April, 1989 Vol. Twenty-two, No. Two

FLUORIDE

OFFICIAL QUARTERLY JOURNAL

OF

INTERNATIONAL

SOCIETY for

FLUORIDE

RESEARCH

Vol. Twenty-two, No. Two April, 1989 Pages 51-100

FLUORIDE

Quarterly Reports

Issued by

THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

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FLUORIDE is published quarterly by the INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH, INC.

SUBSCRIPTION RATES — Price per annum in advance, including postage: $30.00. Single copies, $8.50.

MANUSCRIPTS for publication should be submitted in English, doublespaced with generous margins. References should be arranged according to the order in which they are cited in the text, and written as follows: Author, title, journal, volume, pages and year. Each paper must contain a summary ordinarily not exceeding 15 lines. Papers are accepted for publication after favorable evaluation and recommendation by qualified reviewers.

FLUORIDE is listed in:
Current Contents/Agriculture, Biology & Enviromental Sciences

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FLUORIDE-INDUCED GROWTH STIMULATION IN PLANTS

Over the years a number of reports have indicated that low concentrations of fluoride — in contrast to toxic effects often from only slightly higher levels — may occasionally induce growth stimulations in plants. Many of these have been discussed by Treshow (1) and by Bennett et al. (2). While some of these reports have indicated fluoride-induced increases in length, a few have demonstrated that fluoride treatments may induce increases in dry weight. Such fluoride-induced increases in dry weight, in beans exposed to HF fumigation reported by Treshow and Harner (3), and in maize where fluoride was applied to the root medium as reported by Betrand (4), indicate that perhaps such fluoride-induced growth may at least involve increased photosynthetic yields, if not a direct involvement in the photosynthetic process. More recently Doley (5) has reported experiments with a variety of Pinus caribaea where low concentrations of HF were associated with an increase in both the rate of apparent photosynthesis and also in an increase in the levels of chlorophylls a and b. Hopefully, further research on this possible connection between fluoride and components of photosynthesis may provide interesting data.

Many of the growth stimulations induced by fluoride tend to mimic the effects of gibberellin — that is by an increase in elongation of plant tissues. Garrec and Chopin (6) have presented results suggesting that calcium migrates towards sites of fluoride accumulation. Thus, fluoride may act by removing calcium. Crozier and Turnbull (7) have suggested that gibberellin may act by inducing Ca^{2+} ions to move from the plant cell wall to the protoplast, resulting in increases in cell wall plasticity and growth. Calcium is a constituent of cell wall pectins. Perhaps by tying up cell wall calcium, fluoride induces more growth. On the other hand, fluoride may act by inducing the formation of new gibberellins.

While fluoride-induced growth has proven difficult to replicate, some of the problem is probably due to the broad range of toxic concentrations of fluoride and the narrow range of growth stimulating concentrations. At least fluoride-induced growth may prove to be a fruitful line of investigation as new techniques become available and new investigators enter the field.

Fluoride could never be applied on a large scale and especially not to food crops, but it may prove to be a useful application one day to conifer (forestry) seedlings in a greenhouse or nursery, or to woody or greenhouse ornamentals at an early stage in their commercial development. For example, recently, Bunce (8) reported on possible stimulations of hemlock growth induced by exposure to HF.

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CORRECTION: (Vol. 22, No. 1, 1989. Paragraph 3, line 12). In the "Report of a Meeting," International Fluoridation Symposium in Brazil the word "agreed" should have been "argued."

ANGLESEY FLUORIDATION TRIALS RE-EXAMINED

by

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SUMMARY: Although recognized by several national authorities as a potential environmental and health hazard, water fluoridation is usually justified on the grounds of its reputedly enormous dental benefits. Recent evidence suggests that the benefits from fluoridation in reducing dental caries may have been overestimated; consequently, there is need for scientific evaluation of the experimental design of previous fluoridation trials.

The often-cited Anglesey fluoridation surveys are re-examined as a case study. In the 1974 and 1983 surveys, the non-random choice of a "control," 19 years after fluoridation, negated the benefit of blind examinations. Instead of a longitudinal controlled trial, there remain two cross-sectional surveys for which the test population was mainly rural while the "control" population was entirely urban. Two different categories of secular reduction in caries, which cannot be attributed to fluoridation, occurred between 1974 and 1983. So, it is doubtful that these Anglesey studies, or the earlier 1955-1967 study, provide evidence of large benefits from fluoridation.

KEY WORDS: Anglesey; Dental caries; Experimental design; Fluoridation.

Introduction

The fluoridation of water supplies has been recognized by several national authorities as a potential environmental and health hazard. For instance, the National Agency for Environmental Protection in Denmark recommended to the Minister for the Environment "not to permit fluoridation of drinking water in Denmark. The recommendation of the Agency is among other things based upon the fact that a number of questions on human health and environment are not and hardly can be clarified" (1).

The French Ministry responsible for the Environment "has confirmed that France is opposed to fluoridation . . . The fluor put in the drinking water ducts is therefore essentially put back, one way or the other, in the natural environment where it can create some important problems" (2).

The Associate Committee on Scientific Criteria for Environmental Quality of the Canadian National Research Council has pointed out that "Fluoride is a persistent bioaccumulator, and is entering into human food-and-beverage chains in increasing amounts. Careful consideration of all available data indicates that the amount of fluoride ingested daily in foods and beverages by adult humans living in fluoridated communities currently ranges between 3.5

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and 5.5 mg. For a 70 kg human adult, this range is close to the 0.03 to 0.07 mg/kg/day estimated for 'an acceptable daily intake.'" Moreover: "Long-term ingestion, with accumulation of fluoride in animals and man, induces metabolic and biochemical changes, the significance of which has not yet been fully assessed" (3).

