posing a severe public health problem.^{3,13} In skeletal fluorosis, the spinal column is the site mainly affected by chronic fluoride intoxication.^{2,14}

1,25-(OH)₂D₃ (active form of vitamin D), along with PTH and CT plays an important role in the maintenance of normal serum concentrations of calcium and phosphorus by mobilizing these minerals from bone.⁸ But whether vitamin D deficiency may be one of the factors responsible for bone deformities seen in endemic fluorosis and endemic genu valgum has not been studied so far.

Of the several biochemical parameters studied in endemic genu valgum, fluorosis and their respective control subjects, it was observed that there was a slight elevation of serum alkaline phosphatase activity. Increased serum alkaline phosphatase levels in skeletal fluorosis have been reported earlier.¹⁵ The increase in serum alkaline phosphatase seems to be higher in genu valgum than in skeletal fluorosis.

It was interesting to observe that the serum 25-OH-D₃ levels were significantly increased in endemic genu valgum subjects. Also the serum 25-OH-D₃ levels indicate that none of the groups studied suffered from vitamin D inadequacy. The values even in the control groups were either within the normal range or higher than the reported values for normal subjects.^{16,17} In addition, as all the subjects studied in the present investigation were drawn from low socioeconomic groups who were habitually exposed to adequate sunlight throughout the year, the normal vitamin D nutritional status in these subjects can be attributed solely to the photochemical synthesis of this vitamin by the skin. Moreover, since the diets of these subjects are essentially cereal based, the contribution of vitamin D through dietary sources may be considered negligible. Even children born to parents of similar socio-economic status were found to have normal or above normal values of serum 25-OH-D₃ levels¹⁸ as compared to Asian immigrant children¹⁹.

One striking observation in the present study is that serum levels of 25-OH-D₃ in endemic genu valgum are significantly higher ($P < 0.05$) than in other groups. Such an increase was observed in skeletal fluorosis, though both the groups of subjects were exposed to high fluoride intake for prolonged periods,

and perhaps similarly exposed to sunlight. Teotia *et al*²⁰ reported normal values of serum 25-OH-D₃ in subjects with endemic skeletal fluorosis, and that there was no evidence of defective conversion of vitamin D₃. However, subjects with endemic genu valgum were not studied.

The significance of elevated serum 25-OH-D₃ levels in the genu valgum patients is not clear. Another parameter known to be different between fluorosis and endemic genu valgum is serum PTH levels. It has been observed that in both conditions PTH levels were significantly higher than in control subjects.²¹ However, the increase was much greater in genu valgum than in fluorosis. It is possible that increased 25-OH-D₃ in genu valgum may be mediated through elevated PTH levels. The present study, however, does not rule out the possibility of altered conversion of 25-OH-D₃ to 1,25-(OH)₂D₃ in subjects with endemic genu valgum.

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SODIUM FLUORIDE INDUCED CHROMOSOME ABERRATIONS AND SISTER CHROMATID EXCHANGE IN CULTURED HUMAN LYMPHOCYTES

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SUMMARY: Experimental sodium fluoride (NaF) up to 30 times the level recommended in drinking water (1 ppm) was compared with an inorganic salt for its ability to induce chromosome aberrations and sister chromatid exchange (SCE) in cultured human lymphocytes. An increase in the frequencies of chromosome aberrations but not of SCE was found.

Key words: Chromosome aberration; Human lymphocytes; Sister chromatid exchange; Sodium fluoride.

INTRODUCTION

Fluoride is a ubiquitous substance found in food and water and extensively utilised for industrial purposes. Fluoride emissions have a direct or indirect effect upon living organisms and have been subjected to many genotoxicity experiments.

An early study on bean (*Vicia faba*) reported no fluoride-induced chromosomal aberrations.¹ On the other hand, A H Mohamed later reported positive results with tomato,^{2,3} corn,⁴ and onion⁵. In subsequent *in vivo* experiments Mohamed reported a significant increase in chromosome aberrations in mice fed on drinking water containing fluoride concentrations as low as 1.0 ppm,⁶ but later apparently joined in rebutting such finding.⁷

It was observed that fluoride induces morphological and neoplastic transformations, chromosome aberrations, sister chromatid exchanges and unscheduled DNA synthesis in cultured Syrian hamster embryo (SHE) cells.⁸ No increase in chromosome aberrations in human fibroblasts was reported following prolonged exposure to low concentrations of sodium fluoride.⁹

However, in cultured human lymphocytes sodium fluoride induced chromosomal aberration,¹⁰ though Klein *et al* found no effect of fluoride on lymphocytes.¹¹ More recently, the significant increase in sister chromatid exchange (SCE) rate of persons exposed to fluoride in the endemic (1.98 to 2.2 ppm) area of North Gujarat has been reported.¹²

In view of such conflicting reports, it was decided to compare the *in vitro* effects of sodium fluoride and another inorganic salt upon the induction of chromosome aberrations and frequencies of SCE in cultured human lymphocytes.

MATERIALS AND METHODS

Peripheral blood samples were obtained from 50 individuals. Blood cultures set up each containing 0.6 mL of RPML 1640 supplemented with 20% fetal calf serum and 0.2 mL reconstituted PHA (Sigma). After 24-hours of incubation NaF (10, 20 and 30 µg/mL), NaCl (Chemically related control, concentration 10, 20 and 30 µg/mL) and Mytomycin C (Positive control, 25 and 50 ng/mL) were added to the cultures. Colcemid (0.5 µg/mL) added at 69-hours. After two hours the cultures were given hypotonic treatment (0.075M KCL). The cells

TABLE 1. Chromosome aberration categories

Treatment	Duration	Dose per mL	No. of people sampled	No. of cells scored	Mean age	Aberrations/100 cells					Total aberration -gaps	
						G	D	E	Dic	O		F
Control	72 hrs	-	50	5000	32.6	42.5	4.5	0.0	0.0	0.0	0.0	4.5
NaCl (NS)	48 hrs (24-72 h)	10 µg	50	4950		73.5	3.5	0.0	0.0	0.0	1.0	4.5
		20 µg	50	4910	32.6	77.0	5.0	0.0	0.0	0.0	0.0	6.0
		30 µg	50	4880		81.5	6.0	0.0	0.0	0.0	0.0	8.0
NaF (NS)	48 hrs (24-72 h)	10 µg	50	4920		88.5	5.5	0.0	0.0	1.0	0.0	6.5
		20 µg	50	4930	32.6	92.0	5.0	0.0	0.0	2.0	1.0	8.0
		30 µg	50	4870		97.5	4.0	2.0	1.0	1.0	1.0	9.0
MMC *	48 hrs (24-72 h)	25 ng	50	4810	32.6	78.0	93.5	43.0	1.0	3.0	5.0	145.5
		50ng	50	4790		95.5	115.0	55.0	3.0	5.0	9.0	187.0
G = Gaps, D = Deletion E = Exchange Dic = Dicentric O = Ring F = Fragments NS = Not Significant												

G = Gaps, D = Deletion, E = Exchange, Dic = Divalent, O = Ring, F = Fragments, NS = Not Significant

* Significantly different from control ($p < 0.01$)

were fixed (3:1 methanol:acetic acid) and air-dried chromosome preparations was done. The slides were stained in 3% Giesma. For SCE metaphase chromosome preparations from blood samples of treatment and controls were carried out by routine phytohaemagglutinin (PHA) stimulated cultures. 5-Bromodeoxyuridine (BrdU) was added at final concentration of 10 $\mu\text{g/mL}$ after 24 hours of stimulation. The fluorescence plus Giesma method was used for scoring sister chromatid exchanges.¹³

All slides were randomised with code numbers and were scanned by a single individual. A total of 100 first division metaphases from each culture was scored for chromosome aberrations and 25 second division metaphases were scored for SCE.

RESULTS

The frequencies of chromosome aberrations are presented in Table 1. The highest concentration (30 $\mu\text{g/mL}$) of sodium chloride (NaCl) showed higher frequencies of chromatid aberrations as compared to control, but these were mainly chromatid gaps. Similarly at the highest concentration (30 $\mu\text{g/mL}$) of sodium fluoride used in the present study also showed a slight elevation in the level of chromosome aberrations, over both the control and chemically related control, that includes mainly chromatid gaps one dicentric and one ring. The NaF induced treatment of gaps was similar to the MMC positive control.

The frequencies of sister SCEs after various treatments are shown in Table 2. Sodium fluoride and sodium chloride did not induce any significant increase in SCE level as compared to controls.

TABLE 2. Sister Chromatid exchange frequencies after various treatments

Treatment	Duration	Dose per mL	No. of People sampled	No. of cells scored	No. of metaphases with 2nd cell cycle	SCE/CELL
Control	72 hrs	-	50	1250	420	7.3 \pm 0.54
NaCl (NS)	48 hrs (24-72 h)	10 μg	50	1220	410	7.4 \pm 0.70
		20 μg	50	1205	395	7.5 \pm 0.60
		30 μg	50	1195	380	7.9 \pm 0.70
NaF (NS)	48 hrs (24-72 h)	10 μg	50	1210	405	7.7 \pm 0.60
		20 μg	50	1195	390	7.9 \pm 0.70
		30 μg	50	1180	390	7.9 \pm 0.70
MMC	48 hrs (24-72 h)	25 ng	50	1150	320	24.5 \pm 2.10*
		50 ng	50	1110	300	31.0 \pm 3.00**

Significantly different from control: * $p < 0.01$ ** $p < 0.001$

DISCUSSION

Fluoride is generally admitted to cause chromosome damage *in vitro*. *In vivo* studies are where the controversy exists.¹⁴ The conclusion derived from the report⁸ that fluoride induces chromosome aberrations was based solely on the presence of chromatid gaps and breaks, which have been reported to be unreliable indicators of real damage to the genome.¹⁵

The concentration of sodium fluoride tested in the present study was 10, 20 and 30 $\mu\text{g/mL}$ which was 10-30 times the level used in the fluoridation of drinking water (1 ppm). An increase in the frequencies of chromosome aberrations (whether gaps are excluded or not), but no appreciable increase of SCEs was noticed after treatment with sodium fluoride (Tables 1 and 2). As expected, exposure of culture to MMC proved to be clastogenic and carcinogenic and produced significant increase in chromosome aberrations and SCEs.

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INFLUENCE OF LONG-TERM SODIUM FLUORIDE ADMINISTRATION ON SELECTED PARAMETERS OF RAT BLOOD SERUM AND LIVER FUNCTION

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SUMMARY: We investigated the effect of fluoride ion (F^-) on selected parameters of blood serum and liver function in rats. Sodium fluoride was administered for periods of 3 and 6 months, respectively, via drinking water containing 10 and 30 ppm F^- . We demonstrate that concentrations of fluoride and glucose in blood serum are both universal indicators for monitoring fluoride poisoning in rats at the two F^- concentrations and exposure times used. Also, activities of some liver enzymes are of similar indicative value. These enzymes are: aspartate aminotransferase -AST [GOT], alanine aminotransferase -ALT [GPT], ornithine carbamoyltransferase [OTC], arginase [ARG], malate dehydrogenase [MDH] and isocitrate dehydrogenase [ICD].

Key words: Biochemical parameters; Blood; Liver function; Rat; Serum; Sodium fluoride.

INTRODUCTION

Fluorine is one of many noxious environmental factors affecting humans and animals. It is present in varying amounts in air, water and food. After crossing the intestinal barrier fluoride ions are distributed throughout the body by blood and deposited in various tissues.¹ The ions quickly reach the liver, the function of which becomes affected. The present report assesses fluoride influence on selected parameters of blood serum and liver efficiency.

MATERIALS AND METHODS

The study was carried out on 60 one-month-old male Wistar FL rats weighing 106 ± 7 g. The animals were obtained from the Central Experimental Animal Facility at the Silesian Medical Academy in Katowice.

The animals were divided into 4 study groups and 2 control groups. In the first, three-month long, experiment rats in two study groups received 10 and 30 ppm F^- via drinking water while rats in one control group received tap water containing 0.15 ppm F^- . The second, six-month long, experiment also included two study groups (receiving 10 and 30 ppm F^-) as well as one control group.

During the experiment the rats received standard fodder containing 0.7 mg F^- /kg on average.² After 3 and 6 months the rats were anesthetized with ether, and blood from the heart as well as samples of liver and kidneys were taken. Homogenates (10% w/v) were prepared according to Singh and Kanwar³ and were then used for biochemical determinations. Liver and kidneys were also examined morphologically.

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The following parameters were determined in blood serum: protein and protein fractions, fluorides, glucose, creatinine, cholesterol, triglycerides. In liver, homogenates activity of the following enzymes was determined:^{4,5}

aspartate aminotransferase -AST [GOT] - EC 2.6.1.1.

alanine aminotransferase - ALT [GPT] - EC 2.6.1.2.

malate dehydrogenase [MDH] - EC 1.1.1.37.

isocitrate dehydrogenase [ICD] - EC 1.1.1.41.

ornithine carbamoyltransferase [OTC] - EC 2.1.3.3.

arginase [ARG] - EC 3.5.3.1.

Glucose, creatinine, cholesterol and triglycerides were determined in the Cobas Mira Roche analyzer (Switzerland). Enzymatic activity was assayed by routine methods.⁴

Serum protein concentration was determined by the Biuret method⁶ with Cobas Mira Roche analyzer. Serum protein fractions were separated electrophoretically by the Paragon Electrophoresis System (USA). Fluorides were determined with a selective electrode (Radelkis, Hungary). Liver homogenate protein was assessed according to Lowry.⁷

The results were analyzed statistically (Student's t-test).

RESULTS

The results, presented in four Tables, are averages from groups of 10 rats. Standard deviation and percent of change vs. control were computed. Statistically significant increase tendency is denoted by \nearrow and a decreasing one by \searrow .

Electrophoresis of serum proteins showed no differences among particular groups. Therefore these results were not included in the Tables.

Tables 1 and 2 show the results on fluorides, glucose, total protein and lipid compounds (triglycerides and cholesterol) in the serum of rats exposed to F⁻ during the 3-month period (Table 1) and the 6-month period (Table 2):

Serum fluoride content: fluoride concentration in blood serum of rats exposed to F⁻ increased significantly, after both 3 months (27% for 10 ppm and 67% for 30 ppm) and 6 months (35% for 10 ppm and 81% for 30 ppm).

Serum glucose content: glucose concentration in blood serum was significantly increased after fluoride administration, with the exception of rats which received 30 ppm F⁻ for 3 months (increase insignificant).

Serum protein content: administration of fluoride did not change the total blood protein concentration.

Serum lipids content: concentrations of cholesterol (CH) and triglycerides (TG) did not change, except in the group subjected to 10 ppm F⁻ for 6 months.

TABLE 1
Concentration of compounds in blood serum of rats exposed to NaF
in drinking water for 3 months. Each group consisted of 10 animals;
SD = standard deviation; \nearrow denotes percent increase; \searrow denotes
percent decrease statistically significant vs control group, $p < 0.05$.

