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ABSTRACTS

The 17th ISFR Conference will be held in Budapest, Hungary from June 23 through June 25, 1989 in the Sporthall, which is centrally located. The official language will be English.

The meeting, which will be organized by **Dr. Miklós Bély,** will stress problems with fluoride: chemistry; toxicology; biological effect; instruments and measuring techniques; effect of fluoride on plants, animals, humans; osteofluorosis.

It will be held as a separate conference but at the same time as one sponsored by the Hungarian Society for Rheumatism, which will bring specialists from Hungary, Poland, West and East Germany. The Hungarian Society sponsors and covers costs thanks to the President of the Society, Prof. Dr. Béla Gömör.

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FLUORIDATION: THE AUSTRALIAN EXPERIENCE

Among recent critical assessments of water fluoridation, one of the most penetrating and challenging is undoubtedly that to be found in the book, Fluoride in Australia – A Case to Answer, authored by a talented young Australian journalist, Wendy Varney.* Based on her honors master's thesis in political science at the University of Sydney, this clearly-written, well-documented analysis probes deeply into many of the important scientific issues as well as her main theme, the ever-present "politics" of fluoridation. Although comprising only 128 pages of actual text, the book has over 450 reference citations to support her account and interpretation of the Australian experience with fluoridation.

In her Introduction, Varney traces the beginning of fluoridation in Australia to the year 1953 in Beaconsfield, Tasmania. Salient features of the current debate are then outlined: "Doubts about fluoridation's safety, far from subsiding, have been fuelled by studies which show, at best, that much more research needs to be done in the area and, at worst, that fluoride could be contributing to more ill health than was previously thought." Continuing, she raises the logical question of the supposed "right of an elite in the dental and 'health' establishment to make decisions on behalf of, but with no accountability to, the community, especially when the ramifications of that decision go way beyond dental health." Of course proponents of fluoridation vigorously reject any such challenge. In their view, fluoridation is strictly their "preserve," and only duly appointed health officials – not members of the public to whom they are responsible – should be involved in considering whether or not to have it.

In the first of four ensuing chapters, Varney reviews the origin and promotion of fluoridation in the United States and subsequently in other countries, including Australia, where at present about two thirds of the population are served by water systems that are fluoridated. At first reluctant to extend general approval, the U.S. Public Health Service soon reversed itself and, in 1950, gave the green light to widespread adoption of the procedure, even though few of the permanent teeth of children exposed from birth in trial studies had erupted.

Varney then proceeds to document how "experts," with close ties to promoting organizations and supporting industries, were named to special committees and study panels to come up with reports that were inevitably favorable to fluoridation. She describes how rejections of fluoridation by voters and local governing bodies in Australia have been overturned by regional and state authorities; how adverse medical and dental findings are ignored or denied, even when they are reported by highly-qualified scientists with no personal gain at stake; how proponents repeatedly refuse to engage in public debate of the evidence (which they nevertheless insist is entirely on their side); how data and studies have been manipulated or misrepresented to "prove" the efficacy and safety of fluoridation; how opponents with good professional credentials are invariably disparaged and/or denigrated; and how evidence showing no significant dental benefits from fluoridated drinking water has been ignored, concealed, or denied.

* Published by Hale & Iremonger Pty, Limited, GPO Box 2552, Sydney 2001, N.S.W., Australia, November, 1986 ($12.95 p/b; $25.95 h/b, Australian dollars).

Disturbing and shocking though they are, the foregoing aspects of "Fluoride in Australia" are only part of the picture. In the succeeding chapters, Varney takes a still closer look at the lucrative role of industrial beneficiaries, the misleading and even false pronouncements of prestigious medical and dental organizations, and the back-stage as well as up-front promotional activities and actions of various branches and commissions of government.

Industrial beneficiaries are, in her words, firstly, "those [industries] which supply the by-product to the water supply authorities (along with nonsuppliers who produce, as a by-product of their industries, a fluoride compound in some form); secondly, food manufacturers whose products are conducive to tooth decay; and thirdly, those companies which have entered the fluoride market with products such as fluoridated toothpastes, which are rendered 'beneficial' by the [carefully engineered] prevailing public acceptance of fluoride as a harmless and effective prophylactic against dental decay."

Like many other polluting industries, fluoride emitters "are on the defensive unless they can attach social benefits to their operations or products." Ironically, with fluoridation, they have been able to go one step better. In terms of risk versus benefit: "Their waste is not so much offset by a benefit – their waste is the benefit."

Proponents of fluoridation in the medical-dental establishment naturally want the public to believe that the evidence for the safety and efficacy of fluoridation is so abundant and secure that there never could be any doubt about its overall validity. Yet, when challenged to present such evidence in open debate and to refute contrary findings, they invariably resist and try in every way possible to avoid doing so. "Judging by their fear of dialogue but their penchant for promotion, one can only conclude that fluoridationists have a severe lack of confidence in either their own case or the public, or perhaps both. Certainly promotion does not run counter to the paternalism which has been evident among fluoridationists."

Moreover, contrary to popular impression, the endorsements of fluoridation in Australia have not been arrived at independently but are derived directly from the pre-existent profluoridation stance of the National Health and Medical Research Council. And that body, in turn, based its endorsement on the report of an advisory committee set up in New South Wales in 1954. Although the committee's report claims that opposing submissions were sought and carefully considered, in fact the request resulting in one of the most trenchant of these submissions was not even received by the overseas addressee until only nine days before the committee's report was submitted, thereby casting grave doubt on the committee's assertion that opposing submissions "have been proved to have no basis in fact."

Just because tooth decay has declined in fluoridated communities does not mean that fluoridation is responsible. In the northern suburbs of Sydney, for example, the percentage of children, age 5 to 12, with caries-free teeth – an indisputable index of improved dental health – has increased steadily since 1961, with no evidence of any impact from fluoridation, which began there in 1968. Similar dental improvements, independent of fluoridation, have

been occurring in other parts of Australia as well as in other industralized nations.

In this fact, Varney sees a pertinent parallel in the steady declines in mortality from respiratory tuberculosis and acute rheumatic fever that have occurred in Britain since the last century. Death rates from these and other diseases, such as cholera, dysentery, typhoid, and even scarlet fever, diphtheria, whooping cough, and measles, all showed their greatest declines before the introduction of antibiotics and wide-spread immunization. Improved sanitation, housing, nutrition, and living conditions most likely made the biggest impact, not direct medical intervention.

Therefore, contrary to claims by medical-dental authorities that fluoridation represents "another great health improvement" resulting from something they have done, it is, in fact, a procedure of doubtful utility that is facing an extremely serious "legitimation crisis." Proponents would like these problems to go away, but they will not. "After three decades of fluoridation in Australia, the issue is not only still alive — the major backlash against it is still to come." Strong words indeed, but they need to be taken to heart. The scientific community engaged in fluoride research should take careful note of all these developments and conduct itself and its work in such a manner that it will not deserve to lose public support and confidence.

A.W.B.

CHLOROPHYLL-PROTEIN COMPLEXES IN FLUORIDE-TREATED PEA SHOOTS

by

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SUMMARY: Systemically applied 1 mM KF induced a significant decrease in a chlorophyll-protein complex in pea leaves after 72 hours of treatment.

KEY WORDS: Chlorophyll-protein complex; Pisum sativum; Systemic KF.

Introduction

Fluoride has been recognized as a phytotoxic air pollutant for nearly a century (1). It may induce decreases in chlorophyll levels and in chloroplast electron transport (2).

Chlorophyll-protein complexes have been extensively studied by polyacrylamide gel electrophoresis. These complexes have been characterized for a variety of plants including peas (3,4), spinach (5), maize (6,7) and tobacco (8,9). A relationship between chlorphyll accumulation and the formation of chlorophyll-protein complexes has been shown to be affected by such environmental factors as light (3,10) and water stress (11). Also, fluorides have been shown to cause decreases in chlorophyll content of chloroplasts and in photosystem II (12) activity as well as photophosphorylation (13), so studies are necessary to determine systems first affected. To this end we have investigated chlorophyll-protein complexes. The method might provide some insight into the effects of fluoride on the photosynthetic process.

Materials and Methods

Pea plants (Pisum sativum L. cv. Tall Telephone) were grown and systemically treated as previously described (2). Treatment solutions consisted of two control solutions containing either 1 mM KCl or distilled water and KF at 1 mM. Each treatment consisted of 7 beakers (8 shoots per beaker) arranged in a randomized block design. They were maintained at ambient temperature and humidity under cool white fluorescent light and incandescent lights at a PAR of 15 $\mu E/(m^2 \text{ sec})$ for eight hours per day. The two youngest fully-expanded leaflets of a shoot provided material for the isolation of chlorophyll-protein complexes. All extraction procedures were carried out in mid-morning, and all operations were performed at 0-5°C. Isolation procedures for chloroplasts followed the methods of Ballantyne and Glover (2). One gram of leaf material was chopped into 6 mL of extraction buffer consisting of 400 mM sorbitol and 100 mM tricine, pH 7.8. Samples were homogenized with a polytron blender with 2-3 second bursts. The brei was filtered through two, then eight layers of cheesecloth. The filtrate was centrifuged to 6,000 X g and brought to rest in approximately 5 minutes. The pellet was washed with unbuffered 10 mM NaCl and suspended in 1 mL of pH 7.8 buffer composed of 33 mM sorbitol and 100 mM tricine. An aliquot was used for chlorophyll

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determination and the balance was reserved for the isolation of chlorophyll-protein complexes. Chlorophyll content was determined in subdued light using the methods of Walker (14).

The isolation of chlorophyll-protein complexes was based on methods adapted from Markwell et al. (4) and changes from the procedures of Markwell et al for peas are as follows. Suspended chloroplasts were centrifuged up to 12,000 X g and then brought to rest. The resultant pellet was re-suspended in 1 mL of 6.2 mM Tris and 48 mM glycine buffer at pH 8.3. Sodium dodecyl sulfate (SDS) was employed, using a ratio of 10:1 (w/w) SDS: chlorophyll; and 50 mM mercaptoethanol and 10% glycerol (v/v) were added. Solutions were incubated at room temperature for ten minutes prior to electrophoresis.

The polyacrylamide tube gel electrophoretic system descibed by Markwell et al was used for the separation of chlorophyll-protein complexes (9). Complexes were separated for three time periods: 1, at 24 hours of treatment; 2, at 48 hours of treatment and 3, at 72 hours of treatment. Chlorophyll samples of 25 µL (8.1-12.5 µg of chlorophyll) were routinely electrophoresed on each tube gel. A constant current of 36 mA was applied. Electrophoresis was carried out until the front running band had run approximately 60 cm in the tubes. Immediately following electrophoresis, gels were scanned at 672 nm in a densitometer. A planimeter was used to measure the area of the chlorophyll-containing peaks obtained from tracings. Each peak was measured three times, and measurements were averaged. Percentage areas were calculated.

Gels were immersed in a solution of 0.25% Coomassie brilliant blue R in 50% methanol and 7% acetic acid to detect polypeptides associated with separated chlorophyll-containing bands. Excess stain was removed by circulating a solution of 30% methanol and 7% acetic acid over the gels.

All statistical analyses were at the 5% level of significance and were based on four replicates.

Results and Discussion

Electrophoretic fractionation of the chlorophyll-containing complexes resulted in four separated bands (Figure 1). The same chlorophyll-containing bands were separated in all treatments, at all times, and were designated L, M, N and P for convenience. In all cases, all chlorophyll-containing material entered the gels. Because bands N and P had been observed to coalesce comparisons were made by combining the areas of peaks N and P. The chlorophyll-containing complex from KF-treated shoots was found to be significantly different in area from that of the KCl or water treated shoots by 72 hours (Table 1).

Coomassie brilliant blue staining confirmed that the chlorophyll-containing bands were protein-containing complexes. Also, there were polypeptide bands present not associated with chlorophylls.

The same chlorophyll-containing complexes were found in pea shoots treated with KF, KCl and water with time. It would be purely speculative to attempt identification of photosystem I and/or II constituents based on the evidence obtained here. However, the two most rapidly moving bands, N and

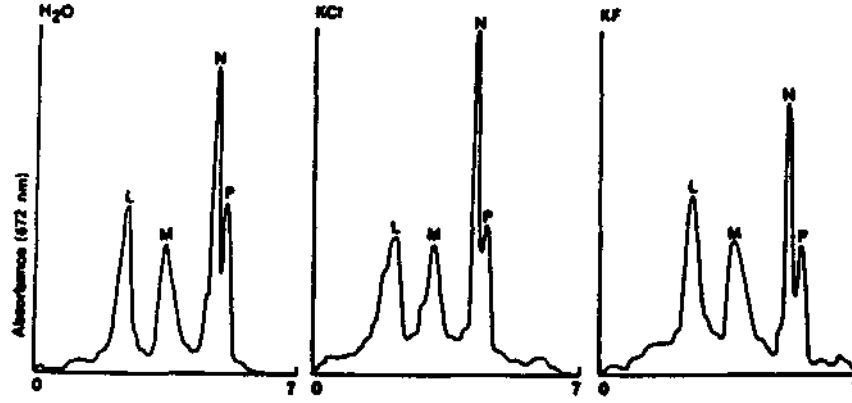

Figure 1

Chlorophyll-containing complexes: two separated and two partially separated peaks. Densitometer scan (672 nm) of complexes separated in 5% polyacrylamide gels. Complexes were extracted from the youngest leaflet pair of Tall Telephone pea shoots after systemic treatment for 72 hours with H_2O, 1 mM KCl and 1 mM KF. (Gel length was 10.0 cm; length scanned was 7.0 cm).

P, seem best interpreted as representative of a single complex. We feel that this can be justified on the basis of inconsistent movement displayed by these bands during electrophoresis. Erratic migration patterns seem to be an inherant flaw of SDS-PAGE separations and have been noted by others (9). On no occasion did the L and M bands meld in the same fashion. They always separated as two distinct bands.

Table 1

Percentage Area of Chlorophyll-containing Peaks [1,2,3]

Treatment	Day 1	Day 2	Day 3
H_2O	50.8^{ab}	52.3^{ab}	50.3^{ab}
1 mM KCl	50.4^{ab}	56.5^{a}	46.3^{b}
1 mM KF	46.8^{b}	51.8^{ab}	38.3^{c}

[1] Percentage area = $\frac{\text{Area under peaks N and P}}{\text{Area under peaks L, M, N and P}} \times 100$; see Figure 1 for peaks L, M, N and P.

[2] Areas of peaks N and P were combined from densitometer scans at 672 nm.

[3] Chlorophyll-containing peaks were separated in 5% polyacrylamide tube gels. They were isolated from the youngest leaflet pair of Tall Telephone pea shoots after systemic treatment with H_2O, 1 mM KCl and 1 mM KF. Means are significant different at the 5% level if not followed by the same letter or letters. Analyses were performed on the arcsine transformed data of four replicates.

Changes observed in the N/P complex may or may not be a consequence of other changes that have been observed after KF treatment of pea shoots. A significant decrease in electron transport was observed after 72 hours of systemic treatment (2) but fluoride-induced decreases in growth and increases in ATP could be detected after 24 hrs. of treatment (15).

Acknowledgements

We are grateful for financial support for these studies from the National Sciences and Engineering Council of Canada and the Faculty Research Fund of the University of Victoria.