The Swedish Fluoride commission found that: "The combined and long-term environmental effects of fluoride are insufficiently known, which is yet another reason for rejecting fluoridation of water. The Commission also found that no adequate survey exists concerning the possible effects of fluoride administration via breast milk substitutes" (4).

A representative of the West German Government has written to the Australian Dental Association, concerning a pamphlet which was being distributed to the public, that: "It is not true 'West Germany . . . discontinued fluoridation because of general political or legal reasons . . . .' but that, as was pointed out to you in earlier letters, fluoridation of drinking water was not permitted in the Federal Republic of Germany except for a special permission granted to the City of Kassel in a test case in the district Wahlershausen of Kassel-Wilhelmshöhe. As it was also stated in earlier letters, the fluoridation plant of the municipal water works was taken out of service on April 1, 1971, as a result of legal and health considerations" (5).

A representative of the Government of Greece has written that "It has been proved that fluoridation of water results in many pathological disorders" (6).

Despite these official environmental and health grounds for concern, water fluoridation has been extensively implemented in North America, Ireland, Australia and New Zealand, and justified there on the grounds that it has enormous dental benefits. Specifically, it is stated by dental/medical authorities that fluoridation reduces dental caries by about 50-60% compared with controls. However, there is a growing body of evidence, summarized in a recent editorial in Fluoride (7), which suggests that the magnitude of these benefits may have been overestimated (8-14).

This evidence suggests that there are factors other than fluoridation which are playing an equal or greater role in the reduction of caries. Among these possible factors are changed patterns of nutrition, improved oral hygiene, the use of topical fluorides and possible changes in the immune status of populations (10). Therefore:

(i) It is likely that much funding of those dental health education and research programs which place strong emphasis on fluoridation is being wasted.

(ii) Until the non-fluoride factors are properly understood, it is possible that a reversion to the "bad old days" of high caries prevalence could occur, despite the widespread fluoridation of water supplies in several English-speaking countries.

(iii) Environmental and health concerns should carry more weight in decision-making about fluoridation.

As a consequence of the scientific questioning of the alleged enormous benefits of fluoridation, re-examinations of past studies have commenced, and have already revealed serious flaws in some of the major fluoridation "trials" (15-17).

Two recent studies of fluoridation in Anglesey, North Wales (18-19), have been described as being "carefully controlled surveys" (20) which were "conducted under strictly blind conditions" (21). These studies have been frequently cited in Britain, Australia and New Zealand as demonstrating or proving the "enormous benefits" of fluoridation. In this paper it is shown that there are serious problems with placing such an interpretation on the results of these studies. Hence greater weight should be placed on the evidence of environmental and health hazards from water fluoridation (22-26).

The Anglesey Studies, 1974 and 1983

The island of Anglesey was partly fluoridated in 1955 and completely fluoridated in 1964 (27). But the "control" population introduced by the authors of the 1974 and 1983 studies (18-19), non-fluoridated Bangor and Caernarfon, was only chosen and examined officially in 1974. By this time, all of Anglesey's children up to age 10 years and many, if not most, of its children up to age 19 years had become "optimally exposed," i.e. had consumed fluoridated water since birth (10). Over the period 1974 to 1983 secular (temporal) reductions in caries were observed in both fluoridated Anglesey and unfluoridated Bangor/Caernarfon, but it is incorrect to assume (20) that the large magnitude of the secular reduction in Anglesey was due to fluoridation. In 1974, all the children chosen to be in the Anglesey test group were "optimally exposed." For them the maximum benefit of fluoridation, if any, would already have been achieved by 1974, and the secular changes in caries prevalence after 1974 in given age groups must be the result of factors other than fluoridation.

So, instead of a longitudinal controlled trial, we are left with two separate time-independent comparisons (18,19) between Anglesey and Bangor/Caernarfon in 1974 and 1983. It should be noted that the test population was mainly rural in character, while the unfluoridated comparison population was drawn from towns. Hence, a difference in caries prevalence between the two groups, resulting from environment and lifestyle factors unrelated to fluoride (such as nutrition), would not be unexpected.

Moreover, it would have been surprising if the high level of caries prevalence in Bangor/Caernarfon had not been known, at least to a rough approximation, before that district was designated as "control" in 1974. In other words, although the examination of children's teeth was "blind," the selection of the "control" was not. The failure to take the latter precaution negated the benefit of taking the former, a point made previously by Colquhoun (28).

The First Anglesey Study: 1955-67

In November 1955, part of the island of Anglesey, Gwalchmai, was fluoridated to 1 ppm fluoride, while another part, Bodafon, was left unfluoridated until 1964. Dental examinations were conducted annually for the first five years of fluoridation from 1955-6 to 1961, then discontinued for a period, then continued annually from 1965 to 1967 inclusive (27).

In several ways, the first Anglesey study was better designed than the subsequent ones in 1974 and 1983 discussed above. The test and control areas were located on the same island, adjacent to each other, and both had the same rural character. Since regular dental examinations commenced in 1955, a longitudinal controlled trial could be and was performed.

But there were already two experimental shortcomings at this stage:

(i) The examinations were not "blind," and so unconscious examiner bias cannot be ruled out;

(ii) there was only the single baseline examination in 1955-6, and so the secular trends in the test and control areas before fluoridation could not be determined and compared.

Over the ten-year period, tooth decay as measured by DMFT (the number of decayed, missing and filled permanent teeth per child), remained essentially constant in the test group. However, in the control group there was a large increase in DMFT in all age groups. In order to argue that fluoridation was effective, it was necessary to assume that a similar large increase in DMFT would have occurred in the test group if it had not been fluoridated in 1955 (27). But because of the experimental shortcomings i and ii (above), the validity of this assumption cannot be checked. Evidence suggesting that the assumption may be invalid is that in 3-7 year-olds in the control group, dmft (the number of decayed, missing and filled deciduous teeth) declined significantly over the decade, although to a smaller extent than in the test group. Hence it cannot be assumed that the "natural" trend was for caries to increase in this period.