Group	Fluorides M/L	Glucose mM/L	Protein g/L	CH mM/L	TG mg/dL
Control					
Average	4.3×10^{-6}	5.15	72.8	0.82	41.3
SD	4.1×10^{-7}	0.67	5.6	0.22	6.2
10 ppm					
Average	5.5×10^{-6}	5.82	73.2	0.80	43.3
SD	2.6×10^{-7}	0.17	2.6	0.12	15.2
% change	$\nearrow 27\%$	$\nearrow 13\%$			
30 ppm					
Average	7.2×10^{-6}	5.25	73.0	0.76	43.5
SD	2.2×10^{-6}	0.48	3.5	0.20	6.5
% change	$\nearrow 67\%$				

TABLE 2
Concentration of compounds in blood serum of rats exposed to NaF
in drinking water for 6 months. Description the same as in Table 1.

Group	Fluorides M/L	Glucose mM/L	Protein g/L	CH mM/L	TG mg/dL
Control					
Average	4.53×10^{-6}	7.94	74.5	0.92	103.3
SD	3.4×10^{-7}	0.84	2.5	0.067	33.6
10 ppm					
Average	6.11×10^{-6}	8.85	71.9	0.84	102.9
SD	5.4×10^{-7}	0.58	2.7	0.09	17.8
% change	$\nearrow 35\%$	$\nearrow 11\%$	$\searrow 4\%$	$\searrow 9\%$	
30 ppm					
Average	8.2×10^{-6}	9.60	74.5	0.90	102.7
SD	1.5×10^{-6}	0.94	5.3	0.10	20.8
% change	$\nearrow 81\%$	$\nearrow 21\%$			

Tables 3 and 4 show results of liver homogenate studies for rats subjected to a 3-month exposure (Table 3) and to a 6-month exposure (Table 4):

Protein content in liver: protein concentration did not change with the exception of the group of rats subjected to 30 ppm of F^- for 3 months where a small but significant 12% decrease was observed.

Glutamate oxaloacetate transaminase [GOT] activity in liver: a significant dose-dependent increase of GOT activity was observed after 3 months of F^- administration. This increase of GOT activity was less visible after 6 months of rats' treatment with F^- .

Glutamate pyruvate transaminase [GPT] activity in liver: activity of GPT was also significantly increased as a result of F^- administration.

Ornithine carbamoyltransferase [OTC] and arginase [ARG] activities in liver: the activities of OTC-ase and arginase were reduced by F^- treatment. The effect observed was dose-dependent.

Malate dehydrogenase [MDH] and isocitrate dehydrogenase [ICD] activities in liver: the selected Krebs cycle enzymes [MDH and ICD] did not change following the 3-month-long experiment but after 6 months of F^- administration significant decrease of these enzymes' activity was observed.

Determination of creatinine level showed no differences among particular groups (not included in the Tables). Pathomorphological examination of liver and kidney did not reveal any morphological changes.

DISCUSSION

Studies of the effect of fluorine compounds on living organisms have been performed on body fluids, hard and soft tissues.⁸ Results depend on several parameters, among them age of animals, dose, means of administering toxic compounds, experiment duration, fodder and other factors.

Fluorides usually were administered via drinking water^{9,10} with dose varying from 3 ppm¹¹ to 150 ppm¹². In the present study doses of 10 ppm and 30 ppm were used. The same doses were used by Zhi-zong.¹³ Experiments lasted from 1 month¹⁴ to 20 months¹¹. Our studies were conducted within 3- and 6-month periods.

Concerning rat blood serum, the values obtained for fluoride and glucose in our investigation are noteworthy (Tables 1 and 2). Fluoride content in both control groups was $4.3 \cdot 10^{-6}$ M/dm³ (corresponding to 4.3 μ M or 0.082 ppm; various authors have used different units such as μ M, mM, μ g/mL, ppm, which make comparisons a little complicated). Nonetheless, published results show considerable variation for control groups: 0.009 mg,¹ 0.45 μ M,⁸ 1.2 μ M,¹⁵ 0.02 ppm / 1.05 μ M,⁹ 0.58 ppm / 30.5 μ M,¹⁶ 0.46 μ g/mL / 24 μ M.¹⁷ It is worth mentioning that fluoride administered via drinking water is assimilated better than that administered with fodder. In our studies the fluoride content in serum increased in both groups studied (10 and 30 ppm) which does agree with the results of other investigators.⁹ Serum glucose determination is related to the functioning of the liver. It is known that fluoride administration increases

TABLE 3
Protein content in rat liver homogenates and activity of liver enzymes
after exposing rats to NaF in drinking water for 3 months.
Description the same as in Table 1.

Group	Protein mg/g fresh tissue	GOT U/g protein	GPT U/g protein	OTC mM citrulline /min/g protein	ARG mM urea/ min/g protein	MDH U/g protein	ICD U/g protein
Control							
Average	142.3	147.0	330.7	0.47	3.77	9.03	0.80
SD	9.39	37.9	18.0	0.14	1.13	0.6	0.08
10 ppm							
Average	143.1	289.6	334.1	0.32	2.95	8.98	0.77
SD	11.9	63.2	23.6	0.05	0.78	0.55	0.10
% change	-	↗ 97%	-	↘ 32%	↘ 22%	-	-
30 ppm							
Average	125.6	530.4	359.0	0.23	1.22	9.22	0.78
SD	18.8	65.5	37.6	0.09	0.26	1.35	0.10
% change	↘ 12%	↗ 260%	↗ 8%	↘ 51%	↘ 68%		

TABLE 4
Protein content in rat liver homogenates and activity of liver enzymes
after exposing rats to NaF in drinking water for 6 months.
Description the same as in Table 1.

Group	Protein mg/g fresh tissue	GOT U/g protein	GPT U/g protein	OTC mM citrulline /min/g protein	ARG mM urea/ min/g protein	MDH U/g protein	ICD U/g protein
Control							
Average	136.2	137.1	286.4	0.515	3.49	13.64	0.88
SD	16.9	41.1	62.1	0.04	0.99	1.63	0.24
10 ppm							
Average	135.7	152.4	352.4	0.242	2.37	9.29	0.68
SD	23.3	20.9	48.4	0.04	0.83	1.41	0.13
% change	-	↗ 11%	↗ 23%	↘ 53%	↘ 32%	↘ 32%	↘ 23%
30 ppm							
Average	143.6	200.0	343.9	0.186	1.61	9.38	0.68
SD	12.7	27.1	28.2	0.03	0.60	0.69	0.11
% change	-	↗ 46%	↗ 20%	↘ 64%	↘ 54%	↘ 31%	↘ 23%

glycemia in rats¹⁸ and other animals¹⁹. The mechanism of these changes was partially explained^{20,21} and corroborates the effect of fluorides disturbing the carbohydrate metabolism. Since the liver regulates glycemia, hence the supposition that fluorides affect its functioning. Our results pertaining to glucose content of rat blood serum following a 6-month experiment are similar to those of Zhi-zong *et al.*¹³

The determinations of serum cholesterol (CH) and triglyceride (TG) content showed no difference which has been attested by others,^{16,22,23} although there are also controversies regarding this subject.^{10,23}

Tables 3 and 4 show results from liver homogenate studies. They were performed in order to yield information on changes of liver enzymes. Changes in enzyme activity are a sensitive indicator of functional disturbances despite lack of morphological changes in this organ. And thus, for the urea cycle which takes place exclusively in the liver, its two participating enzymes, OTC-ase and arginase, had their activities lowered in all groups. Inhibition of activity was higher in the groups of animals poisoned with 30 ppm fluoride as compared to the 10 ppm group. Liver decarboxylase inhibition was previously noted by Hogslo.²⁴ The same was observed for arginase by Eagers.²⁵

The mechanism of arginase inhibition by fluoride is unknown. Both enzymes may be considered metal-dependent since arginase is activated by Mn^{2+} ions while OTC-ase activity depends on Ca^{2+} ions.²⁶ In such enzymes the fluoride may bind to the metal thus decreasing enzyme activity.²⁷ Our parallel determination of two urea cycle enzymes makes our conclusion concerning inhibition more trustworthy. The behavior of two liver enzymes, GOT and GPT was also analyzed. The increase of their activity is characteristic for liver ailments, inflammations and poisonings. In this respect GOT turned out to be a more sensitive indicator than GPT. However, after a 6-month long exposure to fluoride a decreasing leveling tendency for GOT was noted, in contrast to GPT. No changes in protein metabolism were observed. The protein content of liver homogenates and blood serum as well as electrophoretically separated serum protein fractions did not differ among various study groups (with the exception of the 10 ppm group summarized in Table 2). Thus, protein synthesis by the liver was not disturbed.

Two Krebs cycle enzymes also showed decrease of their activity albeit only after the animals had been intoxicated with fluoride for 6 months. The enzymes in question are MDH and ICD. Krebs cycle inhibition by fluoroacetate was reported by Bobylewa-Guarriero.²⁸ According to this author the activity of Krebs cycle enzymes is lowered in isolated hepatocytes. This multidirectional fluoroacetate interaction²⁹ is related to disturbances of carbohydrate metabolism in the liver. However, pathological examination of liver and kidneys did not reveal any morphological changes. No disturbances in protein metabolism (liver) were detected biochemically and no changes in creatinine level were noted.

In summary, the intoxication of rats by sodium fluoride at 10 and 30 ppm does not cause morphological changes in liver or kidney but it does disturb certain liver functions. It pertains to enzymes regulating amino acid metabolism

(GOT and GPT), urea cycle (OTC, ARG), Krebs cycle (MDH and ICD) and carbohydrate metabolism (hyperglycemia). A differential increase in intensity of these biochemical changes, as observed in our experiment, allows us to monitor prolonged intoxication processes more precisely.

The results presented in this paper agree with the conclusions reached in a somewhat more general study,⁴ i.e. that fluoride intoxication in rats activates adaptive mechanisms over periods lasting several months. These mechanisms can be seen as diminishing differences among results obtained when varying dose and/or experiment duration. As the animals grow they become more resistant while younger ones are more sensitive to intoxication by fluoride compounds.³⁰

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EFFECT OF SUPPLEMENTAL BORON ON NUTRIENT UTILIZATION, MINERAL STATUS AND BLOOD BIOCHEMICAL CONSTITUENTS IN LAMBS FED HIGH FLUORINE DIET

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SUMMARY: To investigate the effect of supplemental boron (B) as an antidote of fluorine (F), on nutrient utilization, mineral status and blood biochemical constituents in lambs, two experiments were conducted: the first with 20 male lambs (about 4 months old) over 190 days, the second with 24 male lambs of similar age over 120 days. In both experiments the lambs, divided into four equal groups, were fed conventional concentrate mixtures and gram straw, to which were added F (sodium fluoride) for Groups 2, 3 and 4, and B (sodium tetraborate) for Groups 3 and 4. F intakes were similar in both experiments, but B supplementation of Groups 3 and 4 was higher in Experiment II (0.56 and 0.79 mg/mgF) than in Experiment I (0.40 and 0.50 mg/mgF). Body weights, dry matter intakes, mineral status and blood biochemical constituents were recorded. The results indicate that supplemental B increased faecal F excretion, and might also reduce the blood F level, but could not overcome the adverse effect of F on dry matter intake. However, the reduction in body weight gain caused by F was improved by increasing levels of dietary B. Absorption of F was greater with high F diets, but supplemental B reduced the absorption of F by increasing its faecal excretion. Faecal excretion of copper increased with increased F intake, but appeared to be counteracted by B supplementation. The dietary treatments had no apparent adverse effects on blood mineral status and blood biochemical constituents. Supplemental B had a beneficial effect in counteracting adverse effects of F on body weights and feed efficiency.

Key words: Antidote; Blood; Boron; Diet; Fluorine intake; Lambs; Minerals; Nutrition.

INTRODUCTION

Higher fluorine (F) intake through various sources is potentially dangerous for human and animal health as it leads to fluorosis. Animals normally ingest excessive F through underground water and mineral supplements. In addition, in recent years, rapid growth of industries coupled with lack of strict implementation of environment protection laws and little use of modern technologies to recapture and recycle the fluoride compounds in the smelting process in developing countries, has resulted in increased incidence of fluorosis in livestock due to F emission.¹⁻³ Several attempts have been made in the past to alleviate F toxicity with vitamin C, magnesium, calcium, molybdenum, copper, iron, boron etc. In the present study, boron (B) was selected, since according to the available literature,⁴ fluoride-boron complexes $[BF_4]^-$ formed in the body are completely eliminated and it has been tried with success in rabbit. Since the literature is silent on the use of B in ruminants, the present study was planned to elucidate the effect of high F ingestion along with supplemental B on growth, nutrient utilization, mineral retention and blood biochemical constituents in lambs.

MATERIALS AND METHODS

In experiment I, 20 cross-bred male lambs (about 4 months old) were randomly divided into four equal groups on mean body weight basis. They were kept for 190 days on conventional concentrate (maize 31%, groundnut cake 34%, wheat bean 32%, mineral mixture 2% and common salt 1%; crude protein (CP) 20% and total digestible nutrients (TDN) 72%) and gram straw.⁵ The dietary variables were F and B contents and their levels were achieved by addition of sodium fluoride in Groups 2, 3, and 4, and sodium tetraborate in Groups 3 and 4. The dietary F levels in Groups 1 to 4 were 58, 276, 284, and 291 ppm and the corresponding levels of B were 11, 11, 117, and 149 ppm, respectively. The dietary levels of B/mgF thus achieved were 0.4 and 0.5 in Groups 3 and 4, respectively.

In Experiment II, 24 cross-bred male lambs (3-4 months old), divided into four equal groups, were fed conventional concentrate mixture (barley 40, groundnut cake 30, rice polish 27, mineral mixture 2 and common salt 1 part; CP 19% and TDN 71%) and gram straw.⁵ By similar addition of F (Groups 2, 3, and 4) and B (Groups 3 and 4), the dietary levels of F were 41, 267, 272 and 265 ppm and of B were 15, 16, 152 and 209 ppm in the respective groups. In Groups 3 and 4 the levels of B/mgF thus achieved were 0.56 and 0.79 mg, respectively.

Biweekly body weights and daily dry matter (DM) intake were recorded. At the end of each experiment, a metabolism trial of seven days duration was conducted. Representative samples of weigh-back, faeces and urine collected during the metabolism trials were analysed for proximate nutrients.⁶ F content in the samples was determined by the specific ion electrode following procedures described by Villa⁷ and Tusl^{8,9}. Blood samples collected from the jugular vein in the beginning, and subsequently at monthly intervals, were analysed for mineral (calcium, phosphorus, iron, zinc and copper),¹⁰ alkaline phosphatase,¹¹ SGPT,¹² glucose,¹³ and thyroxine level (Radio-immuno assay). The data were statistically analysed as per Snedecor and Cochran.¹⁴

RESULTS

Experiment I

Data on mineral retention in experimental lambs (Table 1) indicated lower intake of Ca, P and Cu in Group 2 (F alone) and supplemental B at both levels (Groups 3 and 4) could not improve it. This was associated with lower feed intake (g/d) in Groups 2 (424 ± 6.24), 3 (432 ± 14.82) and 4 (422 ± 10.60) than in Group 1 (485 ± 8.92). However, retention (% of intake) of various minerals, Ca, P, Cu and Zn remained similar in all the groups. Excretion pattern of Ca and P was proportional to the intake. Apparent digestibility of Cu decreased in the group fed F alone (43.5% against 60.1% in T_1) which improved upon B supplementation at both the levels (59.1 and 58.4%). Data also indicated that intake of F in Groups 2, 3, and 4 was similar and significantly higher than in Group 1. Excretion of F through faeces was 17.08, 44.39, 52.76 and 70.44 mg/d in Groups 1 to 4, respectively.