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THE EFFECT OF pH AND FLUORIDE ON LEACHING OF ALUMINUM FROM KITCHEN UTENSILS

by

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SUMMARY: We tested the effect of boiling acidic solutions at pH values of 1, 2 and 3 with fluoride concentrations of 0, 1 and 5 ppm on the leaching of aluminum from used aluminum pans. The amount of aluminum leached from the pans was inversely related to the pH, but was unaffected by fluoride concentrations up to 5 ppm.

KEY WORDS: Aluminum; Fluoride in water.

Introduction

A recent report (1) that fluoride at 1 ppm in boiled acidified cooking water causes a 1,000-3,000-fold increase in aluminum leaching from aluminum utensils has created considerable concern because of its implications for water fluoridation.

Although the claim has now been retracted (2), in neither report did the authors give any information about the method of aluminum analysis. Savory et al (3) found no effect of sodium fluoride at a concentration of 0.024 mM. However, this is equivalent to 1 ppm NaF, but only to 0.46 ppm F.

Our objective was to test the effect of pH and fluoride concentration on the leaching of aluminum from aluminum pans.

Materials and Methods

We boiled for 10 min, in used aluminum pans, 500 mL samples of distilled-deionized (DD)-water or Winnipeg tap water, the fluoride content of which is 1 ppm, and also acidic solutions containing 50 mM oxalic acid (pK = 1.23), maleic acid (pK, 1.83) and citric acid (pK, 3.14) adjusted with NaOH to pH values of 1, 2 and 3, respectively. The acidic solutions were made up with both DD-water and tap water. We also used 50 mM maleic acid (pH 2) and citric acid (pH 3) in DD-water containing either 1 or 5 ppm fluoride as NaF, or, as a control, equivalent molar concentrations of NaCl (0.053 and 0.263 mM, respectively). After the boiling period, the volumes of the samples were readjusted to 500 mL with DD-water and analyzed for aluminum with a Perkin Elmer model 4000 atomic absorption spectrophotometer, with a nitrous oxide/acetylene flame. Both the samples and certified (Fisher Scientific) aluminum standards were adjusted to contain 1% HCl and 0.1% KCl, as recommended by Perkin Elmer.

Results and Discussion

The results are shown in Sections A and B of Table 1. They indicate,

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Table 1

Aluminum Concentrations (mM) After Boiling 500 mL for 10 min (mean ±S.D. for n > 3)

A

pH acid type	– none	3 citric	2 maleic	1 oxalic
DD-water	0.02 ±0.03	0.79 ±0.39	1.33 ±0.37	8.01 ±1.27
tap water (1 ppm F)	0.28 ±0.19	0.67 ±0.20	1.45 ±0.12	8.17 ±1.51

B

	Salt	0.053 mM	0.263 mM
pH 3	NaCl	0.35 ±0.10	0.47 ±0.01*
	NaF	0.30 ±0.06	0.73 ±0.15*
pH 2	NaCl	1.25 ±0.20	1.36 ±0.01
	NaF	1.24 ±0.13	1.40 ±0.13

* Difference significant at $p < 0.01$ by Student's t-test ($n = 6$)

in Section A, that the amount of aluminum leached from the pans was inversely proportional to the pH and was independent of whether DD-water or tap water was used. As seen in Section B, the aluminum concentration in the citric acid solution containing 0.263 mM NaCl at pH 3 (0.47 ±0.01 mM) was significantly less than that in the solution containing NaF at the same molar concentration (0.73 ±0.15 mM). However, the aluminum concentration in the latter was not significantly different from that in citric acid solution at pH 3, made up with DD-water but no NaCl (0.79 ±0.39 mM).

Thus, our studies provide no evidence that fluoride promotes the leaching of aluminum from aluminum cooking utensils. Consumption of the aluminum leached into acidified solutions during boiling in aluminum cooking utensils may well be undesirable on medical grounds, but we have found no evidence that the problem will be exacerbated by use of drinking water fluoridated at 1 ppm.

Acknowledgement

We thank the MRC of Canada for financial support.

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THE EFFECT OF 30 mg/L FLUORIDE IN DRINKING WATER ON EWES AND THEIR LAMBS AND CURRENT BONE LEVELS OF SHEEP IN N.S.W., AUSTRALIA

by

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SUMMARY: Pregnant ewes were given 1 (control) or 31 (treated) mgF/L in drinking water. Weekly blood serum samples from ewes and later from lambs were measured for physiological variables. Treated ewes had elevated milk fluoride (22.0 ±1.48 µmol/L compared with 7.11 ±0.403 µmol/L). At birth, serum fluoride of lambs was 1.72 ±0.167 µmol/L (treated) and 0.50 ±0.042 µmol/L (control); for treated lambs birthweight was reduced; serum T_4, calcium and magnesium was elevated. At 3-4 weeks, coccygeal bone fluoride was 580 ±117 mg/kg (treated) and 105 ±26.9 mg/kg (control). Serum phosphate in treated lambs showed an upward trend. At 17 weeks wool production of treated lambs was reduced due to decreased staple length.

Fluoride was measured in 270 metatarsal bones collected from nine zones in NSW. The range for ewes was 67-938 mg/kg, for lambs 7-97 mg/kg. Regional differences were observed. Sheep in NSW could be ingesting sub-acute levels of fluoride from water and superphosphate, and wool production could be adversely affected.

KEY WORDS: Australia; Lambs; Maternal transfer of F^-; New South Wales; Sheep.

Introduction

Endemic fluorosis of sheep due to high-fluoride bore water was noted in the 1950's and in research carried out at that time (1-5). Subsequently little interest has been shown in this potential problem, and the possibility of sub-clinical production effects has not been addressed despite the wide and continuing use of superphosphates, and the high evaporation rates experienced during summer from stock water supplies, especially in the western districts of NSW. It is known that sheep may ingest fluoride via soil or superphosphate (6-8). Therefore during drought periods, when sheep are foraging on bare soil and their nutritional plane can be extremely low, they could be ingesting more fluoride than is safe. However, 10-20 mgF/kg feed was thought to increase wool production (9), 30 mgF/L drinking water reduced the growth rate of lambs (10). Whereas fluoride is known to traverse the placental barrier of ewes (11), the effects on lambs are not established (1,2). Nor is it known whether fluoride traverses the mammary barrier in sheep.

Therefore, an experiment was planned to give pregnant ewes a fluoride intake comparable to that from some bore-waters, and to measure a range

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of physiological parameters in both ewes and their lambs (12). Also, in order to investigate the fluoride status of ewes and lambs in NSW, metatarsal bones were collected from nine agricultural areas and analyzed for fluoride (13).

Materials and Methods

Experiment. Twenty healthy Merino x Border Leichester ewes were synchronised in oestrus, mated with Growmark rams at the second oestrus. Three weeks later they were divided randomly into 2 equal groups, each group was penned separately in the same large shed. The control group received tap water (aprox. 1 mgF/L); for the treated 30 mgF/L was added to their water. Both groups were fed equal amounts of sheep pellets. They underwent the same management practices (e.g. drenching). A 24-hour watch was kept at lambing (over 1 week) and lambs tagged at birth. At 4-5 weeks old, lambs were tail-docked (bones were kept for later analysis) and castrated; at 6 weeks they were weaned onto 18% protein pellets fed ad lib.

Blood samples were taken weekly by venipuncture following 4 h deprivation of feed and water, and from both ewes and lambs at parturition. Blood and milk samples were taken from the ewes 2½-3½ weeks after parturition. The lambs were weighed weekly and wool samples taken 3, 9 and 16 weeks from birth. At 17 weeks they were shorn and the fleeces collected individually.

Blood serum was measured for fluoride (14); thyroxine (T_4), triiodothyronine (T_3) and cortisol (radioimmunoassay kits from Farmos Diagnostica, Finland); parathyroid hormone (PTH) radioimmunoassay kit from Cambridge Diagnostics, Inc.); phosphate (automated colorimetric method); calcium and magnesium (flame emission spectrometry). Tail-bones were measured for fluoride (Technicon Auto-Analyser) as was milk (15). Heparinised blood was measured for micro-haematocrit (MCHC). Wool samples were measured for staple length, fiber fineness and both greasy and clean dry fleece weight.

Survey. Fifteen metatarsal bones of both ewes (>4 yr) and lambs (<1 yr) were collected from abattoirs serving nine agricultural areas in NSW during November, 1984–January, 1985. Wherever possible Merino x Border Leichester animals were chosen when appropriate sheep were available, but the extent to which the sheep represented more than one flock is not known.

Bones were skinned, cleaned and dried at 105°C for 2 h. A dorsal/plantar sawcut (16) was made on each bone; dust was collected, ashed and analyzed for fluoride (17). Differences between regions were determined by analysis of variance.

Results

Experiment. Eight ewes lambed in each group, producing 9 lambs in each group. Figure 1 shows the mean weekly blood serum concentrations for the ewes. Minima and maxima were 0.6 ±0.06 and 1.9 ±0.018 µmol/L (control group); 8.7 ±0.63 and 35.4 ±1.22 µmol/L (treated group).

Figure 2 shows the mean weekly serum fluoride concentrations for the lambs. At birth, serum fluoride of the treated lambs (1.72 ±0.167 µmol/L) was three-fold that of control lambs (0.50 ±0.042 µmol/L. Values for both groups

Figure 1

Serum Fluoride (mean ±S.E.) of Control (----) and Treated (. . . .) Groups of Ewes.

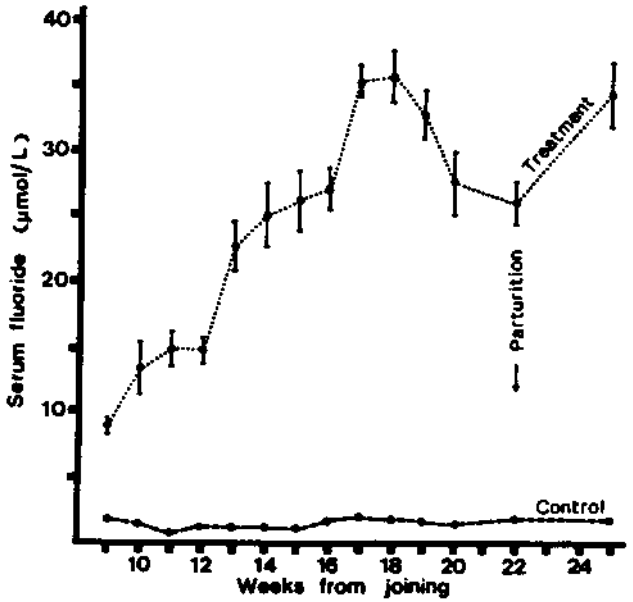
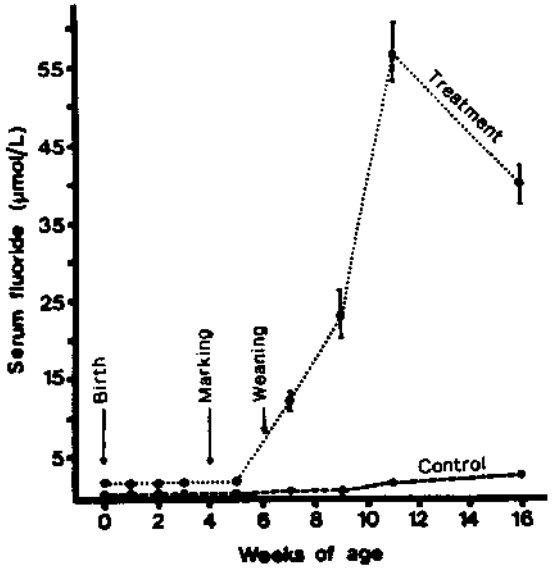


Figure 2

Serum Fluoride (mean ±S.E.) of Control (----) and Treated (. . . .) Lambs.



reached a maximum of 57.1 ±3.71 µmol/L. Treated animals drank less water than controls (Table 1); a higher proportion of ingested fluoride entered the serum of control animals. The mean total fluoride concentrations of milk from the treated group (22.0 ±1.48 µmol/L was three times that from the controls (7.11 ±0.403 µmol/L; cortisol was higher in the control group namely 9.3 ±2.43 nmol/L vs 4.0 ±0.54 nmol/L in the treated group.

Table 1

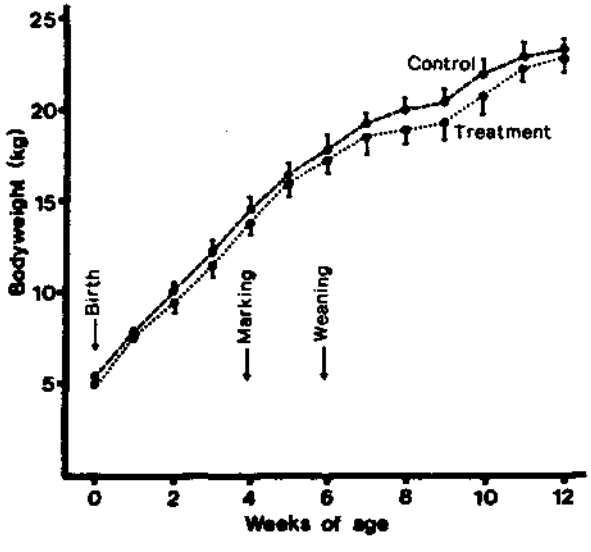
Water Intake and Blood Serum Fluoride of Ewes and Lambs

Group	Mean volume of water drunk (l/animal/day) (A)	Approximate fluoride concentration of water (µmol/L) (B)	Approximate total fluoride ingested per day (µmol) (A x B = C)	Mean serum fluoride of animals (µmol/L)	Proportion of serum fluoride concentration to fluoride ingested (%) (D/C x 100)
Ewes during pregnancy					
Control	4.2	52.6	220.9	1.35 ±0.054	0.61
Treated	3.7	1631.5	6036.6	23.63 ±0.934	0.39
During lactation					
Control	6.7	52.6	352.4	1.74 ±0.088	0.49
Treated	6.0	1631.5	9789.0	30.34 ±1.718	0.31
Lambs					
Control	3.0	52.6	157.8	1.34 ±0.143	0.85
Treated	2.5	1631.5	4078.8	33.12 ±3.144	0.81

Tail bones from treated lambs contained more fluoride (580 ±117 mg/kg bone ash) than controls (105 ±26.9 mg/kg). Bone fluoride was positively related to mean serum fluoride ($p < 0.001$). Treated lambs weighed less at birth (4.8 ±0.44 kg) than control lambs (5.4 ±0.47 kg); their mean weight remained slightly lower throughout the experiment (Figure 3).

Figure 3

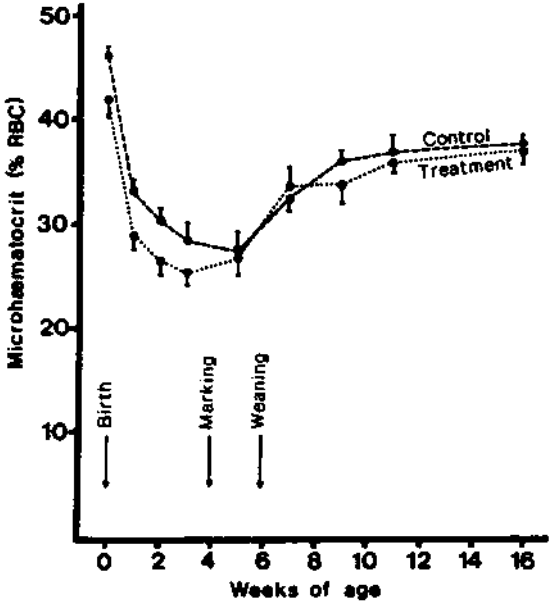
Bodyweight (mean ±S.E.) of Control (----) and Treated (. . . .) Lambs.