It should also be noted that some results which might clarify this and other questions were not published in the official report (27) or, as far as I can determine, anywhere else. The missing results include:

- DMFT in 3-7-year-olds in the years 1957-1960;
- DMFT in 8-14-year-olds in the years 1957-1960;
- DMFT in 12-14-year-olds in all the years 1961, 1965-1967. (For these age groups, the results have only been published for 1955-6 and either 1965 or 1967.)

Discussion

There are serious design flaws in the 1974 and 1983 studies of fluoridation in Anglesey. In some ways, their designs are inferior to that of the first fluoridation study over the period 1955-1967, which was unfortunately terminated prematurely as the result of the fluoridation of the original control group. But the more recent Anglesey studies (18,19) provide evidence of two kinds of caries reduction between 1974 and 1983 which cannot be attributed to fluoridation:

(a) the reduction in unfluoridated areas — e.g. 33% in 15-year-olds in unfluoridated Bangor-Caernarfon;

(b) the reduction in optimally exposed age-groups of children in fluoridated areas — e.g. 43% in 5-year-olds in Anglesey.

For a scientific test of the dental effects of fluoridation, it is necessary to perform several properly controlled longitudinal studies, with annual, blind examinations of children's teeth, including several years of baseline examinations before the test communities have been fluoridated, and follow-up measurements conducted for several years after all age groups have been "optimally exposed." To reduce the possibility of examiner bias, the choice of which community is to become the test group and which the control should be made randomly at the end of the baseline period. Moreover, each test and control group to be compared should be similar in environment, lifestyle and socio-economic parameters. These simple measures, it has been suggested, are the minimum basic requirements for a scientific fluoridation trial (16).

Conclusion

A re-examination of the experimental design and results of the much-quoted Anglesey fluoridation trials indicates that at least a substantial part of the observed secular reductions in dental caries cannot be the result of water fluoridation. Furthermore, there is no scientific evidence that the differences in caries prevalence at a fixed time between the test and "control" groups is due to water fluoridation, rather than (say) differences in nutrition in rural and town communities. These conclusions are consistent with the results of earlier studies (15-17) which find that the experimental design of several other major fluoridation trials is inadequate for determining the magnitude of the alleged dental benefits, which may have been over-estimated.

Hence, the official concerns and scientific evidence that water fluoridation may have adverse environmental and health effects (1-6,22-26) deserve greater weight in the decision-making process.

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ACCUMULATION OF VOLCANOGENIC FLUORIDES BY LICHENS

by

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SUMMARY: A comparative study was carried out to determine the fluoride level in lichens grown on the island of La Palma, in the Canary Islands. The island is characterized by infrequent and small scale volcanic activities. The results show that lichens accumulate fluorides produced from minor volcanic eruptions in relatively high concentrations, with a maximum of 23.3 $\mu g\ g^{-1}$ compared with a background level of less than 1.0 $\mu g\ g^{-1}$. Lichens appear to be sensitive and effective monitors of the dispersal of volcanic gasses.

KEY WORDS: Canary Islands; Fluorides; La Palma; Lichens; Mt. Etna; Volcanos, eruption of.

Introduction

Previous studies of the volcano Mt. Etna (1) have shown that lichens can be used as effective monitors of the dispersal of volcanic gases, particularly fluorides. The aim of this study is to determine whether lichens can also be used to monitor fluoride emissions from other volcanic eruptions which contrast with Etna in terms of the magnitude and length of time of eruption. The area selected for comparison is the island of La Palma, on which there have been six minor eruptions in the last 400 years, the most recent being in 1971.

Mt. Etna

Mt. Etna is a large volcano, 1600 km^2 in area and rising to over 3,300 meters above sea level. It is one of the world's most active volcanos, erupting lava every 2 to 3 years recently, and a continuous emission of a plume of gases from the summit area (2). Estimates of the amount of HF discharged by the plume range from 30 to 2230 tonnes day^{-1}(3,4). A consequence of these emissions is that lichens growing on the downwind side of the volcano at a distance of 5 km from the summit have fluoride levels in excess of 140 $\mu g\ g^{-1}$, in comparison with values of < 3 $\mu g\ g^{-1}$ in specimens taken from the upwind side 15 km from the summit.

La Palma

La Palma is the most north-westerly island of the Canaries archipelago. 730 km^2 in area, it rises to 6,500 m above the sea floor, and was created by Pleistocene and recent volcanic eruptions overlying a basment complex of pillow lavas extruded by dyke swarms. The north of the island is mainly formed by the remnants of a large extinct volcano, the Caldera de Taburiente, whereas the southern part is dominated by a north-south trending ridge, the