The corresponding values for urinary F excretion were 7.78, 10.41, 6.48 and 8.20 mg/d, respectively. Retention (% of intake) of F in the four treatment groups

TABLE 1. Mineral balances and retention in lambs under different dietary treatments (Experiment I)

	Treatments			
	1	2	3	4
Calcium				
Intake (g/d)	4.78 ^a ±0.11	3.63 ^b ±0.16	4.08 ^{ab} ±0.20	3.71 ^b ±0.40
Voided (g/d)				
Faeces	3.34 ^a	2.39 ^b	2.46 ^b	2.35 ^b
Urine	0.06	0.07	0.02	0.03
Balance (g/d)	1.38 ± 0.07	1.17 ± 0.06	1.61 ± 0.09	1.34 ± 0.23
Balance (% of intake)	28.82 ± 1.14	32.34 ± 1.24	39.41 ± 2.05	36.22 ± 4.39
Phosphorus				
Intake (g/d)	3.07 ^a ±0.06	2.60 ^b ±0.08	2.46 ^b ±0.16	2.78 ^{ab} ±0.14
Voided (g/d)				
Faeces	1.62	1.27	0.90	1.17
Urine	0.013	0.009	0.005	0.05
Balance (g/d)	1.44 ± 0.08	1.33 ± 0.18	1.56 ± 0.08	1.57 ± 0.10
Balance (% of intake)	46.80 ± 2.50	51.16 ± 6.91	53.56 ± 2.45	52.12 ± 3.52
Iron				
Intake (mg/d)	57.65 ^a ±1.38	48.60 ^{ab} ±2.28	41.04 ^b ±4.21	47.89 ^{ab} ±4.23
Voided (mg/d)				
Faeces	32.34	34.39	29.08	35.48
Urine	0.89	0.48	0.39	1.13
Balance (mg/d)	24.42 ^a ±1.37	13.73 ^b ±1.81	11.57 ^b ±1.53	13.91 ^b ±2.87
Balance (% of intake)	42.27 ^a ±1.48	27.89 ^b ±2.67	27.95 ^b ±1.07	28.93 ^b ±5.78
Copper				
Intake (mg/d)	23.08 ^a ±0.52	17.55 ^b ±0.95	17.94 ^b ±2.10	21.33 ^{ab} ±1.03
Voided (mg/d)				
Faeces	9.21	9.92	7.33	8.87
Urine	0.62 ^a	0.41 ^{ab}	0.21 ^b	0.54 ^a
Balance (mg/d)	13.09 ^a ±1.89	7.23 ^b ±1.37	10.40 ^{ab} ±0.91	11.93 ^a ±0.44
Balance (% of intake)	56.44 ± 7.34	40.35 ± 5.24	57.12 ± 3.10	56.64 ± 5.12
Zinc				
Intake (mg/d)	48.63 ± 1.30	37.97 ± 1.86	37.80 ± 2.72	40.17 ± 4.26
Voided (mg/d)				
Faeces	19.35	22.76	19.24	22.22
Urine	16.06 ^a	5.50 ^b	8.60 ^b	7.44 ^b
Balance (mg/d)	13.23 ± 3.06	9.71 ± 0.68	9.96 ± 2.25	10.52 ± 2.95
Balance (% of intake)	26.89 ± 5.67	25.55 ± 1.12	25.69 ± 4.39	25.46 ± 4.98
Fluorine				
Intake (mg/d)	32.65 ^b ±0.91	120.34 ^a ±8.54	121.28 ^a ±12.11	139.02 ^a ±15.28
Voided (mg/d)				
Faeces	17.08 ^c	44.39 ^b	52.76 ^b	70.44 ^a
Urine	7.78 ^b	10.41 ^a	6.48 ^c	8.20 ^b
Balance (mg/d)	7.79 ^b ±0.92	65.54 ^a ±2.96	61.56 ^a ±3.75	60.38 ^a ±2.14
Balance (% of intake)	23.86 ^c ±2.56	54.46 ^a ±3.30	50.75 ^{ab} ±4.29	43.43 ^b ±3.73

Means ± standard error (SE) bearing different superscripts in a row differ significantly ($P < 0.05$)

groups was 23.86, 54.46, 50.75 and 43.43, respectively. Data on serum mineral status and biochemical constituents (Table 2) indicated that feeding of F alone or in combination with B did not affect either Ca, P, Fe, and Zn status or the activity of alkaline phosphatase, SGPT, glucose level or thyroxine level. However, lower value of serum Cu was recorded in the F fed group which was improved upon B supplementation. It is evident from the results that lambs fed diet containing 291 ppm F for a period of 190 days exhibited no gross adverse effect on retention of minerals, mineral status and various blood biochemical constituents.

TABLE 2. Serum mineral status and biochemical constituents in lambs under different dietary treatments (Experiment I)

	Treatments			
	1	2	3	4
Calcium(mg/100 mL)	10.57 ± 0.71	12.43 ± 0.85	10.72 ± 0.78	10.16 ± 0.88
Phosphorus (mg/100 mL)	3.85 ± 0.20	3.77 ± 0.20	3.76 ± 0.21	3.43 ± 0.16
Iron (ppm)	1.77 ± 0.30	1.52 ± 0.15	1.89 ± 0.24	1.77 ± 0.16
Zinc (ppm)	3.27 ± 0.29	3.18 ± 0.32	3.21 ± 0.33	3.23 ± 0.31
Copper (ppm)	1.60 ^a ± 0.14	1.14 ^b ± 0.12	1.52 ^a ± 0.10	1.37 ^{ab} ± 0.1
Alkaline phosphatase (IU/L)	172.61 ± 14.73	187.80 ± 16.07	176.18 ± 15.15	162.96 ± 16.88
SGPT (IU/L)	14.30 ± 0.56	14.19 ± 0.61	15.78 ± 0.63	14.46 ± 0.59
Blood glucose (mg/100 mL)	44.86 ± 1.75	40.13 ± 1.50	44.04 ± 1.90	45.61 ± 1.33
Thyroxine (ng/mL)	43.49 ± 2.26	48.06 ± 3.31	43.20 ± 3.26	38.66 ± 2.26

Means ± standard error(SE) bearing different superscripts in a row differ significantly ($P < 0.05$)

Experiment II

High F diet (Treatment 2) depressed ($P < 0.05$) body weight gain of lambs (Table 3). Supplemental B at 0.79 mg/mg F (Treatment 4) improved the gain to the level of control statistically. Feed intake in Treatment 2 was lower ($P < 0.01$) than in Treatment 1 and supplemental B in Groups 3 and 4 did not improve it.

The average feed conversion ratio (dry matter intake: gain) was 10.80, 16.95, 12.09 and 10.43 in Groups 1 to 4, respectively, indicating the adverse effect of F alone in Group 2 and beneficial effect of B given in combination of F in Groups 3 and 4. Data on digestibility coefficient (Table 1) indicated that addition of either F alone or in combination with B did not affect the digestibility coefficients of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), organic matter (OM), nitrogen free extract (NFE) and nutritive value of feed w.r.t DCP (%) and TDN (%). However, digestibility of F increased in the diet added F alone (Group 2) and reduced subsequently by addition of B (Groups 3 and 4). The values in the four treatment groups were 64.64, 83.82, 76.67 and 67.88%, respectively.

Data presented in Table 4 also reflected no visible treatment effect on N retention in experimental animals. However, retention of F was significantly ($P < 0.01$) influenced due to various treatments. Ingestion of F was similar in the treatment Groups 2, 3 and 4 but faecal excretion of F increased significantly

(from Group 2) upon addition of B at both the levels (Groups 3 and 4). Intake of F (mg/d) in the four groups was 20.16, 97.45, 98.35 and 93.22, respectively. The corresponding values of faecal F excretion (mg/d) were 7.14, 15.27, 21.99 and 29.45, and those of urinary F were 8.55, 28.43, 29.32 and 23.59 mg/d in the respective groups. Retention of F (% of intake) reduced significantly ($P < 0.01$) upon addition of B and the values in the respective groups were 22.18, 53.40, 46.62 and 42.19 per cent, respectively.

TABLE 3. Feed intake and nutrient utilization of growing lambs under different dietary treatments (Experiment II)

	Treatments			
	1	2	3	4
Initial body wt. (kg)	9.57 \pm 0.65	9.57 \pm 0.40	9.57 \pm 0.45	9.55 \pm 0.47
Final body wt. (kg)	13.93 \pm 0.35	11.68 \pm 0.40	12.48 \pm 0.20	13.15 \pm 0.37
Body wt. gain (g/d)	43.34 ^a \pm 5.40	20.16 ^c \pm 0.52	27.78 ^{bc} \pm 2.71	34.29 ^{ab} \pm 1.56
DM intake (g/d)	434.29 ^a \pm 11.79	341.12 ^{bc} \pm 16.71	322.07 ^c \pm 15.52	349.98 ^b \pm 19.34
DM intake(% body wt.)	3.70 ^a \pm 0.08	3.25 ^b \pm 0.10	3.06 ^c \pm 0.14	3.16 ^{bc} \pm 0.12
Feed conversion ratio	10.80 ^b \pm 1.32	16.95 ^a \pm 1.07	12.09 ^b \pm 1.20	10.43 ^b \pm 0.91
Nutrient digestibility (%)				
DM	51.08 \pm 3.06	53.60 \pm 1.99	53.24 \pm 2.26	50.06 \pm 2.58
CP	60.35 \pm 2.79	64.61 \pm 1.91	62.45 \pm 1.56	61.52 \pm 2.09
CF	47.15 \pm 2.24	46.55 \pm 0.67	42.98 \pm 2.15	41.50 \pm 1.22
EE	59.58 \pm 1.04	61.00 \pm 1.00	64.33 \pm 1.84	61.70 \pm 3.52
OM	52.24 \pm 3.14	54.85 \pm 1.48	53.63 \pm 2.09	50.65 \pm 2.41
NFE	51.70 \pm 5.03	53.97 \pm 2.84	55.83 \pm 3.29	49.86 \pm 3.68
F	64.64 ^c \pm 0.98	83.82 ^a \pm 2.01	76.67 ^b \pm 4.52	67.88 ^c \pm 1.96
DCP	8.14 \pm 0.38	8.55 \pm 0.25	8.50 \pm 0.21	8.22 \pm 0.28
TDN	50.40 \pm 2.91	52.25 \pm 1.75	52.56 \pm 1.97	48.74 \pm 2.05

Means \pm standard error(SE) bearing different superscripts in a row differ significantly ($P < 0.05$)

TABLE 4. Nitrogen and fluorine balance in growing lambs under different dietary treatments (Experiment II)

	Treatments			
	1	2	3	4
Nitrogen balance				
Intake (g/d)	9.81 \pm 0.50	7.30 \pm 0.70	6.42 \pm 0.53	7.03 \pm 0.78
Voided (g/d)				
Faeces	3.86 \pm 0.19	2.55 \pm 0.13	2.78 \pm 0.24	2.67 \pm 0.23
Urine	2.57 \pm 0.21	2.07 \pm 0.18	2.05 \pm 0.16	1.62 \pm 0.30
Balance (g/d)	3.38 \pm 0.66	2.69 \pm 0.71	2.60 \pm 0.47	2.74 \pm 0.68
Balance (% of intake)	33.85 \pm 5.28	34.85 \pm 6.98	34.31 \pm 4.05	38.24 \pm 5.60
Fluorine balance				
Intake (mg/d)	20.16 ^b \pm 0.95	97.45 ^a \pm 9.99	98.35 ^a \pm 8.81	93.22 ^a \pm 9.35
Voided (mg/d)				
Faeces	7.14 ^d \pm 0.42	15.27 ^c \pm 1.24	21.99 ^b \pm 2.92	29.45 ^a \pm 1.61
Urine	8.55 ^c \pm 0.61	28.43 ^a \pm 1.30	29.32 ^a \pm 1.90	23.59 ^b \pm 1.58
Balance (mg/d)	4.47 ^b \pm 0.68	53.75 ^a \pm 10.91	47.04 ^a \pm 9.24	40.18 ^a \pm 6.76
Balance (% of intake)	22.18 ^c \pm 3.22	53.40 ^a \pm 5.95	46.62 ^{ab} \pm 4.50	42.19 ^b \pm 3.29

Means \pm standard error(SE) bearing different superscripts in a row differ significantly ($P < 0.05$)

DISCUSSION

Though two separate experiments were conducted at different boron levels keeping fluorine more or less similar, because of the great similarity in experimental conditions, both the experiments have been combined and discussed together. Reduced dry matter intake upon F feeding as reported earlier,^{1,15-19} was also observed in both the experiments. It is also evident from the data that addition of B at any level could not overcome the depressed dry matter intake caused by high F feeding. Shortly after exposure to high fluorine through concentrate mixture containing 574 and 487 ppm, in experiment I and II, respectively, the reluctance in voluntary feed intake was observed. Soluble fluorides are rapidly and almost completely absorbed from the gastrointestinal tract even at high intakes.²⁰ This absorption results in high fluorine concentration in blood, and thus in reduced feed intake, as appetite is extremely sensitive to rise in plasma F concentration.²¹ Reduction in feed intake in rats has also been reported on intravenous administration of F rather than its supplementation in the diet.²² It is evident from the data that boron supplementation increased faecal F excretion, and this might also be reducing the blood F level, but could not overcome the adverse effect of F on dry matter intake. The data presented in Table 3 revealed a significant reduction in body weight gain in Group 2, and its improvement with increasing level of B in the diet. Feed intake was depressed in all the three groups with different rates of gain, indicating that limited feed intake was not solely responsible for variable growth, but was associated also with some physiological processes which might have influenced feed utilization efficiency and growth. Boron might have reduced those ill effects in Group 4 (Experiment II, B at 0.79 mg/mg F), exhibiting improved feed conversion efficiency (10.43) to the extent in control (10.80). Fluorine alone or with boron did not affect apparent digestibility coefficients of dry matter, crude protein, crude fibre, ether extract, nitrogen free extract and organic matter, and nutritive value in terms of digestible crude protein and total digestible nutrients (Table 3). Similar observations were recorded in cattle.²³ The absorption of F was more in high F diet. Supplementation of B reduced the absorption of F (Table 3) by increasing its faecal excretion (Tables 1 and 4). Higher faecal F excretion upon B supplementation in the present study further confirmed the probable binding of F with B as $[BF_4]^-$ at the intestinal or metabolic level, as hypothesized by earlier workers.²⁴ The proportion of F retained by the animals apparently decreased with corresponding increase in the B levels. However, statistically, the differences were not significant ($P > 0.05$) due to high variations observed in F balance. Such variations are often noted in biological systems, particularly when the number of animals is limited. Moreover, retention expressed as per cent of its intake was reduced in B-containing diets.