MCHC in the ewes was unaffected by the fluoride treatment; values for both groups declined throughout pregnancy from a maximum of 42.7 ±3.03 in the first sample to 34.8 ±4.12 in the last sample 3-4 weeks post-partum. In lambs, values for the treated group were lower than those for controls until weaning (Figure 4).

Figure 4

MCHC (mean ±S.E.) of Control (----) and Treated (. . . .) Lambs.



During weeks 9-20 of pregnancy, T_3 and T_4 values were the same for both groups of ewes, with a mean T_3 value of 1.5 ±0.17 nmol/L and mean T_4 value of 87.3 ±4.90 nmol/L. At parturition T_3 was lower for the treated group (2.1 ±0.20 nmol/L) than for controls (2.8 ±0.24 nmol/L). No differences were observed for T_4 in either ewes or lambs. T_3 values for the treated lambs fluctuated in comparison to controls; they were higher (4.4 ±0.18 nmol/L vs 4.1 ±0.16 nmol/L) at two weeks of age, lower at six weeks (1.5 ±0.08 nmol/L vs 1.8 ±0.12 nmol/L) and higher at 17 weeks (1.8 ±0.14 nmol/L vs 1.4 ±0.11 nmol/L).

Serum cortisol and PTH were not different in ewes or lambs.

Serum calcium and magnesium in ewes did not differ during pregancy and at parturition; at 3-4 weeks post-partum both calcium and magnesium were higher in the treated group (Ca: 3.01 ±0.091 mmol/L, Mg: 1.75 ± 0.061 mmol/L) than in controls (Ca: 2.18 ±0.127 mmol/L, Mg: 1.36 ± 0.088 mmol/L). Values for the lambs up to five weeks of age are shown in Table 2.

All values for serum phosphate were higher in treated lambs (Table 3).

Table 2

Serum Calcium and Magnesium Concentrations (mmol/L) from Control and Treated Groups of Lambs

Age of lambs (weeks)	Calcium (mmol/L) (mean ±S.E.)		Magnesium (mmol/L) (mean ±S.E.)	
	Control Group	Treated Group	Control Group	Treated Group
Birth	3.02 ±0.091	3.67 ±0.145	0.92 ±0.037	1.07 ±0.038
1	2.74 ±0.078	2.73 ±0.059	0.90 ±0.025	0.80 ±0.030
2	3.17 ±0.145	2.63 ±0.073	1.05 ±0.032	1.00 ±0.013
3	3.50 ±0.086	2.73 ±0.088	1.18 ±0.020	1.00 ±0.016
5	3.06 ±0.031	3.15 ±0.027	1.10 ±0.014	1.17 ±0.019

Table 3

Serum Phosphate Concentrations (mmol/L) from Control and Treated Groups of Lambs

Age of lambs (weeks)	Serum phosphate (mmol/L) (mean ±S.E.)	
	Control Group	Treated Group
1	4.0 ±0.09	4.3 ±0.25
2	3.8 ±0.16	3.9 ±0.09
3	3.5 ±0.13	3.7 ±0.08
5	3.3 ±0.11	3.5 ±0.17
7	2.7 ±0.12	3.2 ±0.10
9	2.6 ±0.14	3.2 ±0.16
11	2.6 ±0.18	3.0 ±0.13
16	2.7 ±0.11	3.7 ±0.17

Table 4 shows the greasy and clean fleece weights from the lambs. Greasy weights were not different, but both clean fleece weight and staple length were lower in treated lambs.

Survey. The results of the bone fluoride survey are shown in Table 5.

Differences were apparent between the regions, with values for ewes being lowest in the N. Tablelands (162 ±4.0 mg/kg) and highest in the S. Tablelands (477 ±10.7 mg/kg); corresponding values for lambs were 22 ±0.8 mg/kg (N. Tablelands) and 50 ±1.2 mg/kg (S. Plains).

Discussion

Maternal transfer of fluoride in sheep has previously been reported with

Table 4

Fleece Weight, Yield and Staple Length of Wool from Control and Treated Lambs (mean ±S.E.)

Group	Greasy fleece weight (g) (A)	Clean fleece weight (g) (B)	Yield (%) (B/A x 100)	Staple length (mm)
Control	1033 ±66.3	669 ±39.0	65.0 ±1.09	58.6 ±1.65
Treated	901 ±65.5	544 ±45.1	60.2 ±1.16	54.3 ±1.92

Table 5

Metatarsal Fluoride Content of Sheep from Agricultural Regions of NSW

Region	Bone Ash Fluoride (mg/kg) Ewes (>4 y.o.) Mean* ±S.E.	Ewes (>4 y.o.) Range	Lambs (<1 y.o.) Mean* ±S.E.	Lambs (<1 y.o.) Range
N. Tablelands	162^{a} ±4.0	90-235	22^{e} ±0.8	7-38
N. Slopes	248^{abc} ±4.3	107-502	28^{e} ±0.9	20-36
N. Plains	209^{ab} ±5.8	144-344	33^{e} ±0.8	27-40
C. Tablelands	322^{c} ±4.2	155-691	49^{ef} ±1.0	24-77
C. Slopes	189^{ab} ±3.6	67-303	35^{f} ±0.9	19-57
W. Plains	322^{c} ±6.1	189-484	49^{f} ±1.8	38-84
S. Tablelands	477^{d} ±10.7	170-938	31^{e} ±1.0	21-47
S. Slopes	299^{bc} ±5.8	153-485	Not available	
S. Plains	302^{bc} ±6.1	203-538	50^{f} ±1.2	25-97

* n = 15 Means with different superscripts differ significantly from one another ($p < 0.05$)

values ranging between 15% (18) and 96% (11) in utero, compared with our values of 33% for controls and 7% for treated animals.

Reports on mammary transfer are conflicting (19). However change in milk fluoride concentration with dietary fluoride intake is clearly shown in our study. The five-fold increase in tail-bone fluoride of treated lambs is indicative of considerable fluoride accumulation from the maternal environment, but the mean of 577 mgF/kg is well below the 4000-6000 mgF/kg range (cancellous bone) suggested as the toxic threshold for sheep (20).

Reduction in birthweight due to maternal fluoride intake has been reported for humans (21) and rabbits (22); it could be an important factor in lamb survival. Reduced growth rate associated with fluoride intake previously reported (10) was confirmed in this study. Lowered MCHC could be related

to anemia development (23); the T_3 changes in lambs warrant further investigation in view of previously postulated fluoride/thyroid interactions (23).

Changes in serum magnesium and calcium in relation to dietary fluoride have been previously noted in sheep; but, unlike our results in lambs, serum phosphate was unaffected (24).

Conclusion

The most obvious production effect was a reduction in clean fleece weight by some 18%, contrary to Purser's calculations (9). The bone survey showed that there were regional differences. The values for ewes (67-938 mg/kg) could be considered twice as high as those obtained in New Zealand (42-990 mg/kg) in cancellous coccygeal vertebrae (17), for lambs (7-97 mg/kg) could be considered intermediate between treated and controls. Therefore, reduced wool production in NSW sheep, due to dietary fluoride intake, is a possiblity which needs further investigation.

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DETERMINATION OF FLUORIDE IN PLANT MATERIAL BY A NEW SPECTROPHOTOMETRIC METHOD BASED ON THE RUTIN-Zr(IV) COMPLEX

by

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SUMMARY: The fluoride content in several types of vegetables and fruits was determined by a new spectrophotometric method based on the formation of rutin-zirconium (IV) complex. Vegetation samples collected from garden plots near the Glassworks at Inowrocław showed fluoride level as high as 2000 mg/kg, dry sample. The results were compared with those obtained by the potentiometric method using an ion-selective electrode and spectrophotometric titration method based on the SPANDS-Th(IV) complex.

KEY WORDS: Fluoride analysis, Plant tissues, Rutin-Zirconium (IV) complex, Spectrophotometric.

Introduction

In a strongly acidic medium, rutin forms a complex with Zr(IV) ions used for determination of sodium fluoride in a pharmaceutical preparation "Natrium Fluoratum," manufactured by "Polfa" (1). Optimum conditions for the complex formation were determined to include an aqueous-methanol medium (H_2O : MeOH = 60 : 40, v/v), concentration of perchloric acid 2M, and 8-fold excess of ligand. The molar ratio of the reagents was M + L = 1 + 1; stability, log K = 5.90 ±0.23; and stability in time, t < 30 min. of the complex formed under the above-described conditions, were determined. Within the concentration range of 0.0-1.0 µgF/mL, an inversely proportional relationship exists between fluoride concentration and the absorbance (λ = 410 nm) of the complex. This relationship is the basis for the quantitative determination of this element with a molar absorbancy, $\Sigma = 5.3 \times 10^3$ liter mol^{-1} cm^{-1}, and Stendell's sensitivity, $s = 3.5 \times 10^{-3}$ µg cm^{-2}.

The purpose of this work was to employ the above-mentioned composition and properties of the rutin-Zr (IV) complex formed in aqueous-methanol solution for the determination of fluorine in plant tissues. Vegetables and fruit samples were collected in 1983 from garden plots near the Glassworks at Inowrocław, which emitted large amounts of fluoride into the atmosphere.

Materials and Methods

Samples of plant material were taken from plots 100-300 m away from the fluoride emitters on the axis of the prevailing winds, that is, in the south-west, south and north-west directions. The samples were washed under running water, dried and then milled.

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Two methods were used for fluoride isolation: the microdiffusion method (2-4) and the steam distillation method (5-8). For the potentiometric method (9), two methods of sample preparation were used. The first method was based on isolating fluoride from the samples by microdiffusion, whereas the second method was based on ashing the samples in the presence of CaO, fusing the ashes with NaOH, and dissolving the resulting fusion in redistilled water.

Four different methods were used in the analysis of the plant samples:

Method I. Isolation of F (by microdiffusion) followed by spectrophotometric method. A portion of the ground sample, about 0.1-0.25 g, was placed in the middle compartment of the Obrink's chamber, which contained 1 mL of 2M NaOH used as absorbing solution in the inner compartment and 1.5 mL of 80% H_2SO_4 containing 0.02% sodium docecylsulfate in the outer compartment. About 2.5 mL of 72% $HClO_4$, saturated by Ag_2SO_4 and silicon GE-SF-98 was then introduced into the middle compartment. The chamber was closed and maintained at 60°C for 24 h. The absorbing solution was then transferred with the aid of 7-8 mL of redistilled water to a 25 mL volumetric flask and neutralized with 2M $HClO_4$. Into the solution, were then added consecutively 5 mL of 10M $HClO_4$, 1 mL of 2M $HClO_4$ containing 125 µg Zr(IV) and 10 mL of 1.1 x 10^{-3}M rutin stock solution in methanol. After making up to volume with redistilled water, the absorbancy of the solution was measured at 410 nm against a reagent blank prepared in the same manner except for the addition of zirconium. When the fluorine concentration was too high, the absorbing solution was diluted in the required proportion by redistilled water, and for further analysis a required amount, but not more than 8 mL, was taken. Fluoride concentration was determined from a calibration curve, which was linear within the range of 0.0 to 1.0 µgF/mL.

Method II. Isolation of F by microdiffusion followed by potentiometric method was carried out in the same way as described in the above section. The absorbing solution was transferred to a 50 mL volumetric flask, followed by the addition of 10 mL of 2.25M citric acid, and about 5 mL of 12M NaOH to adjust the pH to 5.0-5.5. Fluoride was determined by the ion-selective electrode method. For plant samples containing low fluoride levels, a 25 mL graduated flask was used, with reagents reduced by one-half accordingly.

Method III. For ashing followed by potentiometric method, about 4-10 g of the plant material was placed in a nickel crucible followed by addition of 0.2-0.4 g of CaO. The mixture was then ashed at 550°C. The resulting ash was fused with about 4 g of NaOH, and then the fusion transferred to a 100 mL volumetric flask. After the addition of 20 mL of 2.25M citric acid (pH 5.0-5.5) and cooling the flask, the potential of the resulting solution was measured.

An ion-selective electrode OPF-7113, Radelkis (Hungary) a calomel electrode K-60 (Poland) and digital pH meter N-512, "Mera Elmat" (Poland) were used. The calibration curve was linear within the range of 1 x 10^{-2}-2.5 x 10^{5}M F^-.

Method IV. Isolation of F by distillation followed by Spectrophotometric titration method (5,6,10). Plant sample was ashed in the same way as described in section 3. The ash was then transferred to a distilling flask, followed by the addition of about 40 mL of 72% $HClO_4$ and 0.1-0.2 g of Ag_2SO_4. Steam

distillation then carried out, with the temperature of the distilling flask maintained at 135-140°C. About 500 mL of the distillate were collected, and after neutralization, they were transferred to a 500 mL volumetric flask and made up to a volume with redistilled water. 20 mL of the neutralized distillate was taken into a 30 mL quartz cuvette followed by the addition of 1 mL of 0.02% SPANDS solution, and 1 mL of acetate buffer (prepared by mixing 200 mL of 1M CH_3COONa and 196 mL of 1M HCl and diluting to 1 liter. The mixture was then titrated with a Carl Zeiss-Jena Specol with a titration set Ti. A 4.0×10^{-4}M $Th(NO_3)_4 \times 10\ H_2O$ solution was used as a titrant (λ = 562 nm). A calibration curve was prepared using solutions of known fluoride concentrations. The calibration curve was linear within the range of 0.0 to 1.0 µgF/mL.

Table 1

Fluorine Content in Edible Portions of Vegetables and Fruits Grown near Glassworks at Inowrocław (mg/kg dry sample).

Vegetation	Spectrophotometric method	Potentiometric method	
Sorrel	149	142[1]	-[2]
Leek	135	141	-
Parsley leaves	1910	1890	-
Parsley root	19.6	19.2	-
Carrot	7.4	-	7.0
Celery leaves	195	197	-
Celery root	9.0	-	8.8
Chives	29.3	28.5	-
Red beet root	18.1	17.0	-
Red beet leaves	2030	1780	-
Young red beet leaves	322	300	-
Cabbages	8.2	-	6.6
Cucumbers	13.7	-	14.6
Pears	9.5	-	10.4
Potatoes	3.4	-	3.5
Apples	4.4	-	4.6
Plums	7.8	-	7.2
Onions	3.4	-	3.2
Pumpkin	5.9	-	5.6
Marjoram	2430	2600	-

[1] Based on method II

[2] Based on method III.

Results and Conclusions

The results of fluoride determination obtained by the spectrophotometric and potentiometric methods are presented in Table 1. Fluoride contents of the experimental plant samples were considerably higher than those of the vegetation grown in non-contaminated areas.

Table 2 shows the fluoride contents in several species of plants determined by four different methods. In the case of carrot and celery roots, it was not possible to determine the fluoride content by potentiometric method following fluoride isolation by the microdiffusion method in Obrink's chambers. Because of low fluoride content and only a limited amount of plant material that could be placed in the Obrink's chamber, the concentrations of the sample solutions were too low to be determined by this method.