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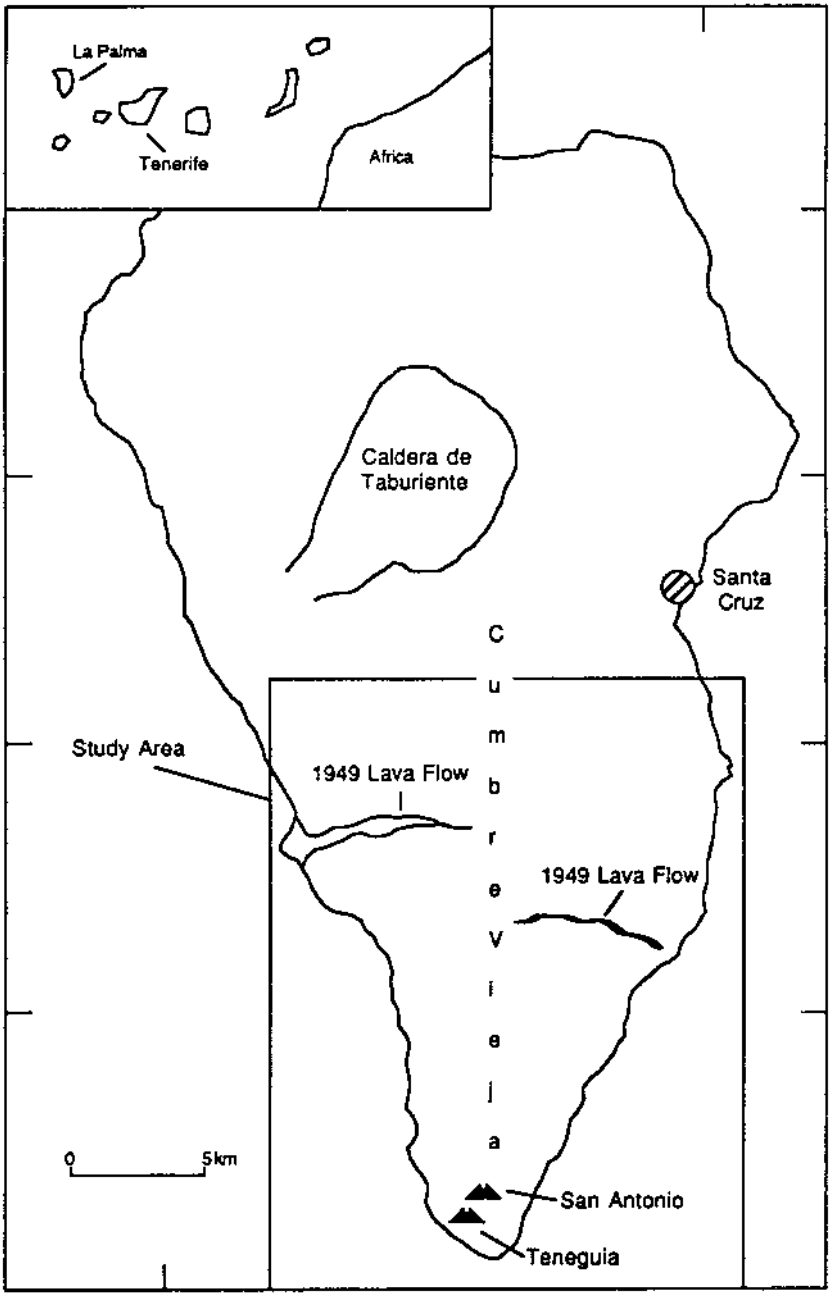
Figure 1

Location and Principal Features of La Palma



Cumbre Vieja, which rises from sea level to 1949 m. The ridge marks the line of a major rift zone, and was created by the build up of volcanic material erupted along this rift. In historic times the crest of the Cumbre Vieja has been the site of four intermittent volcanic actitities, i.e., in 1585, 1646, 1712, and 1949, producing a number of small lava flows confined to the narrow valleys which run east-west down the flanks of ridge. In some instances (e.g. the 1949 flow on the eastern side) these flows are no more than a few meters wide, due to the limited volume of materials erupted and to the restrictions imposed by the width of the valleys. Only on some of the lower slopes, where the valleys broaden as the gradients decline, do the lavas spread to widths of the order of a few hundred meters.

At the southern end of the ridge are two volcanic cones, San Antonio, which erupted in 1677, and Teneguia, in 1971. The Teneguia eruption is the most recent on La Palma. It lasted for 24 days, and is typical of the historic eruptions, producing only small volumes of lava and ash. The cone of Teneguia rises to about 100 m above the surrounding area, to a peak 439 meters above sea level.

No measurements have been made of fluoride emissions from the eruptions of La Palma; however, the volcanic products of La Palma, like those of Etna, are basaltic in nature. The principal differences between the two areas, therefore, are the magnitude and frequency of volcanic activity.

Materials and Method

In 1988 a survey of La Palma was carried out, concentrating on the southern part of the island (Figure 1, Study Area). Lichens were sampled from 37 sites, in particular in the vicinity of Teneguia, and also from an area close to the 1949 lava flows. In addition, to establish the background levels of fluoride for the region, two samples were collected from the north of La Palma and one from Tenerife, which is about 120 km east of La Palma and on which there has been no major volcanic activity since 1798.

Two species of lichen were chosen for this study, Xanthoria parietina and Stereocaulon vesuvianum, since these proved to be suitable monitors of the fluoride emissions from Mt. Etna (5). These lichens tend to occupy different environmental niches, and therefore allowed a greater variety of sites to be sampled. At some sites, it was possible to collect both species, for comparison.