The lower intake of minerals in high fluorine groups (Table 1) was due to decreased feed intake. Faecal excretion of iron in the F fed group increased and supplemental B had no effect on its excretion, indicating interaction of iron with F. Data also indicated interaction of F with copper, as faecal excretion of copper increased, an effect which appeared to be counteracted by B supplementation.

Though F seemed to affect the urinary excretion of zinc, retention of the latter element remained unaffected. The balances of various minerals were positive and also the values of blood minerals were within normal range. Thus, the dietary treatments had no apparent adverse effect on blood mineral status, nor on the blood biochemical constituents studied. However, F is a known inhibitor of a number of enzymes,²⁰ affecting the various metabolic pathways which might hinder the performance of animals. It appeared that the amount of F retained in the lambs consuming up to 267 to 291 ppm F could not bring out adverse effect except reduction in body weight and feed efficiency, and supplementation of boron at 0.79 mg/mgF had a beneficial effect in counteracting the adverse effects on body weight gain and feed efficiency.

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EFFECTS OF FLUOROSIS ON INDUCED SECRETION OF RAT PROLACTIN *IN VIVO* AND *IN VITRO*

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SUMMARY: The induced secretion of prolactin (PRL) in rats with fluorosis was studied *in vivo* after administration of quipazine, morphine, and thyrotropin releasing hormone (TRH). The effects of sodium fluoride on basal and induced PRL release by TRH, vasoactive intestinal polypeptide (VIP), and metoclopramide were observed in primary cultures of anterior pituitary cells *in vitro*. The results indicated that the response of PRL release to quipazine, morphine, and TRH was reduced in rats with fluorosis. Sodium fluoride can directly inhibit PRL basal secretion and induces release from cultured anterior pituitary cells. All the observations suggest that PRL release is impaired in fluorosis. This impairment is caused by pituitary inhibition.

Key words: Anterior pituitary; Fluorosis; Prolactin; Rat.

INTRODUCTION

There have been reports of agalactia in cows and silver foxes caused by intake of feed containing high levels of fluoride, and in cows resulting from industrial fluoride pollution of herbage.¹⁻³ Shude Yuan reported that lactation and serum PRL were inhibited in lactating rats with both chronic and subacute fluorosis.^{4,5} However, thorough investigations into the inhibition of PRL release by fluoride, and the mechanism involved, have not been carried out. In the present study, the induced secretion of PRL by some hypothalamic factors and neurotransmitters was observed both *in vivo* and *in vitro* to explore the effects of fluorosis on PRL release and its mechanism.

MATERIALS AND METHODS

In vivo: Fifty six Wistar rats of 40 days (obtained from the Laboratory Animal Center of China Medical University) were divided into two groups. The experimental group ($n = 29$) received water containing 100 ppm F to develop a chronic fluorosis animal model. The control group received tap water (containing 0.26 ppm F). The following experiments began after four months.

An atrial catheter was introduced into free-moving conscious rats to collect blood samples and to inject the drugs: quipazine (5-HT (hydroxytryptamine) receptor agonist), morphine (EOP (endogenous opioid peptide) receptor agonist), and thyrotropin releasing hormone (TRH). Blood was collected before injection and at 5, 15, 30 and 60 minutes after administration of drugs. The doses of quipazine and morphine were 1 mg/100g body weight and the dose of TRH was 1 μ g/rat.

In vitro: *Pituitary cells culture:* Monolayer culture was used in the experiment. Anterior pituitaries, removed after decapitation, were cut into 1 mm³ pieces, and then enzymatically dispersed by 0.25% trypsin (Difco) according to the method described by Snyder *et al.*^{6,7} The cell suspension was washed by D-Hank's balanced salt solution supplemented with 2.5% bovine serum albumin, and centrifuged at low speed for 6 min, then the procedure was repeated three

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times. The cell concentration was adjusted to 0.5×10^6 cells/mL RPIM-1640 (Gibco) medium supplemented with 20% bovine serum and 2 mM Glutamine (Sigma). Greater than 90% viability of cells was documented by trypan blue exclusion. Approximately 0.5×10^6 cells per mL were plated and cultured in a 24-well plate (Corning) with the medium described above. The cells were grown at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 96 hours. Before starting a PRL release experiment, the medium in which the cell had been growing was removed, and the wells were washed twice with serum-free RPIM-1640 medium. Fresh test serum-free RPIM-1640 was added to each well. Various concentrations of test agents were added immediately. The cultures were then returned to the incubator for three hours. At the end of the incubation period, the test medium was removed for PRL determination.

Grouping (parallel 6 wells):

- | | |
|---------------------------|--|
| A. Control | B. NaF 0.053, 0.26, and 0.53 mM respectively |
| C. TRH, 100 nM | D. TRH/NaF (TRH, 100 nM) |
| E. VIP, 100 nM | F. VIP/NaF (VIP, 100 nM) |
| G. Metoclopramide, 100 nM | H. Metoclopramide/NaF (metoclopramide, 100 nM) |
- (NaF, 0.53 mM in groups D, F and H)

Determinations of PRL in serum and cultured medium: Prolactin was determined by RIA of double antibody. Prolactin RIA rat kits were kindly supplied by NIDDK (standard NIDDK-rPRL-RP-2). Parallel double samples.

Statistical analysis: Results are expressed as the Mean \pm SEM. Student's *t* test was used to compare any two specified groups for a significant difference at $p < 0.05$.

RESULTS

Serum PRL levels were increased after administration of quipazine, morphine, and TRH in control rats *in vivo*. In fluorosed rats, the three treatment drugs stimulated PRL secretion, but the PRL levels were much lower than that of control rats in the same periods after administration. Figure 1 shows that the serum PRL of fluorosed rats was lower than that of controls at 15 min ($p < 0.05$) and 30 min ($p < 0.01$) after injection of quipazine. Figure 2 shows that PRL release in fluorosed rats was significantly reduced at 5 min ($p < 0.05$), 15 min and 30 min ($p < 0.01$) after administration of morphine compared with control rats.

The data of Figure 3 show that the PRL secretion in rats with fluorosis was also decreased compared with the control rats at 5 min and 15 min ($p < 0.05$) after administration of TRH. The *in vivo* results indicated that PRL release response to quipazine, morphine, and TRH was decreased in rats with fluorosis.

The *in vitro* results show that PRL secretion from dispersed anterior pituitary cells was reduced by 0.26 mM ($p < 0.05$) and 0.53 mM ($p < 0.01$) sodium fluoride (Figure 4), while the stimulatory effects of TRH, VIP and metoclopramide on PRL release were inhibited by 0.53 mM sodium fluoride (Figure 5). The *in vitro* results indicate that sodium fluoride can directly inhibit PRL release from pituitary cells.

DISCUSSION

It is well known that pituitary PRL secretion is under the control of hypothalamic factors and neurotransmitters. 5-HT is a stimulating factor of pituitary PRL secretion, acting on the hypothalamus to stimulate the secretion of PRL-releasing-factor (PRF).^{8,9} EOP stimulates PRL release by decreasing the dopamine receptor

turnover rate and increasing the activity of the 5-HT system of the hypothalamus. Dopamine is the major PRL releasing inhibiting factor. From the *in vivo* experiment, we observed that the induced secretion of PRL by quipazine and morphine was reduced in fluorosed rats. This decreased PRL release from fluorosed rats to 5-HT receptor agonist and EOP receptor agonist indicates that the existing PRL regulating obstruction is in the hypothalamus-pituitary system under fluorosis conditions. TRH is the most important stimulating factor of

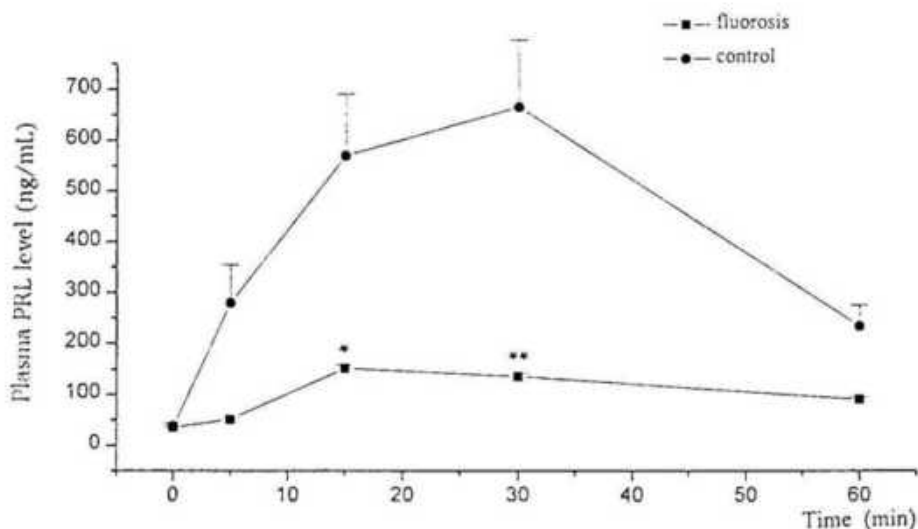


FIGURE 1. Change of serum PRL of rats with fluorosis after administration of quipazine. * $p < 0.05$, ** $p < 0.01$, compared with control

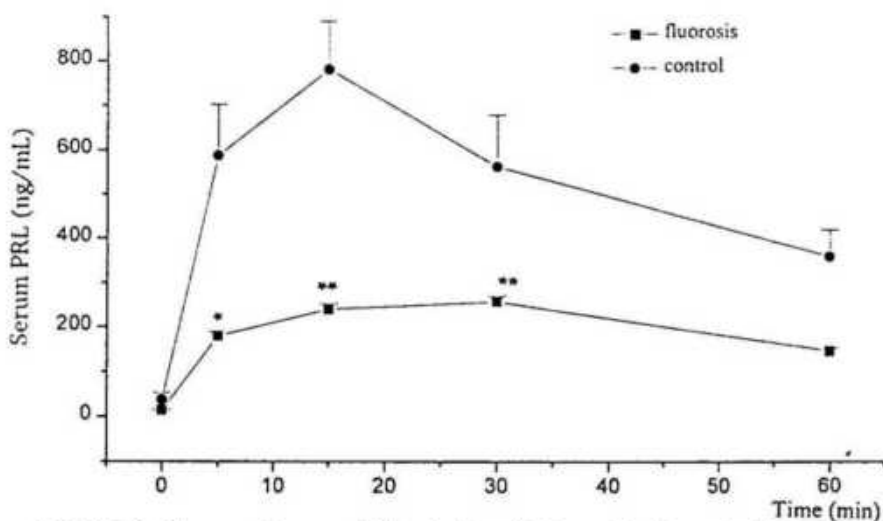


FIGURE 2. Change of serum PRL of rats with fluorosis after administration of morphine. * $p < 0.05$, ** $p < 0.01$, compared with control

pituitary PRL secretion. The mechanism of TRH stimulated PRL release is via direct action on TRH receptors on the pituitary cell membrane.^{10,11} In the present study, the induced PRL secretion by TRH was inhibited in fluorosed rats. The *in vivo* results suggest that PRL release was impaired in the pituitary of fluorosed rats. It may be that hypothalamic changes are the mechanism of inhibition of pituitary PRL secretion in rats with fluorosis.

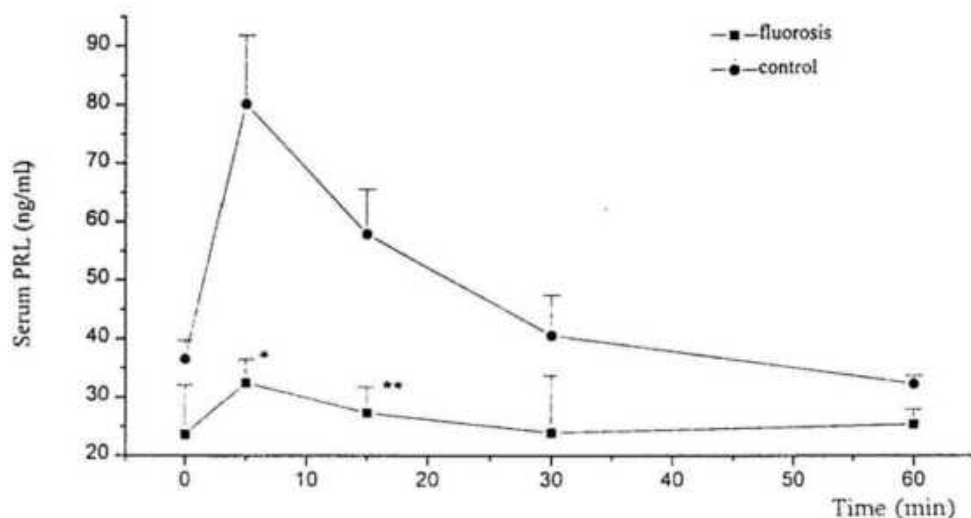


FIGURE 3. Change of serum PRL of rats with fluorosis after administration of TRH. * $p < 0.05$, compared with control

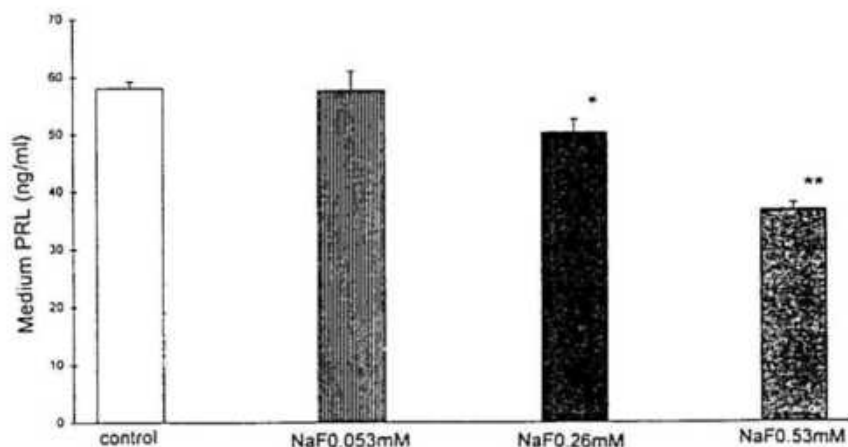


FIGURE 4. The inhibition by NaF on PRL basal secretion in anterior cells *in vitro*. * $p < 0.05$, ** $p < 0.01$, compared with control

In addition, TRH, VIP, and metoclopramide are known to stimulate PRL release from pituitary *in vitro*.¹²⁻¹⁵ The results in the present study were the same as others for normal control rats. But the induced secretion of PRL by TRH, VIP, and metoclopramide was decreased by addition of 0.53 mM sodium fluoride to the culture medium of cultured anterior pituitary cells. The results suggest that the cells' ability to respond to some PRL stimulating factors was lowered by sodium fluoride in dispersed anterior pituitary cells. The basal secretion of PRL from pituitary cells was also inhibited by sodium fluoride. The *in vitro* results were consistent with those *in vivo*.

All the above observations suggests that high level fluoride can work directly on the pituitary to inhibit PRL release. The hypothalamus may be the site at which fluoride blocks PRL secretion. Further research is necessary to determine the effects of fluoride on the hypothalamus and subsequent PRL release.