Table 3 shows the recovery of fluoride added to plant samples obtained by the above-mentioned four analytical methods. In the case of carrot and celery roots, fluoride recovery by the potentiometric method following fluorine isolation was determined, taking for the calculation the fluorine content in these plants, essayed by method III, that is by the potentiometric method without fluoride isolation.

Table 2

Determination of Fluoride in Selected Species of Plants by Four Analytical Methods (mg/kg dry sample)

Method	Tissue					
	Parsley leaves	Parsley root	Celery leaves	Celery root	Red beet leaves	Carrot
Spectrophotometric method using rutin-Zr(IV) complex	1840	18.4	196	8.8	2050	7.1
	1980	20.7	194	9.8	1920	7.4
	1870	19.6	195	8.4	2130	7.3
	1930	19.7	-	-	-	-
Potentiometic method following fluoride isolation	1950	21.6	192	-	1800	-
	1850	17.3	198	-	1820	-
	1900	18.8	202	-	17.5	-
	1850	-	-	-	1850	-
Potentiometric method without fluoride isolation	1790	16.8	200	9.3	1760	7.1
	1750	20.7	194	8.3	1860	6.7
	1750	19.3	195	8.7	1690	7.3
Spectrophotometric titration method using SPANDS-(Th) complex	1760	18.0	193	9.3	1850	7.7
	1790	22.0	179	8.6	1820	7.1
	1860	17.0	202	10.9	1770	6.3

Table 3

Recovery of Fluoride Obtained by Four Analytical Methods

Method	Sample							
	Parsley leaves µg F added/found	Parsley root µg F added/found	Celery leaves µg F added/found	Celery root µg F added/found	Red beet leaves µg F added/found	Carrot µg F added/found
Spectrophotometric method using rutin-Zr(IV) complex	200/211	4.00/4.42	20.0/22.3	4.00/3.42	200/166	3.00/3.16
	/190	/3.75	/19.4	/3.57	/229	/3.16
	/214	/3.62	/20.3	/4.23	/183	/3.10
	/202	/3.88	/19.2	/4.24	/195	/3.17
Potentiometric method following fluoride isolation	200/201	10.0/10.5	25.0/26.4	25.0/24.7	200/185	25.0/23.7
	/198	/10.7	/27.3	/23.0	/205	/25.4
	/200	/ 9.1	/23.2	/22.9	/193	/24.6
	/195	/ 9.0	/25.6	/24.7	/201	/24.6
Potentiometric method without fluoride isolation	1000/ 977	100.0/95.8	500/501	50.0/48.2	1000/951	50.0/50.8
	/ 937	/99.0	/494	/48.2	/998	/50.1
	/1001	/104.3	/477	/47.0	/966	/47.3
Spectrophotometric titration method using SPANDS-(Th) complex	5000/4570	100.0/93.6	1000/956	50.0/47.7	5000/4630	50.0/46.0
	/4680	/93.9	/967	/48.7	/4740	/49.3
	/4790	/92.1	/968	/48.0	/4680	/47.3

Table 4 shows the statistical comparison of the four analytical methods used in this study. The data were compiled based on those shown in Table 2 and 3. The analysis of the data in Table 2, 3 and 4 permits us to draw the following conclusions:

Table 4

Summary of Experimental Data Obtained from Use of Four Analytical Methods

Method I: spectrophotometric method; Method 2: potentiometric method following fluoride isolation; Method 3: potentiometric method without fluoride isolation; Method 4: spectrophotometric titration method

Product	Method	Average Content of F mg/kg (dry product)	S.D.	Variability Coefficient	Average Recovery of F Added/Found/Found µg / µg / %
Parsley leaves	I	1910	62	3.25	200/ 204/102.1
	II	1890	48	2.54	200/ 199/ 99.5
	III	1760	23	1.31	1000/ 971/ 97.1
	IV	1800	51	2.83	5000/4680/ 93.6
Parsley root	I	19.6	0.94	4.80	4.00/ 3.92/ 98.0
	II	19.2	2.18	11.35	10.0/ 9.80/ 98.0
	III	18.9	1.98	10.48	100/ 99.7/ 99.7
	IV	19.0	2.65	13.95	100/ 93.1/ 93.1
Celery leaves	I	195	1.0	0.51	20.0/ 20.3/101.5
	II	197	5.0	2.54	25.0/ 25.6/102.5
	III	196	3.2	1.63	500/ 491/ 98.1
	IV	191	11.6	6.07	1000/ 964/ 96.4
Celery root	I	9.0	0.72	8.00	4.00/ 3.87/ 96.8
	II	-	-	-	25.0/ 23.8/ 95.2
	III	8.8	0.50	5.68	50.0/ 47.8/ 95.6
	IV	9.6	1.18	12.29	50.0/ 48.1/ 96.3
Red beet leaves	I	2030	106	5.22	200/ 193/ 96.5
	II	1810	42	2.32	200/ 196/ 98.0
	III	1770	85	4.80	1000/ 972/ 97.2
	IV	1810	40	2.21	5000/4680/ 93.7
Carrot	I	7.3	0.15	2.05	3.00/ 3.12/104.1
	II	-	-	-	25.0/ 24.6/ 98.4
	III	7.0	0.31	4.43	50.0/ 49.4/ 98.8
	IV	7.0	0.70	10.0	50.0/ 47.6/ 95.2

a. The results obtained from fluoride analysis by use of the spectrophotometric method (method I) are generally higher than those obtained by other methods. This is especially evident with analysis of plant tissues containing high fluoride levels.

b. The results obtained by use of the potentiometric method (method II) in which fluoride was first isolated by the microdiffusion method approximate most of those obtained by the spectrophotometric method (method I). It seems that fluoride isolation by microdiffusion is the most advantageous method because of a lower loss of fluorine than with the distillation method or by ashing (potentiometric, method III).

c. The above-mentioned conclusion is supported by a study of the recovery of added standard fluoride amounts by both these methods, i.e. the spectrophotometric method and the potentiometric method following fluorine isolation. The recovery of fluorine standard with these two methods was 96.5-104.1% and 95.2-102.5%, respectively. A distinctly lower recovery (93.1-96.4%) of fluorine was obtained with the spectrophotometric titration method (method IV), where fluoride was first isolated by steam distillation. In cotnrast the potentiometric method (method III) without prior fluorine isolation yielded a 97.1-99.7% recovery.

Conclusion

From these experimental data it is concluded that the newly developed method for determining fluorine, based on the formation of rutin-Zr(IV) complex, is comparable with other methods already in use and is suitable for fluoride determination in plant material.

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RADIOLOGICAL AND HISTOLOGICAL STUDIES ON BONES OF EXPERIMENTAL RABBITS IN SKELETAL FLUOROSIS

by

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SUMMARY: Thirty albino rabbits, ranging in weight from 1 to 1.5 kg, were segregated into three groups of ten each. Ten were fed 50 mg NaF/day/kg of body weight, ten 10 mg NaF/day/kg body weight, and ten served as controls. Seven months later, after being sacrificed, their bones were studied by light microscopy, scanning electron microscopy, and x-rays.

Compared with controls, the experimental animals, especially those fed 50 mg NaF/day/kg of body weight, showed prominent subperiosteal new bone formation and multiple exostoses. Some showed osteoporosis at the distal end of radius, ulna and phalangeal bones. Unlike human fluorosis, in these animals, radiological changes were prominent only in long tubular bones of the extremities. Nevertheless, no prominent changes were discerned in bones of the trunk. Light microscopy and scanning electron microscopy revealed that both new bone formation and old bone resorption were disturbed and occured more speedily.

KEY WORDS: Bone resorption; Experimental animals; New bone formation; Osteoporosis; Osteosclerosis; Skeletal fluorosis.

Introduction

Endemicity of foodborne skeletal fluorosis in Guizhou, China, and its radiological characteristics were reported by one of the authors in 1981 and in 1984 (1,2). Later, the disease was also found in the neighboring provinces of Hupei, Yunan and Sichuan. At first, it was believed that the high fluoride content in Guizhou food was derived from soil. Shortly thereafter some investigators reported that much of the fluoride in food resulted from air contaminated by baking and drying it in ovens. To disclose some mechanisms of fluorosis, the authors made the following observations.

Material

30 albino rabbits, ranging in weight from 1 to 1.5 kg, were segregated into three groups of ten each. They were all raised under the same experimental conditions except two groups which were fed 50 mg and 10 mg NaF/day/kg of body weight respectively. One group served as a control. After seven months the animals were sacrificed for observations.

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Radiological Observations

Upon radiological examination, the controls demonstrated smooth and clear cortical surfaces without signs of accelerated subperiosteal new bone formation. There were no signs of ossification or calcification of tendons and ligaments, no exostoses, no osteophytes. The demarcation between cortex and medullary cavities was distinct. In long tubular bones of the extremities, the provisional calcification zone appeared as a dense transverse line at the metaphyseal end. Most of the epiphyseal cartilaginous discs were indistinct and could not be discerned.

In the group fed the lower NaF dosage, radiological appearances were essentially like the controls except that subperiosteal new bone formation of minimal degree could occasionally be noted in long tubular bones.

In the higher dosage group, all animals showed prominent subperiosteal new bone formation. This change was accentuated on surfaces of radius, ulna and other long tubular bones of extremities which resulted in formation of multiple exostoses protuding outward from the cortex. The contour of bones became uneven and irregular (Figures 1,2,3). Some osteoporosis with widening of the medullary cavity and thinning of cortex at the distal end of radius, ulna and phalangeal bones was observed (Figures 4,5). Unlike fluorosis in humans, the spine and pelvis on radiograms appeared essentially normal.

Figure 1

Bony prominence on the cortical surface of a higher dosage animal protrudes outward.

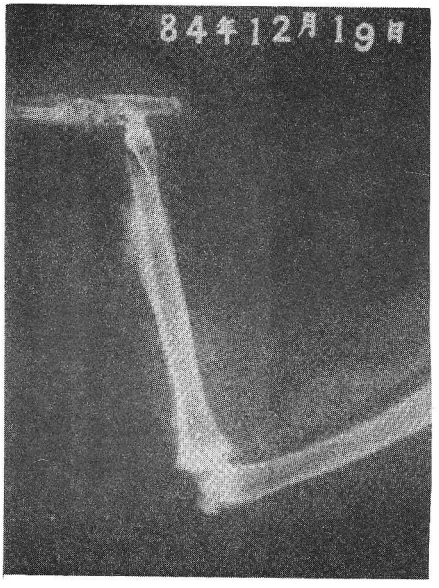


Figure 2

Bony prominences on the anterior and posterior surfaces of a higher dosage animal.

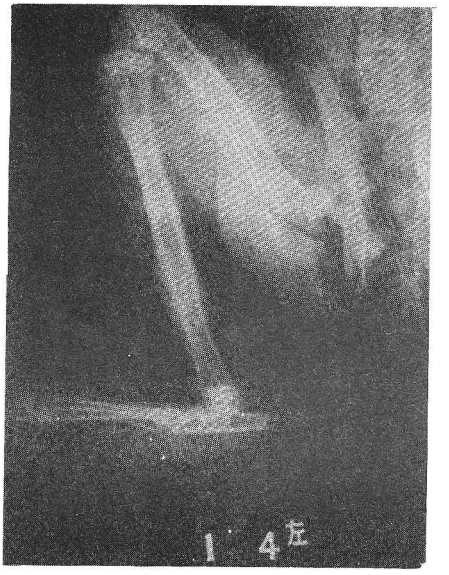


Figure 3

Bony prominence at various sites of long tubular bones.

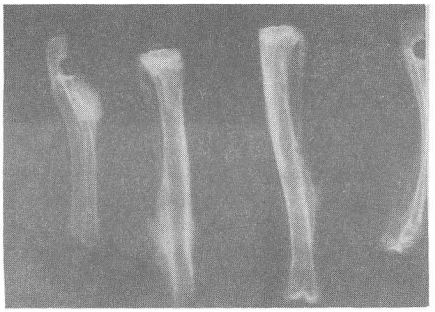


Figure 4

Marked cortical thickening and exostosis on the shaft of radius. Widening of medullar canals and osteoporosis at distal ends of radius and ulna. Fracture at upper end of humerus was a postmortem artefact.

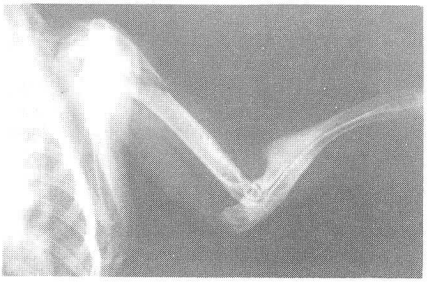


Figure 5

In the bilateral forelegs of a higher dosage rabbit, medullary canals were widened, bones porotic, with some cortical thickening.

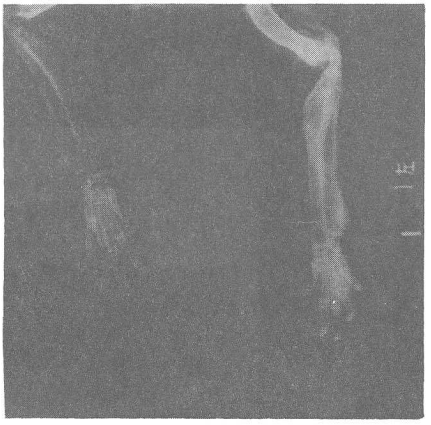
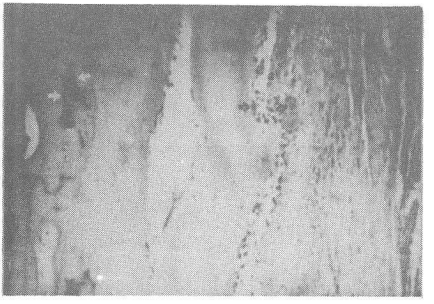


Figure 6

On the cortical surface of a radius, subperiosteal osteoblasts were abundant and conglomerated (black arrow). White arrows indicate blue stained, poorly calcified chondroitin sulfate. LM x240.



Light Microscopic Observations

For light microscopy, bone samples, excised from the carcases, were fixed in 10% formaldehyde. After decalcification in 5% nitric acid, the samples were embedded in paraffin, sliced into 7 micrometer thickness, and stained with either Mallory's trichrome or haematoxylin and eosin. In the controls, a layer of periosteum with its subperiosteal tissue, which was of relatively even thickness, could be detected. The cortex and trabeculae were well calcified.

Light microscopic appearances of the lower dosage animals were essentially like those of the controls except that osteoblasts were increased in number in some areas of the periosteum.

In the higher dosage group, periosteal osteoblasts were prominently increased in number and agglomerated, forming a layer of new bone tissue on the cortical surface (Figure 6). In some areas of the extremities, the newly formed bone tissue was very thick, protruding outward. Osteoclasts were scant and found on the endosteal surface. Many vacuoles could be noted within metaphyseal trabeculae of the radius; presumably they resulted from accelerated resorption of bone tissue in this area (Figure 7). Much chondroitin sulfate, stained blue, was noted in bone tissue (Figure 6). Some trabeculae were thickened, whereas others were thinned. In some areas of the epiphyseal cartilage, chondrocytes were arranged out of alignment.

Figure 7

At the metaphyseal end of radius, osteocytes scant, many trabeculae had vacuoles. LM x240.