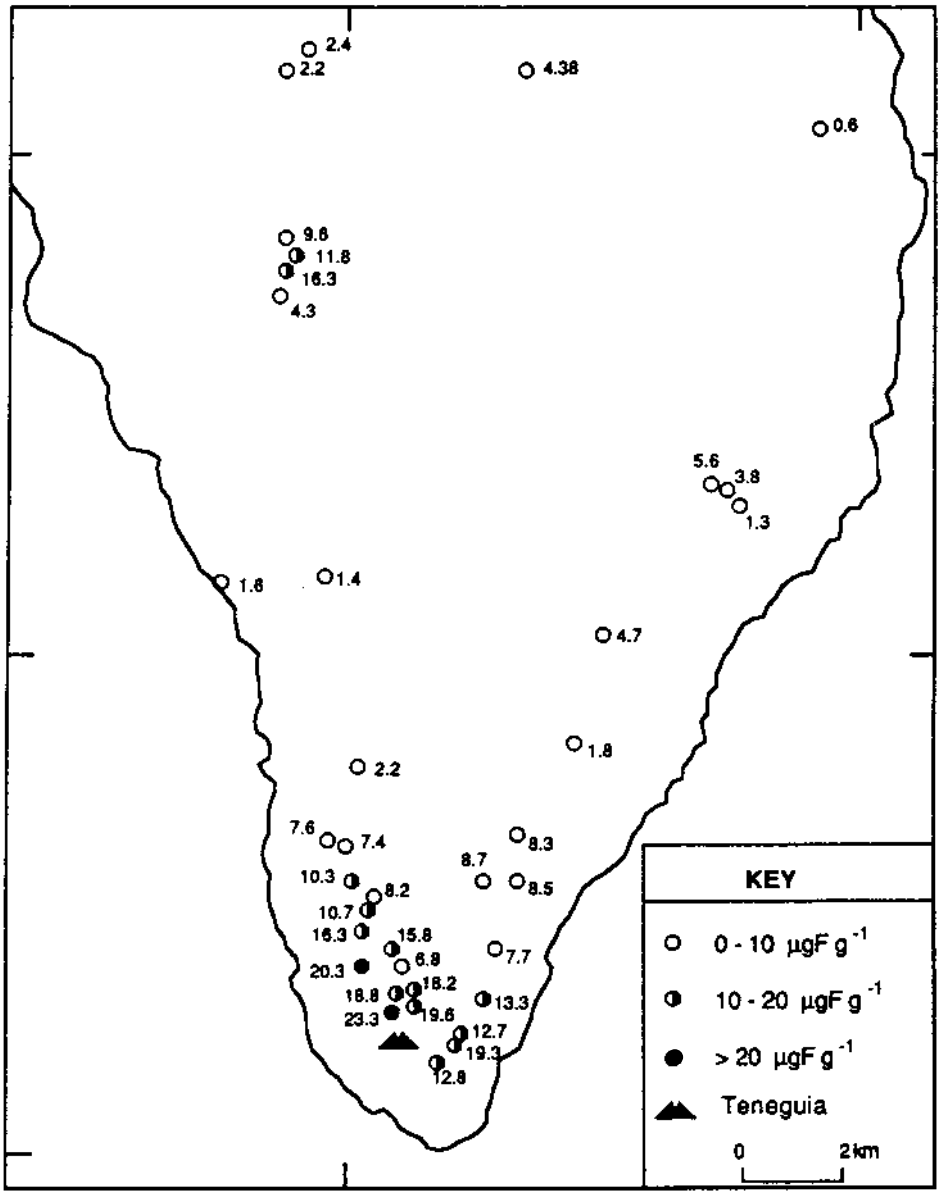
The specimens were washed, dried, and ashed following the procedure of Hall (6), and analyzed for fluoride by fluoride selective ion electrode.

Results

The fluoride levels in the lichens at each sample site in the study area are shown on Figure 2. Fluoride concentrations ranged from 0.6 $\mu g\ g^{-1}$, to 23.3 $\mu g\ g^{-1}$, the highest concentration being recorded 400 m north of Teneguia. For the two samples collected from the north of the island and the one from Tenerife used as controls, the levels were 0.4, 0.7, and 0.9 $\mu gF\ g^{-1}$, respectively. At 12 of the sites on La Palma, both species of lichen were sampled, and the fluoride concentrations in these samples are similar to those obtained from Mt. Etna (5).

Figure 2

Fluoride Concentrations of the Lichens in the Study Area
(expressed as µgF g^{-1} dry weight)



Discussion

The fluoride concentrations in samples from the northern and central parts of the study area (Figure 2) are similar to the background levels, but in samples collected from areas close to the two 1949 flows and from the southern part of the island, in the vicinity of Teneguia, the concentrations are markedly higher. There is no local industry which could account for the raised fluoride levels, and it is clear from their distribution that these are related to the volcanic activities in the three localities.

1949 Flow (West): Four samples were collected from the vicinity of the western 1949 lava flow and the results are shown below:

Table 1

Four Samples Collected from Vicinity of Western 1949 Lava Flow

Sample Number	Distance from Flow (in meters)	Fluoride Concentration ($\mu g\ g^{-1}$)
6	200	4.3
35a	50	9.6
$35b_2$	30	11.8
$35b_1$	0*	16.3

* sample taken from the marginal levee of the flow

Although the 1949 flow is less than 200 m wide at this location, and only about 1-2 m thick, this relatively small volume of lava has degassed sufficiently to cause a very marked increase in the fluoride levels in the lichens close to the flow. Of particular interest is the sample $35b_1$ taken from the northern marginal levee of the flow, which has the highest fluoride concentration of this group. The levee is about 6 m high and was created by rubbly lava being piled up on the margins of the flow. Numerous specimens of Stereocaulon vesuvianum grow on the north facing slope of this levee, and clearly must post-date the flow. The conventional view of lavas is that degassing takes place rapidly as the lava cools, probably within a few weeks, but the high level of fluoride in the lichens taken from the levee indicates that fluorides are released from this flow over a period of tens of years. It is suggested that this is caused by the chemical breakdown of glassy metastable lava material. This releases fluorides, which are dissolved in precipitation, and the lichens absorb the fluorides from rainwater as it washes over the slope of the levee.