CONCLUSIONS

Induced secretion of PRL by quipazine, morphine, and TRH was reduced *in vivo* in rats with fluorosis. Sodium fluoride can directly inhibit PRL basal release. Further, induced secretion by TRH, VIP, and metoclopramide from cultured anterior pituitary cells was inhibited. The results of the present study indicate that PRL release was impaired in fluorosis. This impairment is thought to be caused by pituitary inhibition by sodium fluoride.

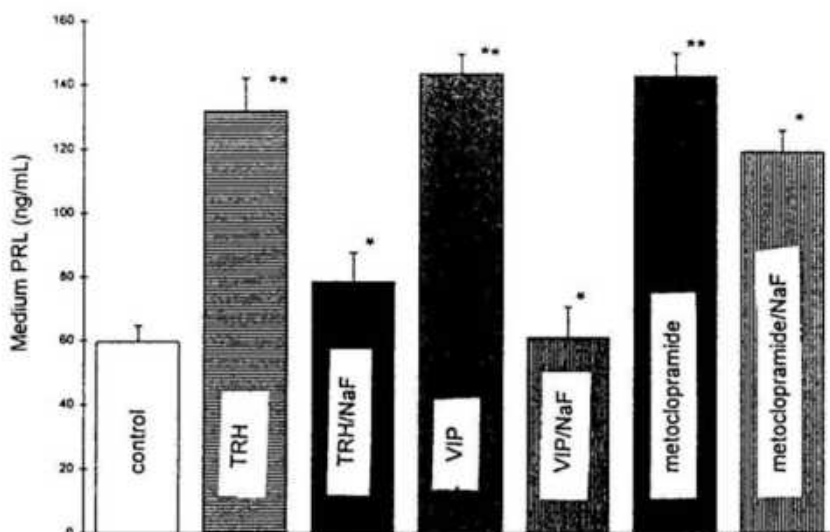


FIGURE 5. The inhibition by NaF on PRL secretion induced by TRH, VIP, and metoclopramide in anterior pituitary cells *in vitro*. * $p < 0.05$ compared with treatment reagent ** $p < 0.01$ compared with control

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NEW EVIDENCE ON FLUORIDATION

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ABSTRACT: A review of recent scientific literature reveals a consistent pattern of evidence – hip fractures, skeletal fluorosis, the effect of fluoride on bone structure, fluoride levels in bones and osteosarcomas – pointing to the existence of causal mechanisms by which fluoride damages bones. In addition, there is evidence, accepted by some eminent dental researchers and at least one leading US proponent of fluoridation, that there is negligible benefit from ingesting fluoride, and that any (small) benefit from fluoridation comes from the action of fluoride at the surface of the teeth before fluoridated water is swallowed. Public health authorities in Australia and New Zealand have appeared reluctant to consider openly and frankly the implications of this and earlier scientific evidence unfavourable to the continuation of the fluoridation of drinking water supplies.

In recent years, new scientific evidence has emerged which suggests that there are significant risks and negligible benefits from ingesting low levels of fluoride. We outline the evidence that fluoridation of water supplies is harmful to bone, while providing negligible benefits when swallowed.

In focussing on the new evidence (mostly since 1989) in just two areas, it is not intended to diminish the importance of earlier evidence for concern about the health hazards of fluoridation: notably dental fluorosis, allergies and intolerance reactions, and genetic damage. These are reviewed elsewhere.¹⁻³

Fluoride damages bones

Since 1990, five major epidemiological studies from three countries – the United States (US), United Kingdom and France – showing a higher rate of hip fractures in fluoridated regions than unfluoridated regions have been reported in leading peer-reviewed journals.⁴⁻⁸ Although two of these reports were published as letters, the first was a correction to a refereed publication⁹ and the second was a supplement to a refereed publication about a prospective study which took account of major individual risk factors.¹⁰ In addition, a prospective study from the US shows a higher rate of hip fractures in a region naturally fluoridated with four parts per million (ppm) fluoride in drinking water than in a comparison region with 1 ppm.¹¹ Although there have been a few studies that have found no difference between fluoridated and unfluoridated regions, they have been either limited to small samples, or the women were not exposed

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to fluoride during the time of their lives when fluoride would be expected to affect bone most, that is, before menopause.¹²

The main weight of the recent evidence on hip fractures is consistent with earlier evidence from naturally fluoridated areas that low levels of fluoride ingested for several decades can cause the disease of bones and joints known as osteofluorosis or skeletal fluorosis. Evidence of skeletal fluorosis has been reported in at least nine studies from five countries with fluoride concentrations in drinking water of 0.7 to 2.5 ppm. These studies, and the inadequacies of studies that assert that there is no skeletal fluorosis in the US at fluoride concentrations below 4 ppm, have been reviewed elsewhere.²

In three to four decades, when people in areas where water is artificially fluoridated have accumulated fluoride in their bones from birth to old age, the increase in rates of hip fractures and skeletal fluorosis will be larger.

Fluoride has been used in high doses (20 to 32 mg a day) for short periods (one to two years) to treat osteoporosis. It is now recognised widely that, while this therapy adds mass to bones, it also damages the bone structure and leads to a higher risk of hip fracture.¹³⁻¹⁸ Bone analyses have shown that elderly women who lived for at least a decade in the town of Kuopio, Finland, with 1 ppm fluoride in its water supply, had high levels of fluoride in bone (typically 900 to 2300 ppm, but for women with impaired kidney function, as high as 3890 ppm).^{19,20} These levels are as high as have been reported in patients who have undergone fluoride therapy for osteoporosis.²¹

In the US National Cancer Institute's Surveillance, Epidemiology and End Results Program, an increase of 79 per cent was found in the incidence of osteosarcomas in young men living in fluoridated areas of Iowa and Seattle, but not in the unfluoridated areas, where the incidence decreased by 4 per cent.²² In fluoridated regions of the State of New Jersey, the incidence of osteosarcoma was three to seven times higher among males aged 10 to 19 years than in unfluoridated regions.²³ Osteosarcoma is a rare disease and so more evidence is required before any conclusions are drawn. But there is already a strong basis for concern, because the human data are supported by an animal experiment: the US National Toxicology Program has recorded a statistically significant, dose-related increase in the incidence of osteosarcoma in male rats ingesting fluoride.^{24,25}

Thus, there is a consistent pattern of evidence – hip fractures, skeletal fluorosis, the effect of fluoride on bone structure, fluoride levels in bones, and osteosarcomas – pointing to the existence of causal mechanisms by which fluoride damages bones.

Negligible benefit from fluoride ingestion

Recent research on the mechanism of action of fluoride in reducing the prevalence of dental caries (tooth decay) in humans shows that fluoride acts topically (at the surface of the teeth) and that there is negligible benefit in actually ingesting it.²⁶⁻²⁸ This is supported by experiments on laboratory rats: a slow-release source of fluoride fixed in the mouth reduced dental caries, but

when the mouth was bypassed by placing the source under the skin, there was no detectable reduction.²⁹ The lack of observed systemic benefit from ingesting fluoridated water at a concentration 1 ppm is not surprising, since the resulting level of fluoride in the saliva is only around 0.01 ppm.³⁰

The evidence that there is negligible systemic benefit from fluoridation is accepted by eminent dental researchers²⁶⁻²⁸ and at least one leading US proponent of fluoridation, Professor Brian Burt.³¹ Therefore, proponents must come to grips with a serious ethical question: is it right to put fluoride in *drinking* water and to mislead the community that fluoride must be ingested, when any small benefit is due to the topical action of fluoride on teeth.³²

Alleged benefit from fluoride

We say 'any small benefit', because the results of recent large-scale studies in at least three countries show that, when similar communities are compared and the traditional *DMFT* (number of decayed, missing and filled teeth) index of dental caries is used, there is no detectable difference in caries prevalence. This has been demonstrated for schoolchildren in the major cities of New Zealand, Australia, the US and elsewhere.³³⁻³⁸ (When the newer *DMFS* (number of decayed, missing and filled surfaces) index was used, a 20% reduction was reported for US,³⁹ but, in absolute terms, this is only a fraction of a cavity per child.)

Of the many studies used by proponents of fluoridation to claim that there are enormous benefits from fluoridation, not one is a randomised controlled trial. Those that have been re-examined have been found to have serious design flaws.^{38,40-44} Indeed, hardly any of the many small-scale studies by enthusiasts of fluoridation are 'blind' and, in the rare cases when they are, the so-called 'control' was selected from a known high-caries area.⁴³ Many studies have also failed to take into account that unfluoridated towns tend to be rural, while fluoridated towns tend to be large cities, and that there is generally more dental caries in rural areas, irrespective of fluoridation status. In general, diet tends to be better in urban areas.

Many other studies have had no controls. Their authors have justified their profluoridation conclusions on the basis of large temporal declines in tooth decay. But, it is now known that equally large declines in caries have taken place in unfluoridated areas,⁴⁵⁻⁴⁸ and that in several cases this decline commenced before fluoride in any form was used to a significant degree.^{47,48}

However, there is now abundant evidence that topical uses of fluoride, extensively practised in Europe instead of water fluoridation, are effective in controlling tooth decay.⁴⁹ We agree that their cautious uses in dentistry are justified and provide an alternative to fluoridation which satisfies ethical concerns.³² However, in the past they have been promoted and practised rather irresponsibly – for example, the provision of highly concentrated fluoride tooth-pastes and mouth rinses to young children who inevitably ingest much of the fluoride. Too often overlooked is the evidence that tooth decay is associated with inadequate diets,⁵⁰ and that dietary control of caries, without the use of fluoride, is possible.⁵¹

Bias of health authorities

In our view, the evidence indicates that fluoridation entails real health risks and at best very small benefits. Therefore, the fluoridation of water supplies should be terminated forthwith. Yet, both in Australia and New Zealand, health authorities appear to be redoubling their efforts to fluoridate the remaining towns that have so far managed to hold fluoridation at bay.

The 1991 report on fluoridation by the National Health and Medical Research Council was published just as the first papers reporting the link between fluoridation and hip fractures were being published.⁵² It acknowledged in its section 6.4 some of the evidence that skeletal fluorosis is a potential health hazard, but created the false impression in its executive summary that there are no health risks. It is the executive summary which is read by decision-makers and the media. The report's profluoridation bias was further demonstrated by its failure to cite any of the studies presenting the evidence against fluoridation published in refereed journals.

The 1995 Report to the Minister by the New Zealand Public Health Commission demonstrated similar bias by failing even to cite any of the published papers on hip fractures, skeletal fluorosis or osteosarcomas.⁵³ However, the 1994 New Zealand Public Health Commission report did include some of these references and did acknowledge that:

It is possible that there is a small increased risk of hip fracture associated with water fluoridation, though the evidence for this is very inconclusive. More research is required to clarify this issue. A large amount of research has failed to provide evidence that exposure to fluoride causes cancer. However, the possibility of a small increased risk of osteosarcoma (a rare type of bone cancer) in young men cannot be ruled out at this stage. Here again, more research is needed. [From the executive summary; there are similar statements on p. 74 and p 78.]⁵⁴

But this information, and the references supporting it, were not forwarded officially to the Minister.

One of us (DE), while Federal Minister for Health in Australia from 1972 to 1975, could not get frank answers from his own department on the risks and benefits of fluoridation. Another of us (JC), while convenor of the New Zealand Fluoridation Promotion Committee, observed at first hand how his then fellow proponents of fluoridation kept from the public and decision makers the evidence that fluoridation is less effective than claimed by proponents and is harmful, and then represented the evidence in a misleading way when it was eventually released. All of us have observed attempts by the medical and dental establishment in profluoridation countries to evade the evidence of concern and to suppress and misrepresent scientists, medical practitioners and dentists who attempt to publish evidence against fluoridation.⁵⁵⁻⁵⁷

For these and other reasons, we have no confidence in the impartiality of those institutions of government and the professions which have endorsed fluoridation for decades. Those who have built their careers and professional status on fluoridation cannot credibly assess the evidence against it. We have submitted this short paper for publication in the hope that at least some kind of scholarly debate will ensue.

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FLUORIDE CONTENT OF FEMORAL CORTICAL AND TRABECULAR BONE IN FEMALE PATIENTS WITH COXARTHROSIS

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In this study, the relationship between bone fluoride concentration, bone quality and age was studied in 48 female patients aged 36-73 years (mean, 60 years) operated on for coxarthrosis. The fluoride concentrations were measured in cortical and trabecular bone samples taken intraoperatively from resected femoral head and neck. Patients receiving NaF in therapeutic doses and professionally exposed to fluorides were not included in the study.

Bone mineral density measurements of femoral neck were performed pre-operatively by dual energy x-ray absorptiometry with Lunar DPX-L apparatus. The fluoride concentration was measured with Orion Fluoride Ion Selective Electrode after dissolving the prepared bone pieces in perchloric acid.

Fluoride concentration in trabecular bone ranged from 369 ppm to 2669 ppm (mean 915 ppm) and was significantly higher than in cortical bone where it ranged from 269 ppm to 2114 ppm (mean 784 ppm) ($p < 0.001$).

Neither the age nor the bone mineral density measured in the Ward triangle and femoral neck correlated with the fluoride concentrations measured in trabecular bone of the femoral head and cortical bone of the femoral neck. On the other hand, the bone mineral density results showed a typical age-related decrease. It remains unknown whether the observed "stabilisation" of fluoride concentrations is due to local pathology (coxarthrosis) or to the patients' age (mean 60 years).

Key words: Bone fluoride content; Coxarthrosis.

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DIAGNOSING FLUOROSIS BY COMPUTER ANALYSIS OF RADIOGRAPH IMAGES

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This study aimed at establishing an objective method for measuring the parameters of bone structure on radiographs and applying it to the diagnosis of fluorosis.

The method is based on digitization of the standard radiograph and then image analysis using computer programs. The radiograph is recorded using either IBM PC equipped with a CCD TV camera or scanner, or alternatively by a professional image analyser. The image is recorded in 265 grey levels with resolution of 0.096 mm/pixel. Two separate programs were developed.

The authors' own program, "Trabecula", was compiled for IBM PC (DOS or Windows). It identifies trabeculae on the image and computes their parameters: numbers, width, height, area and density.

The second program, "Quantitab", was developed by applying machine procedures of the Quantimet 570 Image Analyzer. The program detects "non-

trabecular" zones in the radiographs and computes parameters like number, surface, perimeter, horizontal intercept, vertical intercept, length, and anisotropy.

The survey was conducted on 211 radiographs of the distal metaphysis of the radius, selected from those made in 1988 of employees of the Aluminium Works near Krakow. A complete clinical check-up, orthopaedic examination and set of radiographs were made in each case. Patients in the selected group had worked under high exposure to fluoride for long periods (average 18.4 yr).

Every radiograph was assessed by both programs, Trabecula and Quantitab. The bone structure parameters were analysed for: age, magnitude and duration of exposure from living in the polluted neighbourhood. Comparisons were made of aluminium workers and a control group. Various relationships were found. We concluded that the typical features of fluorosis shown on the radiographs were: widening and decreased number of trabeculae, and lower density. The number of non-trabecular zones is increased, and the area of the trabeculae is decreased, in fluorosis.

Key words: Computer analysis; Fluorosis.