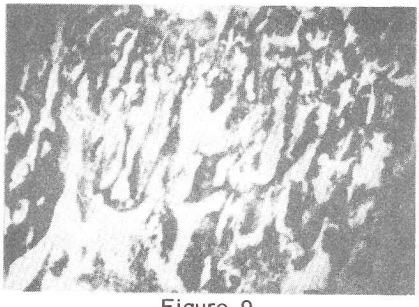


Figure 9

Bony prominences on cortical surface (arrows). SEM x300.

Figure 8

Multiple osteocyte lacunae on cortical surface. SEM x600.

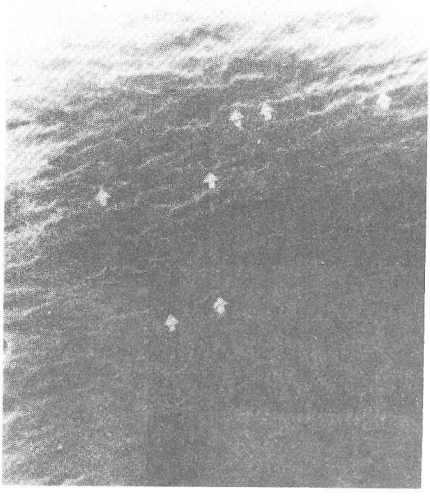
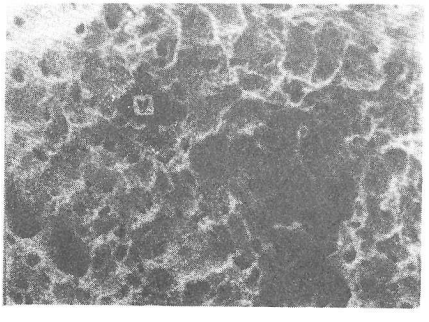


Figure 10

Endosteal medullary surface rough and uneven, with multiple osteocyte lacunae and a blood canal (marked V). SEM x500.



Scanning Electron Microscopic Observations

After fixing in 10% formaldehyde, the bone samples for scanning electron microscopy were immersed in 5% sodium hypochlorite to eliminate organic components after which they were coated with gold and studied under scanning electron microscope.

In the controls, the cortical surface was smooth with osteocyte lacunae regularly scattered. Both cortical and trabecular surfaces were calcified. In animals fed the lower dosage of sodium fluoride, osteocyte lacunae on the cortical surface were slightly increased in number, indicating activated new bone formation. In the higher dosage group, osteocyte lacunae on the cortical surface were further increased in number, forming small prominences protruding outward. The surface was well calcified (Figures 8,9). In contrast, the trabecular surface in this group was rough and poorly calcified. The endosteal surface of the medullary cavity likewise had a similar appearance to that of the trabecular surface (Figure 10). On these surfaces, both osteoblastosis and bone resorption were active.

Discussion

The three groups of animals showed many morphological differences in their radiological and histological appearances, differences which must be attributed to administration of sodium fluoride. Accelerated new bone formation is a noteworthy change, especially in animals given higher dosage, which developed unevenly. In some areas of the cortical surface, it produced prominent exostoses protruding outward, whereas, in other areas, it was indistinct and could hardly be discerned. It was noticeable only in long tubular bones. No significant changes were noticeable in bones of the trunk.

It is well known that, in human fluorosis, bones of the trunk are more severely affected than those of the extremities. This is not the case in animals which suggests that weight bearing and muscular traction might play a role in the development of skeletal fluorosis. Since humans are always in upright position, the spine and pelvis act as the most important weight bearing organs. Rabbits do not maintain an upright position like humans; therefore their spine and pelvis do not bear as much weight. In the presence of toxic levels of fluoride in the blood stream those bones, the function of which is weight bearing or muscular traction, develop osteosclerosis and new bone formation much more prominently than others.

The present communication once again proves that fluoride can stimulate osteoblastosis. Formerly, the authors reported that collagen fibers in fluorotic bone tissues were abnormal (3); they are tortuous, swollen, and irregularly oriented. In fluorosis, large amounts of abnormal collagen fibers, laid down by stimulated and activated osteoblasts, which are subsequently calcified, give rise to the new bone detected on the cortical surface and elsewhere.

The authors presume that vacuoles in trabeculae were caused by accelerated bone resorption which accounts for the porotic areas on radiograms. Normally, bone resorption takes place through osteoclastosis on the endosteal surface and elimination of degenerated bone tissue within trabeculae. Normal resorption of degenerated bone tissue within trabeculae does not leave gaps there, because the space left can soon be filled by other cells. In fluorosis,

the process of bone resorption is also activated and speeded up. In these experimental animals, epiphyseal chondrocytes were out of alignment, signifying that endochondral osteoblastosis was also disburbed.

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CHANGES IN GLYCOGEN CONTENT IN SOME TISSUES DURING FLUOROSIS – AN EXPERIMENTAL STUDY ON RABBITS

by

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SUMMARY: Sodium fluoride significantly decreased the amount of glycogen in spleen, lens, liver and skeletal muscle of young albino rabbits of both sexes during chronic intoxication. By contrast, the level of glycogen was significantly raised in the ovary of all treated rabbits. The decrease/increase in concentration of glycogen was directly correlated with the dosage of fluoride administered and duration of exposure. The results lend support to the involvement of the soft organs in fluorosis.

KEY WORDS: F^- effects; Glycogen content of lens, liver, ovary, skeletal muscle, spleen; Glycogen levels; Rabbits.

Introduction

The metabolic alterations responsible for either acute or chronic fluoride toxicity to animals are not well understood. In contrast, the in vitro effects of fluoride on many enzymes are well known (1). Investigations of the action of fluoride on tissue glycolysis, have been maily concerned with the transformation of carbohydrate in muscle. Embden et al. (2) showed that the breakdown of hexose-phosphate in minced muscle or muscle extract is inhibited by fluoride. Fluoride is claimed to effect the hepatic-glycogenolysis (3). The knowledge of the metabolic changes induced by sodium fluoride poisoning in the issues of animals is far from complete. The present study was undertaken to find out the effect of fluoride on glycogen metabolism in some tissues of albino rabbits of both sexes.

Materials and Methods

Young, albino rabbits of both sexes, obtained from Kaila Scientific Corporation, Agra, used in the present studies, were divided into six groups of 12 animals each. the control group was given 1 cc distilled water/kg body weight; the experimental groups, sodium fluoride injection in the concentrations of 5, 10, 20 and 50 mg/kg body weight/day for 100 days. All groups maintained on a standard laboratory diet, were given drinking water ad libitum. At the end of 100 days experimental period, the rabbits were anesthetized with ether and pleen, lens, ovary liver and skeletal muscle from control and treated rabbits were dissected out and preserved in ethanol for the extraction of glycogen. Glycogen was extracted by digesting the specific organs in 30% KOH; subsequently they were precipitated in 95% ethanol thrice as suggested by Heatley (4). The extract was prepared by disolving the dried mass in a known volume of distilled water. The glycogen content was estimated by the method of Montogery (5). Levels of statistical significance were determined by means of student's test.

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Table 1

Effect of Fluoride on Glycogen Content of Spleen and Lens of Albino Rabbit

Group	Exposure of Fluoride	mg glycogen/g wet tissue wt.			
		Spleen		Lens	
		Male	Female	Male	Female
C	Control	9.500 ±0.860	10.444 ±0.192	26.666 ±1.154	23.410 ±0.260
I	5 mg/kg b.w.	8.950 ±2.610	9.000 ±0.190*	4.120 ±0.550*	3.873 ±0.156*
II	10 mg/kg b.w.	5.630 ±0.310*[2]	8.943 ±0.061*	2.730 ±0.350*[4]	2.230 ±0.110*[4]
III	20 mg/kg b.w.	4.965 ±0.288*[4]	5.490 ±0.084*[4]	1.051 ±0.011*[4]	1.025 ±0.220*[4]
IV	50 mg/kg b.w.	4.890 ±0.310*	4.880 ±0.138*[4]	0.280 ±0.037*[4]	0.680 ±0.041*[3]

* $p < 0.001$ [2] $p < 0.02$ [3] $p < 0.01$ [4] $p < 0.001$

p-values indicate comparison of experimental groups with each other. p-values have been calculated by applying student's t-test of significance (compared with control). Values are expressed as mean ±S.D. (Standard Deviation).

Table 2

Effect of Fluoride on Glycogen Content of Rabbit Ovary, Liver and Skeletal Muscle

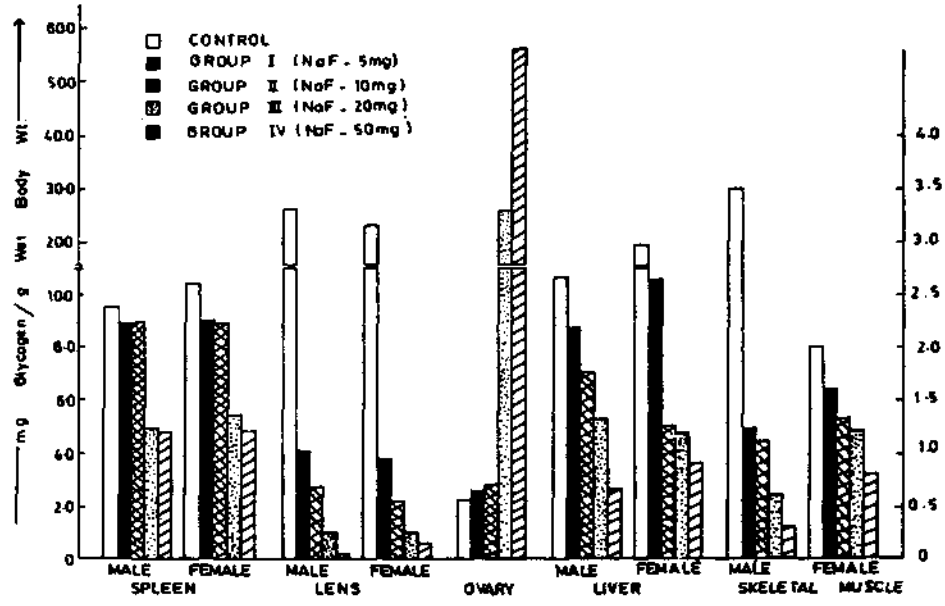
Group	Exposure to Fluoride	mg glycogen/g wet tissue wt.				
		Ovary	Liver		Skeletal Muscle	
			Male	Female	Male	Female
C	Control	2.240 ±0.320	13.676 ±0.110	19.146 ±0.092	3.504 ±0.074	2.081 ±0.110
I	5 mg/kg b.w.	2.600 ±0.490	8.770 ±0.440*	10.340 ±0.180*	1.253 ±0.023*[3]	1.672 ±0.021*
II	10 mg/kg b.w.	2.800 ±0.1928	7.850 ±0.031*[3]	5.175 ±0.665*[3]	1.100 ±0.021*[3]	1.390 ±0.081*[3]
III	20 mg/kg b.w.	26.133 ±0.923*[3]	4.690 ±0.220*[3]	4.797 ±0.042*[3]	0.602 ±0.051*[3]	1.200 ±0.020*[3]
IV	50 mg/kg b.w.	56.666 ±2.886*[3]	2.630 ±0.210*[3]	3.624 ±0.049*[3]	0.370 ±0.021*[3]	0.860 ±0.008*[3]

[5] $p < 0.01$ * $p < 0.001$ [2] $p < 0.01$ [3] $p < 0.001$

p-values indicate comparison of experimental groups with each other. p-values indicate the comparison of treated groups with controls. Values are expressed as mean ±S.D.

Figure 1

The Changes in Glycogen Content in Organs of Rabbits of Both Sexes During Experimental Fluorosis.



Results

Table 1 (Figure 1) indicates the effect of fluoride on the glycogen content of spleen and lens of rabbits of both sexes. The concentration of glycogen in spleen of male rabbits, 9.500 mg/g w.w., decreases insignificantly in Group I followed by significant fall ($p < 0.001$) in subsequent fluoridated experimental groups. The female splen shows significant ($p < 0.001$) reduction in glycogen content in all experimental groups. Decline in glycogen content in 5 mg vs 10 mg F^- group is insignificant.

The amount of glycogen in male lens, which fell from 26.666 mg/g w.w. (control) to 0.280 mg/g w.w. (in 50 mg F^- group), was highly significant ($p < 0.001$). In females the level of glycogen in the lens is 23.410 mg/g w.w. which is less than that for males; it decreased to 0.680 mg/g w.w. in the highest fluoride group.

The ovary which possesses 2.240 mg/g w.w. of glycogen in controls (Table 2) is elevated in all fluoride groups; the highest values were 26.133 mg/g w.w. and 56.666 mg/g w.w. in the 20 and 50 mg F^- groups respectively. Whereas, increase in glycogen content in the 5 mg vs 10 mg F^- group is insignificant, in the 10 mg vs 20 mg F^- group and in the 20 mg vs 50 mg F^- group, it is highly significant ($p < 0.001$).

Table 2 illustrates the depletion in level of glycogen in liver and skeletal muscle of albino rabbits given 5 to 50 mg NaF subcutaneously. The rapid fall of glycogen level in both organs is directly proportional to the amount of fluoride administered. In liver, the concentration of glycogen declines to 2.630 mg/g w.w. (50 mg F^- group) compared to male controls (13.676 mg/g w.w.). In females, glycogen content fell from 19.146 gm/g w.w. to 3.624 mg/g w.w. in the highest F^- group; skeletal muscle also shows a rapid decline in glycogen content in all fluoride groups which is highly significant ($p < 0.001$) in both sexes.

Discussion

Interference with carbohydrate metabolism is frequently noted following acute fluoride intoxication (6). Fluoride poisoning induced dramatic changes in carbohydrate metabolism, which manifest themselves as a rapid decline in glycogen concentration in spleen, lens, liver and skeletal muscle of treated male and female rabbits. The marked changes in the tissue glycogen concentration produced by varying doses of fluoride (5, 10, 20 and 50 mg F) indicate enzyme inhibition of fluoride. Fluorides are recognized as strong inhibitors of enzymes associated with glycolysis (7). The enzyme enolase, present in all tissues, is a dehydrase involved in the anaerobic mechanism of carbohydrates. Activated by magnesium and in the presence of a phosphate buffer, it is inhibited by fluoride with the formation of magnesium fluorophosphate (8-10). In spleen, lens, liver and skeletal muscle, loss of glycogen in the fluoride-treated animals indicated that it caused glycogen breakdown. Handler et al. (6) claim that fluoride poisoning is also accompanied by depletion of liver and muscle glycogen and elevated blood lactate. In rabbits administered 250 mg/kg of NaF in 50 cc distilled water, glycogen content is significantly lowered for 2 hours in skeletal muscle. In chronic fluoride intoxication, produced in rabbits given daily 10-30 mg NaF/kg body wt for 15-159 days, glycogen content decreases in liver and skeletal muscle (11). Annison et al (12) claim a change in glycogen level in liver of sheep given a single lethal dose of fluoroacetate (0.1 mg/kg FAC at 3 day intervals) and a parallel increase in concentration of glucose and lactate in the blood. In hamsters, fluoride decreased glycogen in hepatocytes (13). Decrease in glycogen content in liver and skeletal muscle, during fluoroacetate poisoning, has been attributed to accumulation of citrate and lowering of adenosinetriphosphate in tissues due to blockade in tricarboxylic acid cycle at citrate state (14). McGown and Suttie (3) noted a fall in glycogen level of liver and skeletal muscle in rats administered 20 mg F/kg body wt. over 2 hours. Fluorides are well known stimulators of adenyl cyclase in broken cell preparations (1). Hyperglycemia may result from many factors in addition to hepatic glycogenolysis, including gluconeogenesis, and lactate mobilization from muscle.