1949 Flow (East): The eastern 1949 flow is much smaller; it is only a few meters wide for most of its course, and does not reach the coast. Three samples were taken from the vicinity of the lower part of this flow, where it is less than ten meters wide and no more than 1-2 meters thick:

Table 2

Three Samples Taken from the Vicinity of the 1949 Eastern Flow

Sample Number	Distance from Flow (in meters)	Fluoride Concentration ($\mu g\ g^{-1}$)
2	200	1.3
9	100	3.8
8	50	5.6

Samples 2 and 9 are similar to the background levels for the study area; sample 8, which is the closest of the three to the lava, is slightly higher, which indicates that the degassing of this flow has had some minor local effect. Given the very restricted size of the flow, it is indicative of the sensitivity of the lichens that this effect can be detected.

Teneguia: The highest fluoride levels on the island were recorded in samples collected from close to Teneguia. The 1971 eruption of Teneguia lasted for only 24 days, and was accompanied by both ash falls and small lava flows. Vegetation in only a limited area was destroyed by the eruption, so that plants growing within a few hundred meters of the volcano survived. As a result, lichens which pre-date the eruption were collected from an area close to the volcano.

The maximum fluoride concentration measured was 23.3 $\mu g\ g^{-1}$ in a sample collected 400 m north of Teneguia. The levels declined to the north and east away from the volcano. No samples were available from the area to the south and west of Teneguia, as this was covered by lavas and thick ash falls from the 1971 eruption, which killed all vegetation. As a result, no lichens here were of sufficient size for sampling. This absence of data from the south and west means that it is not possible to establish a definitive pattern for the fluoride distribution around Teneguia. In any case, the wind directions in the Canaries are greatly affected by topography, with an additional modification caused by local land and sea breezes (7), and other these circumstances determining the dominant control on the spread of fluoride from the volcano might well be inconclusive.

The effects of this eruption can be detected in the lichens up to about 4 km to the north of Teneguia. Although there is a steady decline in fluoride concentrations with increasing distance from the volcano, there is an exception to this. Less than 1 km north of Teneguia is an anomalously low value of 6.8 $\mu g\ g^{-1}$, in samples taken from the margins of the crater of San Antonio. These samples, collected at an altitude of over 600 m, are higher than any other samples collected in this area, and over 150 m higher than the present summit of Teneguia. Thus the gases emitted by the eruption of Teneguia were not carried very far up into the atmosphere, but tended to spread out over the lower slopes.

Although the highest fluoride level in the lichens of La Palmas (23.3 $\mu g\ g^{-1}$) is markedly less than that obtained from Etna (141.7 $\mu g\ g^{-1}$), this study has highlighted the sensitivity of lichens to atmospheric volcanogenic fluoride.

It has also demonstrated that they may be used to monitor fluoride emissions from both small scale and short term volcanic eruptions, and from the degassing of minor lava flows.

Acknowledgement

This work was supported by an award from the British Lichen Society. The authors express gratitude for assistance from Luton College of Higher Education, and helpful advice of Dr. A. Duncan in preparation of this manuscript.

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A HIGHLY SELECTIVE METHOD FOR THE SEPARATION OF FLUORIDE ION BY ION EXCHANGE RESIN LOADED WITH A LANTHANUM COMPLEX OF ALIZARIN COMPLEXANE: DEFEROXAMINE AS A MASKING AGENT FOR ALUMINUM

by

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SUMMARY: An effective method for the separation and ion-selective electrode determination of fluoride ion was developed by the use of an anion-exchange resin loaded with alizarin complexane or its sulfonate-lanthanum(III) complex in the presence of deferoxamine as a masking agent for aluminum(III), which seriously interferes with the determination of fluoride ion. The interference caused by iron(III) was not serious when its concentration was not extremely high. Deferoxamine, a chelating agent, selectively forms highly stable chelates with aluminum(III) and iron(III), but not with lanthanum(III).

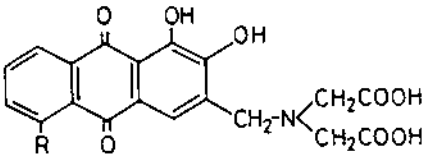
KEY WORDS: Alizarin complexane; Alizarin complexane sulfonate; Deferoxamine; Functionalized resin; Masking agents for aluminum; Preconcentration of fluoride; Separation of fluoride.

Introduction

We have reported previously that anion-exchange resins loaded with alizarin complexane and its sulfonate-lanthanum(III) complexes (abbreviated as ALC-La and ALCS-La resins, respectively) are effective for the selective binding of fluoride ion in the presence of common cations and anions except for aluminum(III) and iron(III) (1). The structure of alizarin complexane (Alizarin Fluorine Blue, 3-aminomethyl-alizarin-N, N-diacetic acid, ALC) and its sulfonate (ALCS) are shown in Figure 1.

Figure 1

Structure of ALC and ALCS



ALC: R=-H
ALCS: R=-SO_3K

Interference from iron(III) is not serious when its concentration is not extremely high, and the effect of iron(III) can be eliminated by the use of common masking agents. However, interference caused by aluminum(III) could not be eliminated completely by use of common chelating agents such as citric acid, 1,2-cyclohexanediamine-N,N,N', N'-tetraacetic acid (CyDTA) and tartaric acid when the concentration of aluminum(III) is higher than that of fluoride ion.