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MACROSCOPIC CHANGES OF RAT STOMACH AND GASTRIC MUCOSA FOLLOWING SINGLE FLUORIDE DOSING

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The first sign of acute fluoride (F) toxicity after ingestion of F is gastric discomfort. Gastric irritations, e.g. nausea, and vomiting, may be caused by direct effects on the gastric mucosa. These changes were investigated by gastroscopy in human cases, and by histological methods in experimental animals. The aim of the present study was to observe the gross changes to the stomach and gastric mucosa following single fluoride dosing. Groups of female Wistar rats, weighing between 160 and 180 g, were given 10, 25, 50, 100 and 200 mM NaF and Na₂PO₃F(MFP) into the stomach by a gastric needle after 15 hours of fasting. All solutions were administered in a dose of 1 mL/100 g body weight. The control group received the same concentration of NaCl. One hour after administration, the rats' abdomens were opened under anesthesia. Color photographic records of the stomach and gastric mucosa were taken.

Macroscopically, the stomachs of the experimental groups administrated over 50 mM of NaF and 100 mM of MFP were distended and filled with a large amount of watery fluid (pH was nearly 7.2 using test paper), and the entire surface of the gastric mucosa showed a bright red color which became darker according to the increase in F concentration. There were no similar changes in the control group. These changes were visible to the naked eye, and suggest that histological changes in the tissues also occurred.

Several microscopic experiments have been reported concerning changes in rat stomachs after F administration, but until now there appear to have been no macroscopic studies.

Key words: Fluoride dosing; Gastric mucosa; Stomach; Rat.

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HALIDE IONS IN RIVER WATERS IN THE KUSATSU-SHIRANE VOLCANO AREA, GUNMA [Japanese]

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Abstract from *Nippon Kagaku Kaishi* (3) 194-200 1997

Concentrations of halide ions in river waters in the Kusatsu-Shirane volcano area, Gunma, were measured by ion chromatography. The decreasing order of concentration among halide ions at various sampling locations is in general: chloride ion > fluoride ion > bromide ion > iodide ion. A strong correlation was observed between the concentrations of the chloride and bromide ions. High halide ion concentrations were observed in river waters indicating that the volcanic activity of Mt. Kusatsu-Shirane is the major source of halide ions in river waters of the area. Measurements of halide ion concentrations along the downward streams of selected rivers revealed that concentration ratios among halide ions did not vary substantially in the absence of any circumstantial change. The amounts of halide ions transported from the Kusatsu-Shirane volcano area by rivers per year were estimated to be 1.4×10^7 mol for the fluoride ion, 3.8×10^8 mol for the chloride ion, 4.1×10^5 mol for the bromide ion and 1.0×10^5 mol for the iodide ion.

Key words: Halide ions; Japan; River waters; Volcano.

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ACCUMULATION OF AIRBORNE FLUORIDES IN FOREST TREES AND VEGETATION

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Abstract from *European Journal of Forest Pathology* 27 (2) 73-82 1997

The accumulation of fluoride in natural vegetation exposed to emissions from active aluminium smelter plants in Norway was studied during the years 1990-93. About 2000 leaf, bark and twig samples of 60 plant species, collected mostly during the growing season, were analysed. Rowan (*Sorbus aucuparia*) was widespread and common in the areas studied, and was used as a reference species. Fluoride concentrations in monthly samples of rowan leaves were linearly related to fluoride exposure (average fluoride concentration in ambient air \times days since leaf emergence). The accumulation coefficient for rowan was estimated to be $1.7 \text{ m}^3/\text{g dry wt. day}$. Most other species had values between 0.3 and 1.5; the median for all species was 0.8. The fern *Dryopteris filix-mas* was exceptional, containing on average three times greater fluoride concentrations than rowan. High background levels indicated that soil uptake contributed significantly to the fluoride accumulation in this species. The fluoride concentrations in bark and shoots of trees were mostly low compared with leaves, but the bark of *Betula pendula* and *B. pubescens* had very high concentrations.

Key words: Airborne fluoride; Forests; Plants.

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A COMPARISON OF THE EFFECTS OF TWO ANABOLIC AGENTS (FLUORIDE AND PARATHYROID HORMONE) ON ASH DENSITY AND BONE STRENGTH ASSESSED IN AN OSTEOPENIC RAT MODEL*

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Abstract from *Bone* 20 (5) 439-449 1997

The aim of this investigation was to compare the effects of sodium fluoride (NaF) and parathyroid hormone (PTH) on ash density and strength in an osteopenic rat model. The study comprised 66 female virgin rats divided into the following 11 groups, each comprising six animals: baseline controls; baseline ovariectomized (ovx); intact controls (5 and 16 weeks), ovx controls (5 and 16 weeks); ovx-treated with PTH (0.02 mg/kg per day, 5 and 16 weeks); ovx treated with NaF (10 mg/kg per day, 5 and 16 weeks); ovx-treated with NaF (1.0 mg/kg per day, 16 weeks). Ovariectomy was performed at 12 weeks of age, 14 weeks prior to start of treatment. Ash density, bone fluoride content, and biomechanical analyses were performed on femoral cortical bone, the right femoral neck, and the sixth lumbar vertebral body. Ovariectomy had no effect on cortical bone, whereas the femoral neck displayed a significantly lower bone strength in ovx baseline animals compared with intact baseline rats ($p < 0.05$). Vertebral ash density was found to be significantly decreased in ovx rats after 5 and 16 weeks ($p < 0.05$). Treatment with fluoride had little effect on the osteopenic rat skeleton. Cortical ash density was significantly lower than ovx and intact groups in the high-dose-treated rats after 5 ($p < 0.01$) but not after 16 weeks. High doses of fluoride for 16 weeks induced a significant increase in maximum load and normalized strength in cortical bone when compared with intact animals ($p < 0.05$), but not at the other bone sites. Cortical bone strength was not different from the ovx animals at either timepoint. In fluoride-treated animals, femoral neck bone strength, vertebral body bone strength, bone quality, and ash density were found to be at about ovx levels and, in the vertebral body, significantly lower than intact animals ($p < 0.05$, $p < 0.01$). In contrast, treatment with PTH increased ash density, bone strength, and bone quality to above ovx levels ($p < 0.01$), and above the level of the intact animals also, although significant values were reached for cortical bone strength only ($p < 0.01$). Additionally, biomechanical competence and ash density measurements were significantly higher in PTH-treated rats compared with fluoride-treated rats. In conclusion: this study has shown that PTH has a highly anabolic effect and is capable of effectively restoring ovx-induced loss of bone mass and biomechanical competence. In addition, in this osteopenic rat model, PTH proved much more advantageous than treatment with fluoride, which failed to restore the ovx-induced loss of bone strength.

Key words: Anabolic effect; Bone quality; Bone strength; Parathyroid hormone (PTH); Sodium fluoride; Rat.

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* For earlier report, see *Fluoride* 30 (2) 119-121 1997

MINERALIZATION OF CANCELLOUS BONE AFTER ALENDRONATE AND SODIUM FLUORIDE TREATMENT - A QUANTITATIVE BACKSCATTERED ELECTRON IMAGING STUDY ON MINIPIG RIBS

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Abstract from *Bone* 20 (5) 393-397 1997

Fluoride stimulates bone formation, whereas bisphosphonates reduce bone resorption. In clinical trials, both treatments increase bone density, although sodium fluoride (NaF) increases and alendronate (bisphosphonate, ALN) decreases bone turnover. In a comparative study using minipigs an inverse correlation has been reported between bone turnover and elastic modulus. Small-angle X-ray scattering (SAXS) measurements of these bones revealed no structural deterioration of the collagen/mineral composite at the nanometer range for ALN-treated vertebra, whereas a slight increase of the average thickness of the mineral crystals as well as changes of the structure of the collagen/mineral composite were found in the bones of NaF-treated animals. In this study we used quantitative backscattered electron imaging (qBSE) to investigate the cancellous bones from ribs of minipigs treated with vehicle, NaF, or ALN. This method provides information on the local mineral concentration in the micrometer range. Mineralization spectra were obtained from each treatment group, and statistically significant differences between ALN and controls were found for the peak position, the peak height, the peak width, and the average calcium (Ca) concentration of the mineral distribution. The results reveal that the cancellous bone matrix was more uniformly mineralized after ALN treatment. The reduced bone turnover induced by ALN, documented histomorphometrically could be at the origin of this phenomenon. No significant differences were detected between NaF and control. Together with the earlier SAXS data these results may explain in part the increase in bone density and the improvement of biomechanical properties observed after ALN treatment in animals and in osteoporotic patients.

Key words: Alendronate; Bone; Cancellous bone; Minipigs; Mineral concentration
Quantitative backscattered electron imaging; Sodium fluoride.

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FLUORIDE AT MITOGENIC DOSES INDUCES A SUSTAINED ACTIVATION OF P44(MAPK), BUT NOT P42(MAPK) IN HUMAN TE85 OSTEOSARCOMA CELLS

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Abstract from *Journal of Clinical Endocrinology and Metabolism* 82 (4) 1126-1135 1997

Fluoride, at micromolar concentrations, stimulates bone cell proliferation *in vitro*. In this study, we sought to test whether fluoride at mitogenic dose increases the tyrosyl phosphorylation level and specific activity of a mitogen activated protein kinase (MAPK) in human TE85 osteosarcoma cells. Analysis by immunoprecipitation with antiphosphotyrosine antibody followed by Western

analysis using an anti-pan extracellular signal-regulated kinase antibody revealed that fluoride at the optimal mitogenic dose (i.e. 100 $\mu\text{mol/L}$) induced a time-dependent increase in the steady-state tyrosyl phosphorylation level of p44(mapk), but not p43(mapk). With the maximal increase (4- to 13-fold) after 1-3 h fluoride treatment. The effect was sustained in that a 9-fold increase was seen after 12 h of the fluoride treatment. The sustained nature of the effect is consistent with an inhibition of dephosphorylation rather than a direct stimulation of phosphorylation. The fluoride effect on the tyrosyl phosphorylation level of p44(mapk) was dose dependent, with the optimal dose being 100 $\mu\text{mol/L}$ fluoride. The mitogenic dose of fluoride also increased the specific activity and the in-gel kinase activity of p44(mapk), but not that of p42(mapk), in a time-dependent manner similar to the effect on the p44(mapk) tyrosyl phosphorylation level. Fluoride at the same micromolar doses did not increase cell proliferation, tyrosyl phosphorylation, or specific activity of any MAPK in human skin foreskin fibroblasts, which are fluoride-nonresponsive cells. Consistent with the interpretation that the effect of fluoride on the steady state tyrosyl phosphorylation level of p44(mapk) is a consequence of an inhibition of a phosphotyrosyl phosphatase (PTP), mitogenic doses of orthovanadate, a bone cell mitogen and a PTP inhibitor, also increased the steady state tyrosyl phosphorylation level of p44(mapk), but not p42(mapk), in a time-dependent sustained manner similar to that observed with fluoride. Together, these findings support the concept that inhibition of a PTP activity in bone cells could lead to an activation of MAPK activity.

Key words: Mitogen-activated protein kinase (MAPK); Mitogenic fluoride dose; Osteosarcoma cells.

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ORGANIC FLUORINE HARDLY EVER ACCEPTS HYDROGEN BONDS

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Abstract from *Chemistry - A European Journal* 3 (1) 89-98 1997

Statistical analysis of structural data and detailed inspection of individual crystal structures culled from the Cambridge Structural Database and the Brookhaven Protein Data Bank show that covalently bound fluorine (in contrast to anionic fluoride) hardly ever acts as a hydrogen-bond acceptor. The weakness of covalently bound fluorine as hydrogen-bond acceptor is backed by results of new molecular orbital calculations on model systems using ab initio intermolecular perturbation theory (IMPT), and is in accord with results of other physico-chemical studies and with the physical properties of fluorinated organic compounds. Factors influencing the strength of hydrogen bonding in extended systems are discussed.

Keywords: Ab initio calculations; Cambridge structural database; Chemistry; Fluorine compounds; Hydrogen bonds; Protein data bank.

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RANDOMIZED CLINICAL TRIAL OF THE EFFECT OF PRENATAL FLUORIDE SUPPLEMENTS IN PREVENTING DENTAL CARIES

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Abstract from *Caries Research* 31 (3) 174-179 1997

This randomized, double-blind study tested the caries-preventive efficacy of prenatal fluoride supplementation in 798 children followed until age 5. Initially, 1,400 women in the first trimester of pregnancy residing in communities served by fluoride-deficient drinking water were randomly assigned to one of two groups. During the last 6 months of pregnancy the treatment group received 1 mg fluoride daily in the form of a tablet and the control group received a placebo. Both treatment and control subjects were encouraged to use postnatal dietary fluoride supplements. Caries was measured in children at ages 3 and 5 while fluorosis was assessed at age 5. Caries activity was very low in both study groups: 92% of children remained caries-free in the treatment group and 91% remained caries-free in the placebo group. Fluorosis was observed in 26 subjects, all classified as very mild. Overall, there were no statistically significant differences in the study groups with respect to caries and fluorosis in deciduous teeth. The study had sufficient power to detect an absolute risk reduction of 5.1% while only a 1.5% reduction was observed. These findings do not support the hypothesis that prenatal fluoride has a strong caries-preventive effect.

Key words: Caries, deciduous; Dental fluorosis; Dietary fluoride supplements; Prenatal fluorides.

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DENTAL FLUOROSIS AND THE USE OF A HIGH FLUORIDE-CONTAINING TRONA TENDERIZER (MAGADI)

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Abstract from *Community Dentistry and Oral Epidemiology* 25 (2) 170-176 1997

It has recently been suggested that magadi, a high-fluoride trona, which is added in cooking to tenderize certain vegetables and beans in two villages in Tanzania, significantly contributed to the prevalence and severity of dental fluorosis. This report aims to substantiate the significance of magadi as a determinant of dental fluorosis. Eighteen villages in four geographical areas (districts) with water supplies containing 0.2 to 0.8 mg/L of fluoride were selected. All schoolchildren aged 12 to 17 years ($n=1566$) who had been born and raised in these villages were examined for dental fluorosis according to the Thylstrup-Fejerskov Index. Dietary history was recorded. The fluoride content of magadi samples was determined and the urinary fluoride excretion of pre-schoolchildren was assessed. The prevalence of dental fluorosis in nine coastal villages where tea and seafish were regularly consumed ranged from 7% to

46%. Severe (pitting) dental fluorosis was rarely seen. The low fluorosis levels observed in non-magadi consuming communities in coastal villages indicate that a fluoride content of up to 0.8 mg/L in drinking water is acceptable under the prevailing conditions of temperature and diet. In contrast, the prevalence of dental fluorosis in nine villages located inland at 1500 m altitude, where fluoride-containing magadi was consumed, ranged from 53% to 100%, and severe (pitting) fluorosis was highly prevalent, ranging from 18% to 97%. The village with the highest fluoride content in the magadi samples collected showed the highest level of fluorosis. The urinary fluoride excretion of pre-schoolchildren from different villages corresponded with the level of fluorosis and the fluoride content in the magadi samples of the respective villages. Data on dental fluorosis from the magadi-consuming communities provide strong evidence that consumption of magadi was the major determinant of the observed high prevalence and severity of fluorosis in inland villages at 1500 m altitude.