Conclusion

The depletion of glycogen in spleen, lens, liver and skeletal muscle of fluoride-treated rabbits, is due to rapid hepatic glycogenolysis, lactate mobilization from muscle and inhibition of enzymes, enolase. Increase in the level of glycogen in the ovary of fluorotic rabbits may be hormonal; this action of fluoride remains to be demonstrated.

Acknowledgement

The material in this paper is part of the work undertaken for a Ph.D. degree by Dr. Shashi. Support from the Indian Council of Medical Research, New Delhi, India, is gratefully acknowledged.

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PHYSICAL DEVELOPMENT OF CHILDREN IN AREAS POLLUTED BY INDUSTRY

by

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SUMMARY: Children aged 4 and 10 years from an area where air is polluted by industry (fluoride and sulfur compounds) were compared with children from a control area without air pollution. The level of F^- in groundwater was identical, namely 0.2 ppm; living conditions of the children were similar. The degree of exposure to fluoride was estimated by its level in the urine. No differences in 20 anthropometric features were discovered between the groups of children, except for more frequent occurrence of less weight in 10 year old boys exposed to changing ecological conditions since birth. The lower level of fluoride in urine of controls was statistically significant.

KEY WORDS: Anthropometric features; Child development; Effects on children; Environmental F; Poland, Police.

Introduction

The purpose of this study, a part of the country-wide investigation sponsored by the Polish Ministry of Health, was to monitor the state of health of the population residing in densely industrialized regions. Here the physical development of children in an area polluted by industry was compared with that of their peers living in an area unaffected by ambient contamination.

Materials and Methods

Two groups of children 4 and 10 years old, chosen at random in kindergarten and grade school, had been living since birth at Police, a town of approximately 20,000 inhabitants situated close to a big chemical factory producing three-component fertilizers. The air in the neighborhood exceeded the limits for admissible concentrations of fluoride and sulfur compounds (Figures 1a and 1b). The pollution led to biological devastation of nearby forests, as well as increased morbidity of the local population which suffered from respiratory tract and superficial eye diseases (1,2). The control group consisted of children in corresponding age groups living in the town of Stargard Szczeciński about 50 km distant from Police, where no excessive concentration of the above-mentioned substances in the air was recorded.

A total of 810 children was studied. All experimental variables, evident within one month since the birth of each child, were taken into account. The measurements for providing anthropometric data were carried out by the same team of investigators using the Martin Saller method (3). The examination

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of 10-year-old children focused on the number of permanent teeth, and the presence of mottled enamel. Concurrently, the fluoride level in the urine in relation to creatinine, phosphorus and hydroxyproline levels was assessed. Urine fluoride concentration was measured with an ion-specific electrode (Orion 9609) in conjunction with pH meter after dilution with an equal volume of Total Ionic Strength Buffer (Orion 940909).

Urinary fluoride concentration measured to monitor fluoride absorption, was corrected on the basis of creatinine concentration by Jackson and Hammersly (4). Hydroxyproline was assessed by the Bergman-Loxley method (5). Parents of the children were interviewed regarding socio-economic status,

Table 1

Mean Values of Fluoride (mM), Creatinine (M) in Urine of Examined Children, Aged 4 and 10 Years

Environment	Age	Sex	Number of Children	Fluoride (mM) Creatinine (M) $\bar{x}$ ± SD	P
Polluted Area	4	Girls	64	4.6 1.4	< 0.01
		Boys	56	4.4 1.3	< 0.01
	10	Girls	61	5.6 2.2	< 0.01
		Boys	42	5.9 2.0	< 0.01
Control Area	4	Girls	50	3.1 1.1	
		Boys	59	2.7 0.8	
	10	Girls	78	3.7 1.3	
		Boys	47	2.6 1.2	

Table 2

Mean Values of Inorganic Phosphorus in Urine of Examined Children, Aged 4 and 10 Years

Environment	Age	Sex	Number of Children	Inorganic Phosphorus (mM) Creatinine (M) $\bar{x}$ ± SD	P
Polluted Area	4	Girls	38	2.45 2.2	> 0.05
		Boys	41	5.14 2.4	
	10	Girls	61	3.7 1.0	< 0.01
		Boys	53	4.6 2.1	< 0.01
Control Area	4	Girls	33	3.8 1.2	
		Boys	30	4.83 2.8	
	10	Girls	76	5.4 2.8	
		Boys	47	5.9 3.7	

educational level, mother's employment and number of siblings. The statistical significance of the difference between the mean values of the studied features was determined by Student's t-test, and the χ^2 test determined the statistical significance between the percentages in children exhibiting disproportions in the basic anthropometric data.

Table 3

Mean Values of Indices of Hydroxyproline in Urine of Examined Children, Aged 4 and 10 Years

Environment	Age	Sex	Number of Children	Proportion Hydroxyproline (mM) to Creatinine (mM)		
				$\bar{x}$ ±	SD	P
Polluted Area	4	Girls	82	0.093	0.052	< 0.05
		Boys	74	0.108	0.047	< 0.05
	10	Girls	61	0.088	0.054	< 0.05
		Boys	53	0.074	0.034	< 0.05
Control Area	4	Girls	68	0.098	0.049	
		Boys	79	0.08	0.039	
	10	Girls	78	0.100	0.046	
		Boys	47	0.078	0.032	

Results

To eliminate the effect exerted by noted differences within social factors, the role of which in this country is still considerable (6,7), the socio-economic data, of the studied children in both environments, were analyzed. In the control region more divorces took place in families and almost four times as many were single mothers. Altogether 13.2% children in the control region were living in incomplete families, while in Police this occurred in only 7.6% of children. This unfavorable finding was compensated by the fact that in the control region 9.2% of parents had secondary or higher education whereas in the polluted area such cases were only 4.9%. More females in the polluted region were not employed professionally which enabled them to give better care to their offspring, but reduced the average family income. No significant differences in the number of siblings in the respective areas was observed. Predominantly in both areas, families consisted of couples with two children; slightly more than 2% were multi-member families, having more than 4 children. The above data do not represent significant socio-economic difference between families living in the polluted and control environments.

Among environmental factors, fluoride compounds constitute the severest life-threatening agent. The average annual norm in the air was greatly exceeded, namely 0.0016 mg/m^3 (Figure 1a) as distinctly illustrated by the urinary fluoride level of the children under investigation. Our results proved to be dependent on the age and place of residence. The lowest mean values were found in 10 year old boys (2.6 ±1.2 F per mol of creatinine 10^{-3}) and in girls (3.7 ±1.3) living in the control area.

Figure 1a.

Frequency and Amount of Fluoride in Excess of Permissible Levels

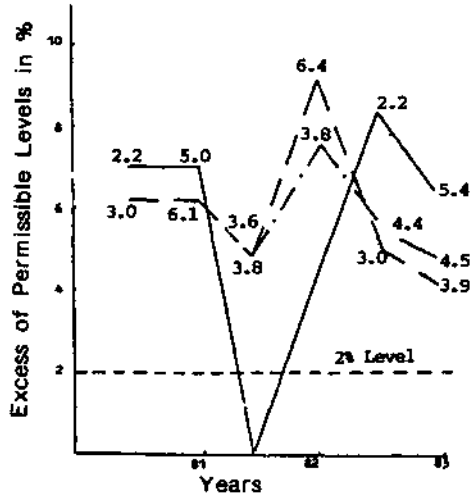
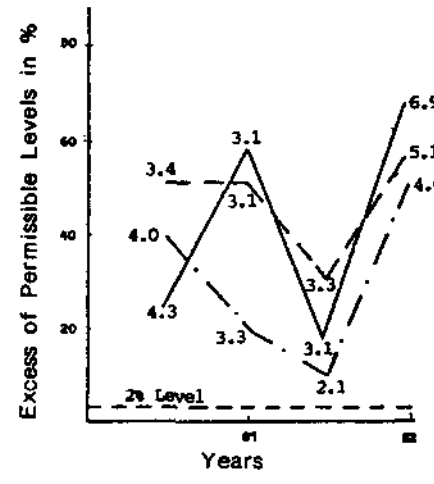


Figure 1b.

Frequency and Amount of Sulphuric Acid Mist in Excess of Permissible Levels



Concentrations in urine were higher in the polluted area. The differences appeared to be statistically significant in the level $p < 0.05$. The urinary fluoride level was higher and in close relationship with the children's age — highest in 10 year olds. Statistically significant differences were also revealed in inorganic phosphorus levels in urine which, in 10 year old children living in the polluted area, was lower compared with controls. Those differences were not detected in four year old children, an observation which may be elucidated by the effect of F on the metabolism of bone and collagen tissues (8). However, nutrition should not be overlooked.

The determination of hydroxyproline levels in urine per mol creatinine may serve as next index designed to evaluate disturbances in the development of bone and collagen tissues. Differences in body weight must also be taken into consideration. However, there were no statistically significant differences in urine hydroxyproline levels in the investigated groups. An additional effect of prolonged exposure to fluoride — changes in dental enamel — was recorded. Mottled enamel, a characteristic sign of fluorosis was visibly more frequent in 10 year old children in polluted regions, involving up to 12.9% of the studied subjects. The mottling might also be due to fluoride added to drinking water as caries preventive (8,9). The more frequent appearance of mottled enamel was accompanied by somewhat better DMF indices, characterizing the caries degree. No differences in the average number of permanent teeth in 10-year-old children which could be associated with accelerated maturation was disclosed.

The averages of the studied features pertaining to the four year old children differed no more than one half of standard deviation, both in plus and minus, from the average values in controls. Circumference and length of lower extremities — the biggest amounted to 64% of standard deviation. In 10 year

old children, the distribution of values of measured features is similarly differentiated; however, differences in favor of girls in the polluted area were preponderant, because 18 out of 20 studied characteristics fit into the zone of positive differences. But these differences were slight; in only two features they exceeded the value, hardly reaching one half of standard deviation. After 10 year's exposure to polluted air, the development of children did not differ significantly from that in controls. Children weighing more than 110% of average in relation to body height were classified overweight; those below 90%, underweight. Underweight occurred at a higher rate in boys (23.8% of the total) in the polluted area. The difference is statistically significant. Underweight, less frequent in girls, was more prevalent in controls.

Table 4

Percentage of Children with Mottled Enamel

Environment	Age	Sex	Number of Children	Mottled Enamel	
				N*	%
Polluted Area	4	Girls	111	1	0.9
		Boys	101	2	1.9
	10	Girls	105	12	11.4
		Boys	101	13	12.9
Control Area	4	Girls	93	0	0
		Boys	117	1	0.8
	10	Girls	109	6	5.5
		Boys	103	5	4.8

*N is number of children with mottled enamel.

Discussion

According to the literature, anthropometric measurements of children are affected by pollution due to industrial emissions (6,7). The toxic effect of fluorine may be explained by the fact that it rapidly decreases the calcium level in plasma, replaces calcium phosphate by calcium fluoride in the bone; simultaneously it affects alkaline phosphatase activity. These changes may influence the child's growth and lower the body weight of those exposed to fluoride. We failed to verify those observations, although urinary fluoride level and increased rate of mottled enamel undoubtedly prove that the studied population is exposed to ever-increasing and harmful emissions of fluorine into the air. Worsening of anthropometric indices for physical development may be countered by adaptation of individuals who survived the fetal period, when the organism is most susceptible to the noxious environmental influence (1,10,11). This is apparent from previous studies on mothers in regions where infants were two to three times more frequently stillborn and the perinatal mortality rate was higher. The extent of adaptation is unknown.

Conclusion

Further monitoring of the health and development status in regions exposed to industrial pollution is indispensable. At the same time, no efforts should be spared in reducing the number of such regions.

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SUBCELLULAR EFFECTS OF FLUORIDE

by

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SUMMARY: Fluoride induces toxicological effects in different tissues when it is administered in vivo and, to a lesser degree, in vitro. The action of fluoride on subcellular components such as membranes, mitochondria, and the systems involved in protein synthesis including the nucleus, polysomes, mitochondria, and hyaloplasm, has been reviewed.

KEY WORDS: F toxic effects; Hyaloplasm; Membranes; Mitochondria; Protein synthesis; Polysomes.

Introduction

Subcellular action of fluoride (F^- administered in vivo and in vitro, has been studied. This paper reviews the effects of F^- on various subcellular systems: membranes, mitochondria, and the systems involved in protein synthesis, including the nucleus, polyribosomes, and hyaloplasm.

Fluoride Effects on Different Subcellular Systems

Effect on membranes (Figure 1). When F^- is administered in vivo, it appears to either increase or decrease intracellular cAMP levels (1-4) due to enhancing or inhibiting the activity of adenylcyclase (3-7). These effects of fluoride are less pronounced when fluoride is administered in vitro. The level of cAMP in the membrane reflects an equilibrium between adenylcyclase-dependent synthesis and phosphodiesterase-catalyzed degradation. One cannot conclude that F^- activates the entire system. Adenylcyclase (Ac) activity can be increased or decreased depending on whether it is present in the inactive (phospho) form or in the active (diphospho) form (1). The membrane Na pump involved intracellular K and extracellular Na can be depressed in the presence of fluoride (8). No information is available concerning the effect of F^- upon the second cyclic nucleotide membrane system, i.e., guanidylcyclase mediated cGMP synthesis. Activation of this system by somatomedins, and of Ac by growth hormone, have been reported (9-11). Little information is available concerning the action of F^- on membrane phospholipids (12), movement of calcium into and out of the membrane in conjunction with the cGMP system (10,11), and internalization of glycoprotein which is transferred from membrane to the intracellular medium. Insulin action has, therefore, been proposed (13).