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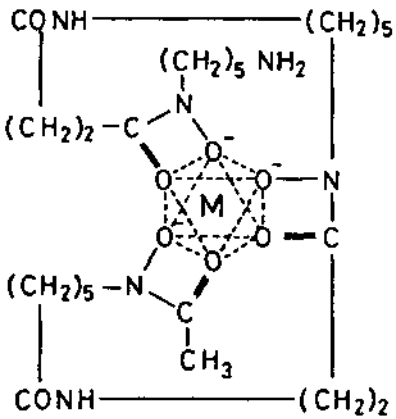
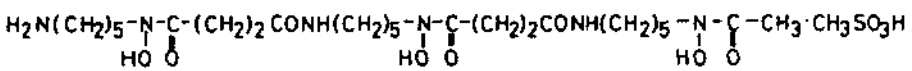
In an attempt to develop an effective method for separation and determination of fluoride ion in the presence of high concentrations of aluminum(III), which is sometimes encountered in environmental water samples, we have examined various chelating agents to mask aluminum(III). Deferoxamine (Figure 2) is an effective masking agent for aluminum(III). Deferoxamine, a metabolic product of Streptomyces pilosus, is a useful therapeutic agent for iron intoxication based on its highly selective chelating ability with iron(III). Recently, deferoxamine which was found to form a stable chelate also with aluminum(III) (2) has been successfully used in the treatment of aluminum encephalopathy (3).

Figure 2

Structure of Deferoxamine and a Possible Structure of Deferoxamine-Metal(III) Complex





This paper deals briefly with the masking ability of deferoxamine for aluminum(III) and iron(III) in the separation and determination of fluoride ion by the use of ALC-La and ALCS-La resins.

Materials and Methods

ALC was obtained from Dojindo Laboratories (Kumamoto, Japan) and ALCS was prepared from potassium alizarin-5-sufonate, formaldehyde and dipotassium iminodiacetate (4). Amberlite IRA 400 (8% divinyl benzene, 100-200 mesh) was used as an anion-exchange resin. Deferoxamine was a gift from Nippon Ciba Geigy Co., Ltd. All other chemicals were of reagent-grade quality.

Preparation of Resin: ALC-La and ALCS-La resins were prepared by a previously reported method (1). Forty μmol of ALC-La or ALCS-La was immobilized on 1 gram of the anion-exchange resin.

ALC-La or ALCS-La resin (0.5 g) was packed into a glass column (1 cm diameter). A 0.02 M acetate buffer (pH 4.2, 100 mL) containing fluoride ion (1.9 ppm), aluminum(III) or iron(III) and deferoxamine was passed through the column at a flow rate of 0.44 mL/min. The sample solution was allowed to stand for 90 and 30 min to complete the complex formation of deferoxamine with aluminum(III) and iron(III) respectively, prior to passing it through the column. The fluoride ions absorbed were eluted from the resin with 1 M NaOH (15 mL). The eluate was neutralized with acetic acid and the fluoride concentration was determined.

Determination of Rate of Formation of Deferoxamine-Aluminum(III) and Iron(III) Complexes in the Presence of Fluoride Ion: To a 0.02 M acetate buffer (pH 4.2) containing fluoride ion (1.9 ppm) and metal ion (aluminum(III), 1×10^{-3} M; iron(III), 1×10^{-2} M), the same concentration of deferoxamine as those of interefering metal ions were added. The concentration of free fluoride ion was determined periodically by the ion selective electrode method using a TISAB solution (1 M acetic acid, 1 M sodium chloride, pH 5.0).

Determination of Fluoride Ion in a Rainwater Sample: A rainwater sample collected in a polypropylene bottle on June 20, 1986 in Osaka, was filtered through a glass fiber filter (Whatman GF/C 47 mm) immediately after sampling. The filtrate (200 mL) was adjusted to pH 4.2 with acetate buffer. The final concentration of acetate was about 0.02 M. Deferoxamine was added to 10^{-6} M. The amount of fluoride ions in the resulting solution was determined in a similar manner to that of the above-mentioned column operation procedure, that of aluminum, with a Shimadzu ICP emission spectrometer 1000.

Determination of Fluoride Ion: Standards were prepared from NaF (Wako Pure Chemical Co., Ltd.). An Orion model EA901 ion-analyzer equipped with an Orion 94-09 fluoride electrode and an Orion 90-02 double junction reference electrode was used throughout this work.

Results and Discussion

As previously reported, effective adsorption of fluoride ions was achieved on ALC-La and ALCS-La resins in the presence of common cations and anions except for aluminum(III) (1). Fluoride ions are selectively adsorbed on the resin, based on the formation of ternary complex between fluoride ion and ALC-La or ALCS-La complex and the optimum pH range was 3.0-4.5. The presence of lanthanum is essential for binding with fluoride ion. We therefore require a masking agent which does not form stable complex with lanthanum(III) but forms a more stable aluminum(III) complex than aluminum-fluoride complexes, such as aluminum-hexafluoride, in order to collect fluoride ions effectively in the presence of aluminum(III).

Deferoxamine forms highly water soluble complexes selectivley with aluminum(III) and iron(III). The values of stability constant (K) of deferoxamine-aluminum(III) and iron(III) complexes are extremely large ($\log K_{Fe(III)} = 30.60$ (5), $\log K_{La(III)} = 20$ (2)), that of its lanthanum(III) complex ($\log K_{Al(III)} = 10.89$ (5)) is much smaller. The masking effect of deferoxamine in the separation of fluoride ion investigated by use of ALC-La and ALC-La resins is summarized in Table 1. Uptakes of fluoride ion on the resins were 63 and 86% at 10^{-4} and 10^{-5} M aluminum(III). The presence of levels of aluminum

Table 1

Effect of Deferoxamine on the Adsorption of Fluoride Ion to ALC-La and ALCS-La* Resins in the Presence of Aluminum(III) and Iron(III)

Metal	Deferoxamine	Uptake of F^-
Al(III), 10^{-5} M	—	86.2
Al(III), 10^{-5} M	10^{-5} M	97.6
Al(III), 10^{-4} M	—	63.4
Al(III), 10^{-4} M	10^{-4} M	98.1
Fe(III), 10^{-4} M	—	97.8
Fe(III), 10^{-4} M	10^{-4} M	96.5
Fe(III), 10^{-3} M	—	98.6*
Fe(III), 10^{-2} M	—	65.6*
Fe(III), 10^{-2} M	10^{-2} M	77.9*

similar to those of fluoride seems to interfere seriously with adsorption of fluoride ion on the resin. On the other hand, uptake of fluoride ion was almost 100% when deferoxamine was added as a masking agent. Aluminum(III) is completely masked by deferoxamine under the conditions.