Key words: Dental fluorosis; F-containing tenderizer; F-trona; Magadi.

Reprints: W H V Helderma, WHO Collaborating Center for Oral Health Care Planning, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands.

RISE AND FALL OF CARIES PREVALENCE IN GERMAN TOWNS WITH DIFFERENT F CONCENTRATIONS IN DRINKING WATER

W Kunzel and T Fischer
Erfurt, Germany

Abstract from *Caries Research* 31 (3) 166-173 1997

The rise and fall of caries prevalence (DMFT) and its relation to changing F concentration of drinking water and other health-related factors is analysed based on dental findings of more than 286,000 subjects of either sex (6-15 years old) from the two industrial towns Chemnitz and Plauen. Water fluoridation (1.0 ± 0.1 ppm F) was implemented in Chemnitz (formerly Karl-Marx-Stadt) in 1959. It was in operation until autumn 1990 with an interruption lasting 22 months around the year 1971. In the F-poor town of comparison, Plauen, 55% of the citizens were supplied with F-enriched drinking water (0.9 ppm F) during the years 1972-1984. Another 20% received F-containing mixed water (0.4- 0.7 ppm F). During the first three decades of the study the level of caries prevalence was strictly correlated with the availability of an optimal caries preventive F concentration in the drinking water. Water fluoridation was followed by a decrease of caries, and interruptions in fluoridation were followed by increasing caries levels. A different caries trend was observed in the years from 1987 to 1995. There was a significant caries decrease down to the lowest DMFT (2.0) since 1959 in spite of the fact that only F-poor water was available over years in both towns. This improvement of oral health is explained by changes in caries-preventive and environmental conditions.

Key words: Caries prevalence; Children; Fluoridation; Germany; Water fluoride.

Reprints: W Kunzel, School of Dentistry, Nordhauser Str 78, D-99089 Erfurt, Germany.

SALIVARY FLUORIDE CONCENTRATION IN ADULTS AFTER DIFFERENT FLUORIDE PROCEDURES

L Seppa, S Salmenkivi and H Hausen
Aapistie, Finland

Abstract from *Acta Odontologica Scandinavica* 55 (2) 84-87 1997

Today, several alternatives for fluoride therapy are available. To give advice on the choice of method, the dentist should have information on how effective different fluoride treatments are in increasing salivary fluoride concentration. The aim of the present study was to measure the fluoride concentration of saliva after the use of four different fluoride methods commonly used in the Nordic countries: F mouthrinse (0.023% F), F toothpaste (1.1% F), F lozenge (0.25 mg F), and F chewing guru (0.25 mg F). In addition, a new method using toothpaste-water mixture as a mouthrinse was included in the study. Fourteen adult volunteers used each of the five methods on separate days. Unstimulated saliva samples were collected at base line and 0, 10, 20, 30, 45, and 60 min after the fluoride procedure. Fluoride was separated by the microdiffusion method and analyzed using a fluoride-specific electrode. Fluoride mouthrinse and fluoride toothpaste increased the fluoride concentration of saliva significantly more than fluoride lozenge and fluoride chewing gum. For both of the latter, salivary fluoride concentration was still increased after 1 h. Toothpaste-water rinse was more effective than brushing with toothpaste. Rinsing with toothpaste-water mixture appears a good alternative for adults who need extra fluoride therapy but are not motivated enough to brush their teeth several times a day.

Key words: Preventive dentistry; Salivary fluoride; Toothpaste.

Reprints: L Seppa, University of Oulu, Institute of Dentistry, Aapistie 3, FIN-90220, Oulu, Finland.

There continue to be published in the dental literature articles on methods of supplying fluoride topically, to reduce tooth decay, which must result also in fluoride ingestion, e.g. J Tenovou, T Hurme, A Ahola, C Svedberg, I Ostela, M Lenanderlumikari, M Neva. Release of cariostatic agents from a new buffering fluoride- and xylitol-containing lozenge to human whole saliva *in vivo*. *Journal of Oral Rehabilitation* 24 (5) 325-331 1997. Reprints: J Tenovou, University of Turku, Institute of Dentistry, Department of Cariology, Lemminkaisenkatu 2, FIN-20520 Turku, Finland.

- Editor

CORRECTIONS: In our last issue (May 1997) the following corrections are required: TOXICITY FROM WATER CONTAINING ARSENIC AND FLUORIDE IN XINJIANG by G Q Wang *et al.* On page 83, 8th line from the top, the number should read 0.6 mg/L, not 0.06 mg/L.

SKELETAL CHANGES WITH TOXICITY FROM FLUORIDE AND ALUMINUM by X G Chen *et al.* In the Introduction (page 85) the name "Yang" should read Wang, as in the reference number 4.

ASSESSING FLUORIDE CONCENTRATIONS OF JUICES AND JUICE-FLAVORED DRINKS by M C Kiritsy *et al.* The address for reprints (page 127) should be: Prof Steven M Levy, Department of Preventive and Community Dentistry, College of Dentistry, The University of Iowa, 329 Dental Science North, Iowa City, Iowa 52242-1010, USA.

POISON PASTE

Warning Labels Will *Make You Brush With Care*

Don Oldenburg

The author, a *Washington Post* Staff Writer, reports on the new warning labels required by the Food and Drug Administration on all fluoride toothpastes and dental care products shipped as of April 7. The warnings include one that reads:

"If you accidentally swallow more than used for brushing, seek professional help or contact a poison control center immediately."

The article points out that none of the caveats that began appearing on toothpaste tubes in 1991 had so candidly broached the risks of ingesting too much fluoride. The general warnings on toothpaste products that displayed the American Dental Association seal of approval had heretofore cautioned: "Don't Swallow - Use only a pea-sized amount for children under six," and "Children under 6 should be supervised while brushing with any toothpaste to prevent swallowing." The word "poison" had not been used.

A director of research and development at the laboratories of one of the toothpaste makers is quoted, commenting on containers of the chemical:

"When I receive the fluoride here, it has a skull-and-bones on it ... If a child was to take a big spoonful of this fluoride, I don't think he could swallow it, but if he did get it down, it is a poison and the child could die. If a child ingested a whole tube of toothpaste, he should be taken right to the emergency room and he would either get his stomach pumped or get some kind of antidote."

She listed three ingredients found in most toothpastes which pose health risks if too much is ingested: sorbitol, a liquid that keeps toothpaste from drying out, is a laxative that could cause diarrhea in children; sodium lauryl sulfate, an ingredient that makes toothpaste foam, can also be a diarrhetic; but the fluoride poses the most danger if too much toothpaste is swallowed - particularly to younger children.

"Small amounts of this material go a long way in causing disruption in their bodies because they are so small. The fluoride in toothpaste is considered a drug. Even though it is an over-the-counter drug, we are altering the body when we brush our teeth with a fluoride toothpaste or tooth gel. ... As normal consumers, you're not aware of these things. But I'm sure our 800 number is going to get more calls as products with the new warnings show up on store shelves."

The article suggests that this summer, as toothpaste shipments with the new labeling replace older inventories, consumers will see nearly twice the warnings displayed on the back of tubes and cartons - the ADA's general warnings along with the new FDA-required statement that starts with: "Keep out of the reach of children under 6 years of age."

Commenting on research which has shown that, because they are not yet in control of their swallowing reflex, children 4 to 6 years old typically swallow toothpaste when brushing, the director stated: "That's why it's recommended that kids get only a pea-size amount of toothpaste, because most of that goes down their throats." A 1995 study at the Medical College of Georgia School of Dentistry had found that about half the children this age do not spit out or rinse out – they swallow the toothpaste instead. Making matters worse, they tend to use too much toothpaste on their own – especially when they use flavored children's toothpastes.

While agreeing that the cavity-preventing effectiveness of fluoride has been demonstrated, the article notes that too much fluoride not only can be dangerous, but can also cause dental fluorosis that discolors or spots developing teeth. Research conducted by the School of Dental Medicine at the University of Connecticut Health Center had concluded that brushing with more than a pea-size amount of toothpaste more than once daily contributed to most of the fluorosis cases it observed in young children. In areas where the drinking water contains fluoride, children who swallow even the pea-size amount of toothpaste are getting too much fluoride and are at risk for fluorosis.

The vice president of "corporate communication and market development" of a small company which in 1975 introduced the first "natural" toothpaste on the market, is quoted: "I haven't heard of problems beyond fluorosis, but that's a valid concern. There are some kids getting too much fluoride ... " Besides its "natural" toothpastes that contain fluoride, the company makes a nonfluoride toothpaste in flavors including "cinnamint" and "fennel." When it recently began marketing its new line of natural toothpaste for children, it left out the synthetic sweeteners, neon colors and bubble gum flavors. But the toothpastes, called Silly Strawberry and Outrageous Orange, contain the same levels of fluoride as competitors' toothpastes. It was explained: "It is always kind of a trade-off. We made a decision to have only fluoride toothpaste for children because that has been proven to be the overall benefit of toothpaste for children. We feel the benefit outweighs the negative ... You have to get the education across to your kids that you don't suck the toothpaste down, just as you have to work with your kids to brush their teeth. The alternative is they don't brush. I guess we don't feel like the risk factor is that high to make that trade-off."

The article reports that many in the toothpaste industry feel the new FDA warnings may be overstating the risks. An ADA spokesman is quoted: "Our position was that they went a little too far. There wasn't really a need for the cautionary statement about the danger of poisoning if you've ingested too much. If children were to sit around the bathroom eating toothpaste, which younger children could do, there is not enough fluoride in the toothpaste to cause them any acute harm. That just doesn't happen ... If you tried to eat a lot of toothpaste, you'd throw it up." He conceded that poison control centers do receive reports of fluoride poisonings every year, but said that the ADA is not aware of any of those cases resulting in adverse effects. "It just hasn't proven to be that kind of a problem We didn't think you needed a label like that because it could unnecessarily scare consumers into not using toothpaste."

CRITIQUE OF STUDY

(Guha-Chowdhury N, Drummond BK, Smillie AC. Total fluoride intake in children aged 3 to 4 years. A longitudinal study. *Journal of Dental Research*. 75 (7) 1451-1457 1996)

An abstract of a New Zealand study of fluoride intake by 3-4 yr old children, by N Guha-Chowdhury and collaborators, was printed in the May 1997 issue of *Fluoride* (pages 126-127). The full study describes how duplicate portions of all food and drink consumed over 24 hours by 66 children resident in fluoridated ($n=32$) and low fluoride ($n=34$) areas of New Zealand were collected on three separate days over a period of 12 months and analysed for fluoride. Fluoride intake from ingestion of toothpaste was also determined for each child. It was concluded that fluoride from diet alone did not exceed 0.04 mg/kg of bodyweight (0.74 mg/day) and from diet plus toothpaste did not exceed 0.07 mg/kg of body weight (1.31 mg/day). Children from low fluoride areas taking currently available dosage of supplements would exceed the intake in fluoridated areas. The results suggested that the recommended fluoride tablet dosage needs to be further reduced if dental fluorosis is to be avoided.

The full study has two tables of information. Table 1 shows mean and standard deviation of the fluoride intakes, in mg/day and mg/kg body weight, for diet alone and diet plus toothpaste for each of the three collections in fluoridated and low fluoride areas. Table 2 shows range, mean, standard deviation and 95% confidence interval for the combined data of the three collections for diet alone and diet + toothpaste, in mg/day and mg/kg body weight, for fluoridated and low fluoride areas. It also includes the range for fluoride intake in low fluoride areas after adding fluoride values of tablets at four dosage levels (1 mg, 0.5 mg, 0.25 mg and 0.1 mg) to the diet plus toothpaste range.

I make the following observations:-

1. It was stated that the combined mean fluoride intakes exceeded the diet only fluoride intakes in low fluoride and fluoridated areas by approximately 70% and 50% respectively. The true values are approximately 226% and 89% respectively.

Table 1. Mean (\pm SD) fluoride intake and body weight of children at baseline and after 6 and 12 months

		Diet Alone		Diet and Toothpaste		
Baseline		6 months	12 months	Baseline	6 months	12 months
Low-fluoride areas (n=34)						
mg/day	0.14 ± 0.08	0.15 ± 0.10	0.15 ± 0.09	0.56 ± 0.33	0.53 ± 0.32	0.39 ± 0.26 ^a
mg/kg bw	0.008±0.005	0.008±0.005	0.008±0.005	0.032±0.018	0.028±0.016	0.019±0.012 ^a
Fluoridated areas (n=32)						
mg/day	0.34 ± 0.22	0.31 ± 0.16	0.44 ± 0.30	0.69 ± 0.37	0.70 ± 0.37	0.64 ± 0.32
mg/kg bw	0.019±0.013	0.016±0.008	0.022±0.015	0.040±0.024	0.037±0.020	0.032±0.017

^a Significant ($p < 0.05$) reduction in fluoride intake over time.

Table 2. Combined (average of baseline, 6 months, and 12 months) values for fluoride intake from diet alone, diet and toothpaste, and extrapolated values for fluoride intake from diet, toothpaste and fluoride tablets

		Low-fluoride areas		Fluoridated areas		
Range		Mean	95% CI	Range	Mean	95% CI
Diet Alone						
mg/day	0.05 to 0.31	0.15 ± 0.06	0.13 to 0.17	0.09 to 0.74	0.36 ± 0.17	0.30 to 0.42
mg/kg bw	0.004 to 0.02	0.008 ± 0.003	0.006 to 0.010	0.004 to 0.04	0.019 ± 0.009	0.015 to 0.023
Diet + Toothpaste						
mg/day	0.17 to 1.21 ^a	0.49 ± 0.25	0.41 to 0.57	0.26 to 1.31	0.68 ± 0.27	0.59 to 0.77
mg/kg bw	0.01 to 0.06	0.027 ± 0.012	0.023 to 0.031	0.01 to 0.07	0.036 ± 0.015	0.030 to 0.042
(Diet + Toothpaste)^a + Fluoride Tablets						
^a + 1.00 mg/day		1.17 to 2.21				
^a + 0.50 mg/day		0.67 to 1.71				
^a + 0.25 mg/day		0.42 to 1.46				
^a + 0.10 mg/day		0.27 to 1.31				

^a Range (0.17 to 1.21 mg/day) of fluoride intake from diet and toothpastes found in the low-fluoride areas

2. The range of fluoride ingested from toothpaste alone is given as 0-1.29 mg/day. In Table 2, the largest value for fluoride from diet + toothpaste is 1.31 mg/day in fluoridated areas, which leaves 0.02 mg/day for diet only. The smallest value, quoted in those areas for diet alone, is 0.09 mg/day, which leaves a discrepancy of 0.07 mg/day. Assuming the other fluoride values are correct, the maximum fluoride value from diet + toothpaste is at least 1.38 mg/day.

3. The maximum fluoride intake from diet + toothpaste DID exceed 0.07 mg/kg body weight (1.31 mg/day) in fluoridated areas, unless the previously described discrepancy can be explained away. The abstract states that fluoride supplement data were determined for each child. These data are not included in any of the total fluoride intakes. The Table 2 values of total fluoride intake for low fluoride areas, assuming four possible fluoride tablet values (1 mg, 0.5 mg, 0.25 mg and 0.1 mg), indicates that the true maximum intake was possibly as high as 2.21 mg (recommended fluoride supplement dosage in New Zealand low fluoride areas is 1.0 mg/day).