Effect on mitochondrial system (Figure 2). Mitochondria are the storage site of cellular energy: ATP is synthesized from ADP and AMP, and the synthesis is catalyzed by Na^+-K^+ ATPase, Mg^{++} ATPase and HCO_3^- ATPase (14). ATP is used for synthetic purposes. Movements of calcium occur between hyaloplasm, mitochondria, and endoplasmic reticulum (10). Fluoride impairs aerobic and anaerobic glycolysis (15), Krebs cycle and, particularly, reduces citrate

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Figure 1

Fluoride and Membrane Receptors

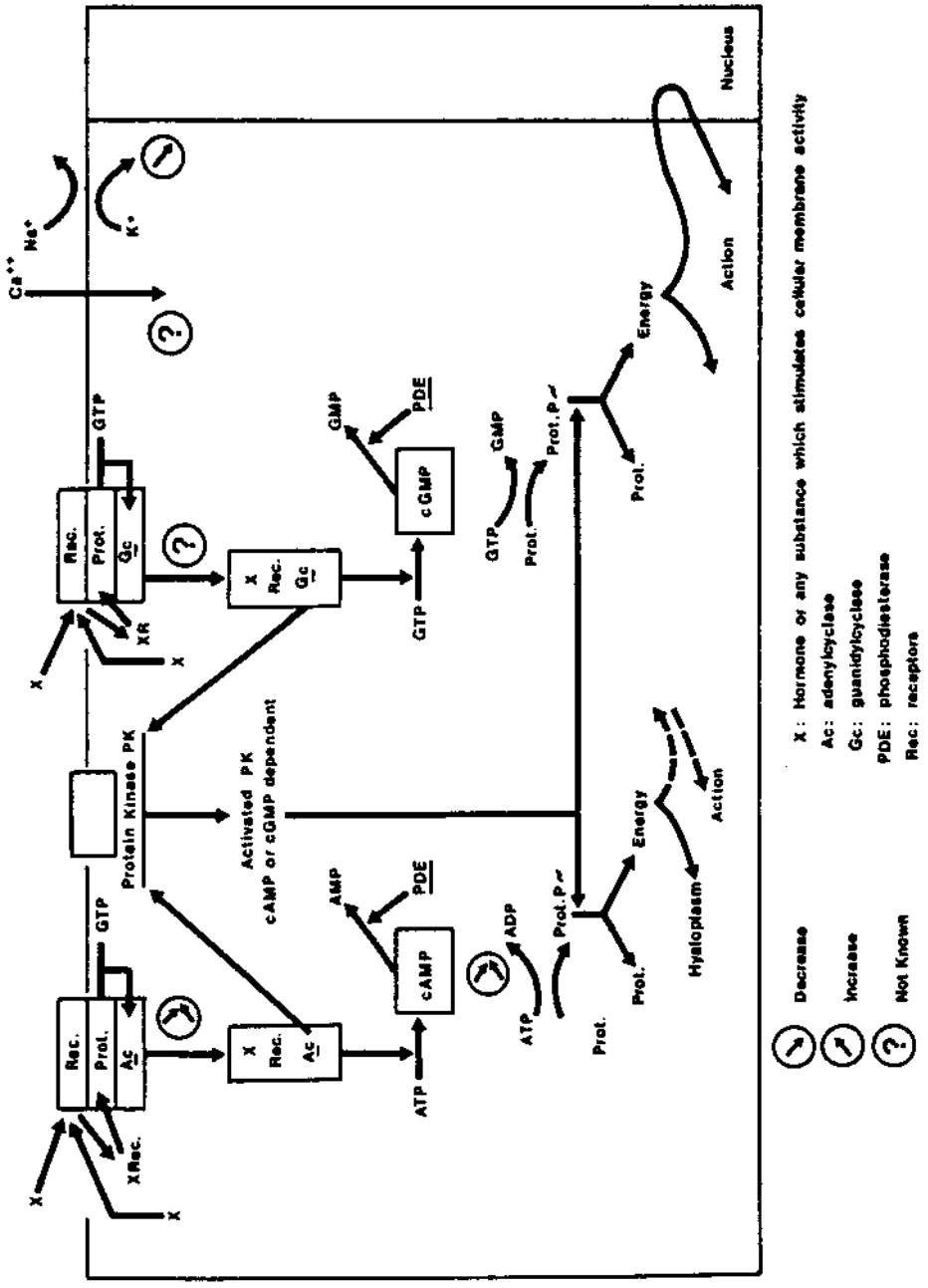
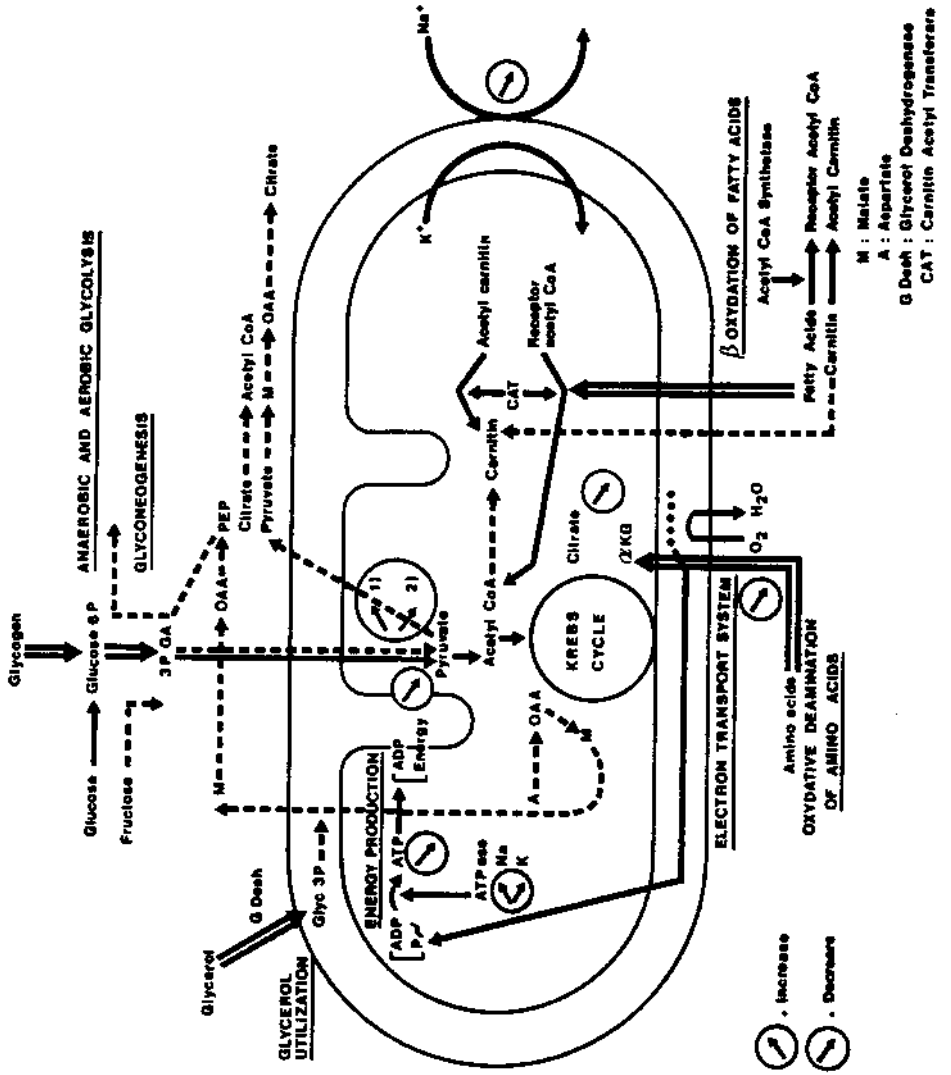


Figure 2

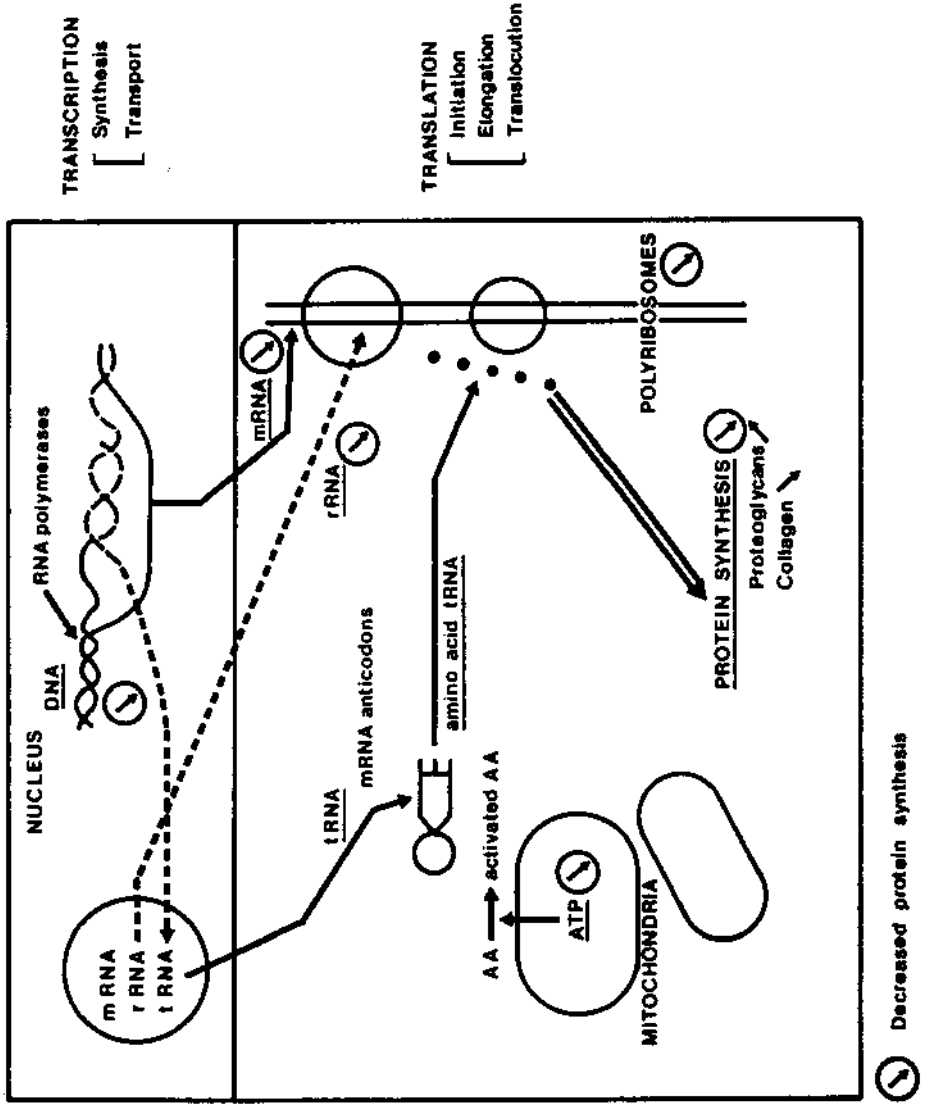
Fluoride and Mitochondrial Activity



(16). In the presence of fluoride, tissue oxygen consumption seems to show a biphasic curve: a slight increase followed by a marked decrease in activity within a few hours following tissue homogenate (17) or mitochondrial preparation (18). An immediate decrease in oxygen consumption may also occur (19). Intracellular ATP level is reduced by fluoride (19-21). The election transport system, particularly cytochromes, is inhibited (22). Na^+-K^+ ATPase level is

Figure 3

Fluoride and Protein Synthesis
Polypeptides Synthesis is dependent on
Nucleus/Polyribosomes/Mitochondria/Hyaloplasm activities



reduced (22,23) and Mg^{++}ATPase level may be decreased (18) or increased (21). Hepatic damage caused by carbon tetrachloride intoxication in rats may not be enhanced by fluoride, which produces major liver alteration itself (21). Mitochondrial levels of ATP and ADP are decreased by fluoride (18,24). Movement of Na^{+} and K^{+} ions across mitochondrial membrane are reduced (25).

Addition of Mg restores the lowered oxygen consumption to normal, as well as Mg^{++}-dependent ATPase, and mitochondrial membrane movements (18). Finally, fluoride induces histological and functional alterations of the energetic activity of mitochondria and the whole cell (25).

Effect on Protein Synthesis (Figure 3). Fluoride is known to reduce protein synthesis (1,26,27). In bone, F^- causes proteoglycans (fundamental substance) to increase, but collagen decreases markedly (28,29). Fluoride inhibits the growth of cellular cultures (1,26,27,30). At the subcellular level, protein synthesis is altered. Chromosomes are altered morphologically (30,31), and a mutagenic effect is sometimes present. Nuclear DNA is either decreased (1,26,27,30) or remains unchanged (32). Intracellular mRNA is decreased (22,25,33). Polyribosomes are partially disintegrated (1,25,33) whereas rRNA is reduced, and ribonucleases are incrased (33). Thymidine uptake by DNA and uridine uptake by RNA are lowered (34). Ribosome translation, which stimulates polypeptides synthesis, is altered. Reduction of mitochondrial ATP could inhibit the activation of hyaloplasmic amino acids, and the functional value of the aminoacyl tRNA. Protein synthesis can be depressed by fluoride at different levels including nucleus, ribosomes, mitochondria, and hyaloplasm (29,35). In bone, a proteic catabolism is present, with an increase of demineralization in spite of relative mineralization (29,36). The effects of F^- on bone, teeth, liver, kidney, pancreas and collagen, may be exhibited by metabolic perturbations leading to their dysfunction at the subcellular level. The in vivo effect may be direct, or mediated by a toxicological complex F-H acting at different levels, or amido-F-H, reducing protein synthesis (37), in particular. In vitro, the minimal toxicological threshold of fluoride affecting different functions is more important (36), because of a decreased toxicological action induced by such complexes. So, different concepts can be better understood by studying the in vivo or in vitro effects of fluoride upon different subcellular fractions.

Conclusion

Different subcellular techniques should also be used to study: a) cytosolic contents of cAMP and cGMP (38), and the peaks at which these compounds appear during the few minutes following in vivo or in vitro administration of fluoride. b) the levels of Ac, Gc, PDE, proteinkinase in membranes or hyaloplasmic extracts (39–41), and c) the determination of calcium movements into or out of the membrane (10,11). It appears necessary, also, to employ other subcellular techniques involved in the study of phospholipid membranes, or glyco-protein (13) that may be affected by fluoride. Still other techniques, such as separation of different subcellular fractions, and use of different substrates following in vivo or in vitro fluoride administration could be studied in comparison with placebos. These techniques should be employed in order to understand fluoride action at subcellualar levels.

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NORMAL FLUORIDE CONCENTRATIONS IN SPOT SAMPLES OF URINE

by

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(Adapted from author's abstract
Fluoride Research 1985; Studies in Environmental Science. 27:401-406, 1985)

To evaluate normal urinary fluoride concentrations, F in spot samples of urine from 1,219 healthy Japanese male adults – considered free from unusual fluoride exposure in their living or working environment – was determined. Mid-morning urine samples were collected from 1,047 Ground Self-Defense Force officials aged 18 to 58, and first-morning urine samples from 172 farmers aged 50 to 69.

Statistical analysis of the data was carried out following logarithmic transformation of the values, since the distribution of fluoride concentrations was nearly logarithmically normal. Geometric mean values of urinary fluoride concentrations of officials and farmers were estimated to be 0.32 mg/L and 0.50 mg/L, respectively.

KEY WORDS: Healthy males; Japan; Urinary fluoride levels.

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SURVEY OF LEAD, CADMIUM AND FLUORIDE IN HUMAN MILK AND CORRELATION OF LEVELS WITH ENVIRONMENTAL AND FOOD FACTORS

by

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(Abstracted from Fd. Chem. Toxic. 24:913-921, 1986)

Lead, cadmium and fluoride were determined in 210 samples of human milk and the mean and median levels and ranges found were 1.04 and 0.55 ng/g (range < 0.05-15.8 ng/g) for lead, 0.08 and 0.06 ng/g (range < 0.002-4.05 ng/g) for cadmium, and 7.08 and <4 ng/g (range <2-97 ng/g) for fluoride. For mothers taking no fluoride supplements and living in communities with fluoride (1 μg/g) in the drinking-water, the mean fluoride level was 9.8 ng/g. Where no fluoride was present in the drinking water, the mean level was

4.4 ng/g. Geometric means for all non-zero lead, cadmium and fluoride concentrations were 0.566, 0.063 and 12 ng/g, respectively. Statistical correlation of levels with some dietary and environmental factors showed that lead levels were most strongly correlated with the age of the house ($p < 0.001$), with maternal exposure to heavy traffic for more than 5 yr ($p = 0.011$), and with coffee consumption ($p = 0.034$). Fluoride levels correlated strongly ($p = 0.007$) with the presence of fluoride in the drinking water. Cadmium levels correlated strongly with exposure to cigarette smoke ($p = 0.005$ if the mother smoked and $p = 0.003$ if the father smoked and the mother did not smoke).

— Author's Abstract

KEY WORDS: Cadmium; Fluoride; Human milk; Lead content.

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PREVENTIVE DENTAL CARE: THE ROLE OF THE PEDIATRICIAN

by

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(Abstracted from Pediatrics 80:107-110, 1987)

In 1966, prenatal fluoride supplements were removed from the market because of insufficient evidence of efficacy. Now they are being recommended: 0.25 mg of fluoride per day until 2 years of age, 0.5 mg/d for ages 2 to 3 years and 1.0 mg/d after age 3 years.

Precautions are urged for the significantly underweight child. "Children younger than 2 years ingesting 0.5 mg/d (double the recommended dosage) can exhibit," the article states, "mild dental fluorosis (mottled or discolored tooth enamel)." Consequently pediatricians are cautioned to take a careful dietary history and to test drinking wate supplies.

KEY WORDS: Dental fluorosis; Prenatal fluoride.

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Editor's Note: According to the Drug News Weekly, Oct. 24, 1986, the question "whether fluoride might aggravate mental retardation and mongolism in offspring was never answered."

TOXIC EFFECTS OF FOOD-BORNE FLUORIDE IN SILVER FOXES

by

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Ithaca, NY, USA

(Abstracted from Cornell Vet. 76:395-402, 1986)

Chronic ingestion of excessive amounts of fluoride from commercial fox food is associated with agalactia in vixens; starvation deaths of large numbers of kits in three fox herds resulted. No evidence of infectious disease or poor management could be found.

The toxic effects of fluoride include dental lesions, arthritis, exostoes, stunting of growth, and decreased milk production. Fluoride is often introduced into lifestock through the feed with mineral supplements.

Initially the farm which involved silver fox pelt production for 7 years, consisted of 49 males, 49 vixens, and a varying number of offspring. The average litter size had been 3.6 kits. Health problems in the herd had been minimal for 5 years. Survival rate for the kits to pelting time was 97.6%; the owners enjoyed premium prices for the pelts.

During the 1985 whelping season, a 67% mortality rate was experienced in kits within 3 days post partum. Vixens were unable to produce the necessary milk to sustain their offspring. Similar compiaints were heard from two other local fox farmers with a total of 62 vixens and their offspring. All these farmers were using reproduction and lactation feeds from the same fox food manufacturer. The feed was suspected as the source of the problem. The highest levels of F in feeds were 136.8 F-ppm for Feed-Reproduction compared to 108.0 F-ppm for Feed-Lactation, and 31.0 in control feeds.

The observed aglactia and subsequent loss of fox pups to starvation directly resulted from this high food-borne fluoride. Fluoride is present in high quantities in fox feeds due to inclusion of fish meal for protein supplementation. No quality control figures have been made available from the fox feed industry on the fluoride levels in fish meal that is used in this supplementation.

This parameter in formation for the canid is largely ignored. The quoted range of tolerance is certainly under 100 ppm, lower for the pregnant or lactating individual. Also evidence in the literature shows high fluoride concentrations in feeds have been associated with perinatal agalactia in the domestic dog. Fluoride ingestion over a period of years results in accumulation in bones, changing calcium hydroxyapatite to calcium fluorapatite.

Fluoride is a known inhibitor of lactation in cattle due to fluoride's toxic effect on bone-resorbing cells. By negatively affecting bone resorption, fluoride reduces calcium availability to the milk producing tissues. Because mammals cannot produce calcium-dilute milk, total milk production is decreased when available calcium is decreased below the requirement for milk production.

Fluoride-induced agalactia in vixens is a here-to-fore undefined disease in the fox industry. A similar syndrome observed in bitches by many veterinarians, has been labelled "fading puppy syndrome." In light of the above-mentioned data on the variability of fluoride in dog foods, some of the "fading puppies" might very well be starving puppies.

KEY WORDS: Agalactia; Fluoride; Fox.

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FLUORIDE CONCENTRATION IN AMNIOTIC FLUID AND FETAL CORD AND MATERNAL PLASMA

by

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To determine the fluoride concentrations in plasma of 50 pregnant women, 44 samples of amniotic fluid and fetal cord blood of 29 fetuses were analyzed at various stages of normal pregnancies: drinking water fluoride was < 0.5 ppm. For all patients in this study, mean fluoride levels of 0.033 ±0.003, 0.017 ±0.003 and 0.028 ±0.005 ppm (±S.E.) were found in maternal plasma, amniotic fluid and plasma from cord blood, respectively.

In patients 36-39 years of age, concentrations in maternal and cord plasma as well as in the amniotic fluid were significantly lower than in two younger age groups, 20-27 and 29-33 years. In amniotic fluid, the mean fluoride concentration was much lower than that in cord or maternal plasma.

In term pregnancies, the fluoride level in maternal plasma and amniotic fluid had significantly increased, whereas the ratio of the mean fluoride concentration in maternal plasma to that in amniotic fluid was significantly decreased.

KEY WORDS: Amniotic fluid; Fetal cord blood; Gestation fluoride; Maternal plasma; Pregnancy, fluoride.

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HIGH-FLUORIDE DRINKING WATER, FLUOROSIS AND DENTAL CARIES IN ADULTS

by

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Ann Arbor, Michigan USA

(Abstracted from J. Am. Dent. Assoc. 114:324-328, 1987)

This report describes the relationship to coronal caries of differing levels of fluoride and fluorosis in two New Mexico communties. Lifelong adult residents of the high-fluoride community, Lordsburg, NM, (3.5 ppm), were chosen for comparison with residents of Deming, NM, (average 0.7 ppm). Although fluorosis increases with rising levels of fluoride, whether increased fluorosis, especially at higher levels, is associated with changes in the amount of dental caries is not clear.

For both communities, records indicate that the same water supply has been in use since at least the early part of the century. The climate in the two communties is virtually identical. Many other characteristics suggest that these cities are a good choice for comparison. Subjects, approximately 30-to-60 years of age, had been born in and had lived for at least the first 6 years of life in the city, and had been consuming city water during that time.

Of 164 Lordsburg residents, 63 or 38.4% were classified as severe fluorosis, 62 or 37.8% very severe, 37 or 22.6% moderately whereas of 151 Deming residents, none fell into the severe or very severe category; 5 or 3.3% were classified as moderate.

Overall, these results suggest that, after controlling for other important variables, the main difference is that the adult participants in Deming have nearly two more restored teeth than the participants in Lordsburg.

KEY WORDS: Adult teeth; Deming, N.M.; Dental caries; Dental Fluorosis; High-fluoride water; Lordsburg, N.M.

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FLUORIDATION: TIME FOR A NEW BASE LINE?

by

A.S. Gray
Victoria, B.C., Canada

(Abstracted from J. Can. Dent. Assoc., 53:763-765, 1987)

The author, who is Director of Dental Health Services for the British Columbia Ministry of Health, reviews current findings indicating an urgent need for the dental profession in Canada to adopt an updated, scientifically appropriate position on water fluoridation. The widely advocated 30-year-old claim that fluoridation reduces dental caries in children by 50-70 percent cannot be maintained, he points out, since tooth decay has been declining dramatically in recent years in nonfluoridated areas as well as in fluoridated ones.

His surveys in British Columbia, with only 11 percent of the population drinking fluoridated water, show lower average DMFT (decayed, missing, filled permanent teeth) rates than in provinces of Canada with 40-70 percent of the population receiving fluoridated water. Moreover, school districts recently reporting the highest rates of caries-free teeth were not fluoridated but, instead, "were totally unfluoridated."

Improved oral hygiene, better and more prompt professional dental care, and expanded, widespread use of fluoridated dental products are held mainly responsible for these large declines in dental caries. With greater use of fluoridated dental products, especially in fluoridated areas, there is growing concern about "fluoride hypoplasias" (dental fluorosis). Pit and fissure cavities, which are now estimated to constitute 83 percent of all cavities in North American children, are considered preventable by sealants not by fluorides. Smooth surface caries, which fluorides are most able to prevent, are now noticeable absent in school-age children.

Hence, although the dental profession may not like to admit it, "we may not need fluoridation as much as we [thought] we once did."

KEY WORDS: British Columbia; Caries decline; Fluoridation re-appraisal

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RELATIONSHIP OF FLUORIDE IN DRINKING WATER TO OTHER DRINKING WATER PARAMETERS

by

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(Abstracted from Arch. of Environ. Health, 42:5-13, 1987)

The purpose of this report is to relate how controlled fluoridation practices and the natural fluoride levels of municipal drinking water are related to other municipal water quality and treatment data. Iron, zinc, and strontium were detectable in every water supply. Strontium appeared easily detectable in the high natural fluoride (HNF) study group. Nickel was more frequently detected among municipalities where fluoride was added to drinking water.

The high-population controlled fluoridation (HPCF) study group most frequently used surface water, whereas the high natural fluoride (HNF) study group generally received its water from deep wells. Shallow well water was most frequently used by the low natural fluoride (LNF) study group.

The high natural fluoride (HNF) study was more likely to have elevated radium 226 and sulfate levels compared to the other study groups. The high percentage of HPCF municipalities with low total hardness concentrations is a reflection of the more common practice of softening drinking water among these municipalities.

The study municipalities with fluoride in water naturally showed a significant negative correlation with barium and nitrogen, ans significant positive correlation with strontium, iodine, lead, groundwater supply sources by well depth, radium 226, and sulfate. Nitrogen and barium are negatively correlated with antural fluoride levels, whereas strontium, lead, and radium 226, which are found in deeper wells, are positively correlated.

KEY WORDS: Barium; Drinking water parameters; fluoride; iodine; lead; nickel; radium; strontium; zinc.

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INHALATION TOXICITY OF AMMONIUM PERFLUOROOCTANOATE

by

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(Abstracted from Food and Chemical Toxicology, 24:1325-1329, 1986)

Ammonium perfluorooctanoate (CAS Registry No. 3825-26-1) is a fine white powder which can become airborne; hence its inhalation toxicity was studied in the male rat. The compound was found to be moderately toxic following single 4-hr. exposures, with an LC_{50} of 980 mg/m^3. This concentration produced both an increase in liver size and corneal opacity. Both findings diminished with increasing time after exposure. Subchronic head-only inhalation exposures (6 hr/day on 5 days/wk for 2 wk to 0, 1, 8 or 84 mg/m^3) suppressed body-weight gain at 84 mg/m^3. Reversible liver-weight increases, reversible increases in serum enzyme activities, and microscopic liver pathology, including necrosis, occurred at exposure of 8 and 84 mg/m^3. No ocular changes were produced. Concentrations of organofluoride in the blood showed a dose relationship with initial levels of 108 ppm in rats treated at 84 mg/m^3 falling to 0.84 ppm after 84 days with a blood half-life of 5-7 days. The no-observed-effect level was 1 mg/m^3 and a mean organofluoride blood level of 13 ppm was detected in rats immediately after the tenth exposure to an atmospheric level of 1 mg ammonium perfluorooctanoate/m^3.

KEY WORDS: Ammonium perfluorooctanoate; Inhalation toxicity; Male rats.

REPRINTS: Central Research and Development Dept., E.I. Dupont de Nemours and Co., Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, Deleware 19714, USA.

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REDUCTION OF AIRBORNE FLUORIDE EMISSIONS FROM CANADIAN ALUMINIUM SMELTERS AS REVEALED BY SNOW CHEMISTRY

by

Marcel Ouellet
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(Abstracted from The Science of the Total Environment, 66:65-72, 1987)

Despite massive atmospheric fluoride emissions from the world's largest aluminum smelter (435,000 tons/year) located in Jonquiere (Arvida), Province of Québec, very few published studies have dealt with its environmental impact. Fluorosis in dairy cattle has been identified in this region since 1951. The highly corrosive hydrofluoric acid (HF) emissions have etched the windows of the local residences and have been accepted for several decades as "normal"

by the residents living within the vicinity of the Arvida plant. A 1985 study of Rampalon disclosed that in the city of Chicoutimi located a few kilometres east of the plant, cancer deaths were more frequent than in 40 other Canadian communities.

The present study compares the estimated gaseous atmospheric fluoride emissions of two old aluminum smelters of the Alcan Company, located within the Saguenay-Lake Saint-John Region (Québec), by measurement of total fluoride concentrations found in the regional surficial snow before (1978) and after (1984), the year of the depollution program was completed. Because of its large hydroelectric power capacity, the hydrological basin of this region has become one of the most important regions for aluminum production in the world (5%).

In March 1978 and 1984, respectively, 344 and 194 surficial snow samples were collected from an area of 4500 km^2 of the regional valley from the eastern shore of Lake Saint-John to "Ha! Ha! Bay." On both occasions, individual sites were in open areas at least 50 m from rural and urban roads. Samples were taken with a small plastic scoop, placed in plastic bags, and frozen (-20°C) until melted for laboratory analyses. The significant reduction of fluorides in the regional snow between 1978 and 1984 is quite evident. Nevertheless, the trend reflects the 1.5-0.7 kg/t gaseous F^- emission reduction at both smelters between the two sampling periods. The difference in magnitude between the reduction of gaseous emissions at the source between 1978 and 1984 (1.5 vs 0.7 kg F/t of Al) and the regional spatial contamination reduction (3000 vs 100 km^2) is most likely related to the concomitant decrease in emission of particulate fluoride. This same factor would also explain the magnitude of reducted concentrations of snowborn fluoride in the vicinity of the smelters (13 vs 1 mg/L). There has been a much greater 10-fold reduction of particulate emissions during the 1975-1983 period.

Thus, fluoride snow chemistry appears to be an inexpensive, rapid and sensitive method to assess the efficiency of an atmospheric environmental pollution control program.

KEY WORDS: Airborne fluoride; Aluminum smelter; Canada; Snow fluoride.

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INSTRUCTIONS TO AUTHORS

Fluoride, the official journal of the International Society for Fluoride Research (ISFR) is published quarterly (January, April, July, October). Its scope is the publication of papers and reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference are published in *Fluoride*. Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

PREPARATION OF PAPERS

1. **General** – No precise limit is given on the length of the paper; it should be written concisely in English, submitted in two copies, doublespaced with generous margins. Measures are given in metric system (SI).

2. **Title** – A concise but informative title should be followed by the name of author(s), the location and state (country) where the research was carried out. The name and address of the institution where the work was done should appear at the bottom of the first page.

3. **Summary** – The paper should begin with a brief, factual summary.

4. **Introduction** – Following the summary, a short introduction should state the reason for the work with a brief review of previous works on the subject. References are given by numbers in parentheses.

5. **Materials and Methods** – should be condensed; however if the methodology is new or developed by the author(s) it can be more detailed.

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8. **Abbreviations or Acronyms** – must be defined either parenthetically or in a footnote when they first appear.

9. **Bibliography** – should be arranged according to the order in which the articles are cited in the text (not alphabetically). An example follows:

> Fiske, C.H. and Subba Row, Y.: The Colorimetric Determination of Phosphorus. J. *Biol. Chem.*, 66:375-400, 1925.

For books, the title, editor, publisher, location and year of publication, and pages should be given.

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