4. True random samples of the population of 3-4 year old children were not possible, because of the exclusion of children with one or two carious lesions. Hence the statistics quoted in the abstract were misleading, because these selection criteria were not given in the abstract.

5. The authors acknowledge that the sample sizes are small.

This study appears to be another small sample size selective study trying to shore up the crumbling fluoride edifice. It attempted and failed to show that fluoride intake was within the 50 year old determined "optimal range". It is today generally accepted that the cariostatic effect of fluoride is predominantly topical and that fluoride tablets should not be given to young children. Toothpaste alone may well be sufficient to minimise caries, if the dubious benefits of fluoride are to be believed. It probably would reduce dental fluorosis, which is certainly not

considered a cosmetic defect by affected people - ask any young woman with discoloured fluorosed teeth. Now seems to be the time to stop water fluoridation and feeding supplements to unsuspecting children. The last sentence of the study begins - "Even if fluoride use were to become totally topical ...". Are the authors feeling their way to a solution to the fluoride question?

Bill Wilson
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North Shore City
New Zealand

The following letter is in response to the critique by Professor Albert Schatz in *Fluoride* 30 (2) 1997 page 131-133. The discussion continues on following pages.

NO ASSOCIATION BETWEEN FLUORIDATION OF WATER AND SIDS

Our ecological study showed there was no linear association between median fluoridation and SIDS mortality rates in New Zealand.¹ Similarly the quadratic curve drawn on our figure is not statistically significant, thus there is no evidence for a paradoxical effect.

We have examined water fluoridation in the New Zealand Cot Death Study,^{2,3} a large nationwide case-control study (379 cases and 1551 controls). 59.9% of SIDS cases occurred in households where the water was fluoridated compared with 61.0% of controls who lived in fluoridated areas (OR = 0.96; 95% confidence interval 0.76, 1.20; not significant). After adjustment for a wide range of potential confounders (n = 25; sociodemographic, pregnancy related, infant related and infant care practices, which includes type of infant feeding), fluoridation was not associated with SIDS (Dick *et al*, unpublished data).

We can be confident that water fluoridation does not cause SIDS.

E A Mitchell, Associate Professor in Paediatrics, University of Auckland

J M D Thompson, Biostatistician, Paediatrics, University of Auckland

B Borman, Epidemiologist, Regional Health Authority, Wellington

R P K Ford, Community paediatrician, Healthlink South

E A Dick, Canterbury Cot Death Fellow, Healthlink South

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- 2 Mitchell EA, Scragg R, Stewart AW, Becroft DMO, Taylor BJ, Ford RPK, Hassall IB, Barry DMJ, Allen EM, Roberts AP. Results from the first year of the New Zealand cot death study. *New Zealand Medical Journal* 104 71-76 1991.
- 3 Mitchell EA, Taylor BJ, Ford RPK, Stewart AW, Becroft DMO, Thompson JMD, Scragg R, Hassall IB, Barry DMJ, Allen EM, Roberts AP. Four modifiable and other major risk factors for cot death: The New Zealand Study. *Journal of Paediatrics and Child Health* 28 (Suppl 1) S3-8 1992.

FLUORIDATION AND INFANT DEATHS

Proponents of fluoridation have yet to provide convincing evidence that it is safe. Until they do that, they are using humans as guinea pigs.¹ It is imperative that the safety of a highly toxic substance such as fluoride be unequivocally established. The responsibility to do that rests squarely on those who allege that fluoridation is safe. The fact that low levels of fluoride exhibit paradoxical effects (as low-level radiation and low levels of many chemical compounds do)² makes it difficult to provide convincing evidence that fluoridation is safe. If the safety of fluoridation cannot be unequivocally established, it should be discontinued. Dental caries is not a life-threatening problem which justifies the use of a highly toxic substance.

One cannot adequately evaluate the role of fluoride in Sudden Infant Death Syndrome (SIDS) without studying significant numbers of malnourished infants because they are most susceptible to fluoride toxicity.³ Until such well-designed research is conducted, one cannot conclude that fluoride is not involved in SIDS. The toxic effect of fluoride in Chilean children was clearly revealed because most children in that country were malnourished.³ Because fluoride was toxic to malnourished Chilean children, it is toxic to malnourished children everywhere, and especially toxic to malnourished infants.

Those who allege that fluoride is not involved in SIDS do not know the total daily intake of fluoride of malnourished infants, whether they are breast fed or not. For some of them, the total daily intake of fluoride may be significantly higher than the total amount of fluoride which they ingest from drinking water.² Those who disregard any important variable "cannot exclude the explanation [they] have not considered."⁴

Albert Schatz PhD
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References

- 1 Franklin J (pen name of Albert Schatz). The opponents of fluoridation are guilty. *National Fluoridation News* 20 (4) 3-4 October-December 1974.
- 2 Schatz A. Paradoxical effects. Critique of a study. *Fluoride* 30 (2) 131-133 1997.
- 3 Schatz, A. Increased death rates in Chile associated with artificial fluoridation of drinking water, with implications for other countries. *Journal of Arts, Science, and Humanities* 2 1-17 1976.
- 4 Datta M. You cannot exclude the explanation you have not considered. *The Lancet* 342 345-347, August 7 1993.

MITCHELL REJOINDER

Less than 20% of sudden infant death syndrome (SIDS) cases occur in infants with low birthweight (LBW, < 2500 g).¹ In 1987-1990 we undertook a large case-control study. The methods have been described in detail,^{1,2} but in brief, the cases (n=485) were all SIDS deaths in the study regions. The controls (n=1800) were randomly selected from all live births and representative of all births in

the study regions. They were not matched by region, hospital of birth, ethnicity or date of birth. We found that 59.9% of SIDS cases occurred in households where the water was fluoridated compared with 61.0% of controls who lived in fluoridated areas. This study included 82 cases and 84 controls of LBW. We adjusted for a wide range of potential confounders including birthweight, and fluoridation was not associated with SIDS, even in infants with LBW.

Professor Schatz's study tells us nothing about SIDS as it relates to infant mortality in a developing country in the 1950s with extraordinary high infant mortality rates ($>100/1000$ live births). SIDS mortality rates are $<2/1000$ live births. Deaths from infections continue to be the major cause of death in infancy in the developing world, and SIDS is relatively rare.

Poisoning has frequently been postulated as the cause of SIDS. The poisons implicated include therapeutic drugs, ammonia, water fluoridation, nappy sterilisers, immunisations and more recently poisonous gases from cot mattresses. It is appropriate to take these claims seriously and fully investigate them. We have undertaken an ecological study³ and a large case-control study and have found no association between fluoridation and SIDS. Continuing to proclaim a causal link causes unnecessary distress to families whose baby has died and diverts attention away from the successful SIDS education campaign which has halved SIDS mortality in many countries throughout the world.⁴

E A Mitchell

References

- 1 See reference no. 4, page 199.
- 2 See reference no. 2, page 199.
- 3 See reference no. 1, page 199.
- 4 Mitchell EA, Brunt JM, Everard C. Reduction in mortality from sudden infant death syndrome in New Zealand: 1986-92. *Archives of Diseases of Children* 70 291-294 1994.

SCHATZ REJOINDER

Mitchell *et al* have not ruled out an association of fluoride and SIDS. They consider only the fluoride content of drinking water, but present no information about the total fluoride ingested by each victim of SIDS. They have therefore disregarded the most basic tenet in forensic toxicology. For example, if the suspected cause of death is an overdose of sleeping pills, it is necessary for the coroner to know the amount of the medication in the body of the particular victim. The amount of medication in each pill in the original bottle of pills does not provide the necessary information. Nor does the number of pills missing from the bottle.

The concentration of fluoride in drinking water may vary significantly from day to day. Even if that concentration were constant, the daily consumption of water varies for different individuals. The ingestion of fluoride from other sources also varies for different individuals. As in forensic medicine, it is necessary to determine the amounts of fluoride in the bodies of SIDS victims. Statistical analysis is no substitute for that quantitative information.

Decades ago Fred Exner MD opposed fluoridation because he said its proponents were, in effect, telling people: "Drink as much water as you want. You'll get the right dose of fluoride with no harmful effects." Exner compared that to a doctor who prescribes a potentially toxic medication and tells the patient: "Take a pill whenever you want, and you'll get the right dose with no harmful effects." If pediatricians prescribed medication for children that way, they would be guilty of malpractice.

Albert Schatz

FINAL WORDS (Each was invited to submit a final summing up)

Mitchell *et al* reported that non-breast feeding significantly increased the risk of SIDS. It is known that an infant formula made with fluoridated water increases an infant's fluoride intake by up to 200 times that of intake from the mother's breast, which supplies milk mostly free of fluoride. Mitchell *et al* provide no data on the ingestion of fluoride from this source of intake, nor include it as a possible risk. It is nonsensical to suppose that every infant will ingest the "mean fluoride intake" of an area. Without recording individual fluoride intakes, and with small samples like 82 cases and 84 controls, it is not possible to rule out a contributing role of fluoride. Also not considered were individual variations in susceptibility to fluoride toxicity. Nonetheless, as was pointed out in my critique, their results are compatible with a paradoxical effect from low doses.

In their study which I criticized they emotively referred to "unethical scare tactics" of opponents of fluoridation. I repeat that proponents of fluoridation have not provided convincing evidence that it is safe. Opponents should not have to provide "conclusive proof" of harm. Many well-documented reports of harm caused by fluoride make the practice of fluoridation unethical.

Albert Schatz

As 90% of SIDS occur before 6 months of age, almost all the SIDS infants' fluoride intake comes from breastmilk or infant formula. The mean fluoride intakes from food and drinks in fluoridated and nonfluoridated areas in New Zealand were 0.263 and 0.082 mg F/day respectively.¹ If fluoridation caused SIDS we would have expected to have seen a higher SIDS rate in fluoridated areas. This was not seen.

Other sources of fluoride need to be considered, especially fluoride tablets and toothpaste. Only one (0.25%) SIDS parent mentioned "possibly half a fluoride tablet". As the first teeth erupt at approximately 6-7 months, toothpaste is not an important source.

Our study clearly shows there is no indication of a relationship between fluoridation of the water supply and SIDS in New Zealand and also that there is no evidence of a paradoxical effect.

E A Mitchell

Reference

- 1 Chowdhury NG, Brown RH, Shepherd MG. Fluoride intake of infants in New Zealand. *Journal of Dental Research* 69 1823-1833 1990.

In his Guest Editorial in *Fluoride* of August 1996 Dr Foulkes described fluoride as a contaminant for which, like radiation, no "safe level" can be determined. In a Guest Editorial in the following issue Professor Jenkins disputed the term "contaminant" and Dr Foulkes replied in the January 1997 issue. Both expressed conflicting views on the "minimum safe dose" in the last (May) issue. The discussion continues:

NO PROOF – EDITOR'S WARNING NEEDED

Dr Foulkes admits (*Fluoride* 30 May p 139) that there is no "proof" that fluoride intake, however small the dose, produces undesirable effects. He points out the difficulties that have prevented the investigation of the possibility that vague symptoms might be caused by chronic F intoxication and I agree with him about these difficulties. In the absence of such investigations, his belief must remain speculative. Back issues of *Fluoride* contain many reports of animal experiments but they provide no answer to the question of whether there is a safe intake below which no symptoms occur. The dosages the animals receive (typically 5 mg to 50 mg/kilo body weight) are usually hugely in excess of human intakes (less than 0.1 mg/kilo body weight). Similarly, the concentrations used to test the response of cells *in vitro* to F usually grossly exceed the concentrations in plasma (0.01 to 0.02 ppm) to which most of the tissues of the body are exposed (the only tissues known to receive higher concentrations are those of the mouth, stomach and the urinary tract).

I think *Fluoride* should copy the tobacco industry and contain an "Editor's Warning: any paper reporting animal experiments in which oral doses exceed 0.5 mg/kilo body weight or expose cells or tissues *in vitro* to concentrations of F exceeding 0.1 ppm may be of academic interest but are irrelevant to the human tissues (except those of the mouth, stomach and the urinary system which do receive higher concentrations)."

I would be very pleased to discuss these questions in my local over a pint of Newcastle Brown Ale (brewed with Newcastle fluoridated water!).

Neil Jenkins
4 Jesmond Dene Terrace
Newcastle upon Tyne
NE2 2ET England

MUCH EVIDENCE – LESSONS FROM HISTORY

"Thus what is produced by anything is injurious, it is doubtful that the thing is not wholesome in itself." ¹

These words stated by the Roman, Vitruvius, in the first century AD about another ubiquitous element, could apply equally to the subject under discussion by Professor Jenkins and myself - fluoride and its adverse effects.

I feel that Professor Jenkins misinterpreted my last letter (*Fluoride* 30 May p 139) when I wrote of the difficulty in obtaining "proof" of fluoride etiology for adverse effects. I was referring, specifically, to the vague symptoms frequently encountered in medical practice. I did not, as Professor Jenkins states, "admit that there is no proof that fluoride intake, however small the dose, produces undesirable effects."

To the contrary, I find that the back issues of *Fluoride*, to which I referred him, report on many examples of undesirable effects in humans that are shown to be caused by fluoride ingestion. I refer him to the studies of Grimbergan, Moolenburgh, Petrabor, Susheela and Lee that were competently reviewed, in 1993, by Bruce Spittle.²

Surely Professor Jenkins accepts that dental fluorosis is an "adverse effect". Fejerskov *et al.* in their *Dental Fluorosis - a handbook for health workers*, state that "... a daily dose of fluoride as low as 0.04 mg/Kg body weight can result in dental fluorosis of the permanent dentition."³

With regard to Professor Jenkins' objections to my statement regarding the problem of no "minimum safe dose", he may wish to ponder the comment in the above book that "a 'magic borderline' below which the signs of dental fluorosis are totally absent from all people does not in reality exist."³

He may also turn the pages of the last issue of *Fluoride* and read Akiniwa's paper concerning the problems in ascertaining an accurate estimate of the minimum toxic dose in acute fluoride toxicity⁴ and the discussions on paradoxical effects by Schatz⁵ and Moolenburgh⁶. These may help his understanding of my statement regarding minimum safe dose and give him pause to reflect on whether the "Editor's Warning" that he recommends is sufficient when it only deals with the errors of traditional linearity rather than pointing the way to a different paradigm.

Lest we forget, earlier orthodoxies held that low doses of arsenic, strychnine, mercury and radiation were "beneficial". The adverse effects of exposure to lead have only been acted upon legislatively in recent times, in spite of the fact that it was this element that Vitruvius warned about nineteen centuries ago with this comment: "Water is much more wholesome when taken from earthenware pipes, than from lead pipes. For it seems to be made injurious by lead."¹

Some historians attribute the collapse of the Roman Empire to the effects of lead poisoning - infertility, dementia and mental instability - familiar themes in contemporary fluoride research literature that I reviewed recently in these pages.⁷ Could the fate of our "civilization", like that of the Roman Empire, be decided by those who, like Professor Jenkins, try to persuade us of the "wholesome" nature of fluoridated public water supplies?

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