In the presence of iron(III) less than 10^{-3} M did not interfere with uptake of fluoride ion on ALC-La and ALCS-La resins. The presence of 10^{-2} M of iron(III) interfered with separation of fluoride ion nor was the interference eliminated by addition of equimolar deferoxamine, probably because ALC-La and ALCS-La complexes on the anion-exchange resin become unstable in the presence of high concentration of iron(III), which is not encountered in common environmental and biological samples.

To determine the reaction rate of deferoxamine with aluminum(III) and iron(III), the time course of the concentration of free fluoride ion was investigated after adding aluminum(III) or iron(III) to a mixture of deferoxamine and fluoride ion (Figure 3). The concentration of fluoride ion, not bound to

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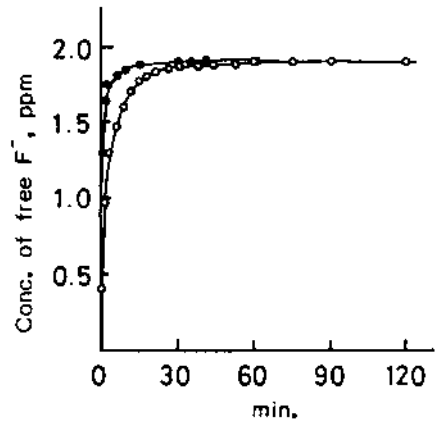
To determine the reaction rate of deferoxamine with aluminum(III) and iron(III), the time course of the concentration of free fluoride ion was investigated after adding aluminum(III) or iron(III) to a mixture of deferoxamine and fluoride ion (Figure 3). The concentration of fluoride ion, not bound to

the metal, increases as the complex formation of deferoxamine with the metal ions proceeds in the presence of fluoride ion. The reaction is expressed as follows:

$$M(III)F_n + \text{deferoxamine} \longrightarrow M(III)\text{-deferoxamine} + nF^-.$$

Figure 3

Rate of the Formation of Deferoxamine-Aluminum(III) and Iron(III) Complexes



(F^-, 1.90 ppm; (o) Aluminum(III) and deferoxamine, 1 x 10^{-3} M; (●) Iron(III) and deferoxamine, 1 x 10^{-2} M.)

The concentration of free fluoride ion becomes constant after 90 and 30 min in the cases of aluminum(III) (Figure 3) and iron(III), respectively. This means that it takes 90 and 30 min for the reactions of deferoxamine with aluminum(III) and iron(III) to reach equilibrium, respectively. The complex formation of deferoxamine is a satisfactory masking agent for aluminum(III), because the time required for 50% release of free fluoride ions by formation of deferoxamine-aluminum(III) complex was less than 5 min (Figure 3).

Accordingly, the masking ability of deferoxamine in the separation of fluoride ion by the use of ALC-La and ALCS-La resins is satisfactory for common environmental and biological samples. For example, the concentration of fluoride ion in rain-water samples has been reported as low as 10^{-7} M and preconcentration is necessary to analyze them by ion-selective electrode method (6). Rain-water samples often contain one or two orders of magnitude higher concentrations of aluminum (7). In fact, the rain-water sample used in this study contained 5.6 x 10^{-7} M aluminum. The concentration of fluoride ion in rain-water, which was determined by the proposed method, was 9.76 ±0.90 ppb (n = 3).

Furthermore, interferences from aluminum(III) and iron(III) in the determination of fluoride ion by the ion selective electrode method were also eliminated by the use of deferoxamine.

Conclusion

Deferoxamine is a potential masking agent for aluminum(III) and iron(III) in the selective collection of fluoride ions by ALC-La and ALCS-La resins and in the determination of fluoride ion by the ion selective electrode method. Elimination of interference from aluminum(III), a serious problem in the determination of fluoride ion, was achieved by use of deferoxamine as a masking agent.

Acknowledgement

We are indebted to Dr. Naotaka Hata for valuable information on deferoxamine-aluminum(III) complex.

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FLUORIDE TOXICITY AND MUSCULAR MANIFESTATIONS: HISTOPATHOLOGICAL EFFECTS IN RABBIT

by

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SUMMARY: To assess the effect of fluoride on skeletal muscle of rabbit during experimental fluorosis, sodium fluoride at 5, 10, 20, and 50 mg/kg body weight/day was injected subcutaneously for 100 days into rabbits of both sexes. Controls were given 1 cc distilled water/kg body weight/day for the same period. Histopathological studies showed retraction of muscle fibres from perimysial sheaths. The sarcoplasm of muscle fibres showed focal areas of necrosis. Nuclear hyperplasia in endomysial connective tissue was observed in animals treated with 20 mg/kg of sodium fluoride. Atrophied and hypertrophied muscle fibres were present. The process of atrophy and hypertrophy of fibres followed a definite pattern as seen in cases of peripheral neuropathies. In animals of 50 mg fluoride group, the muscle fibres showed acute necrosis. These experiments followed a plausible explanation for the marked weakness and fibrillation of the extremities and muscle throughout the body encountered in preskeletal fluorosis.

KEY WORDS: Atrophy; Fluoride; Hypertrophy; Muscle fibre; Necrosis; Rabbit; Skeletal muscle.

Introduction

Chronic fluoride poisoning is a form of geographical and occupational pathology. Its importance has increased sharply in recent years with the use of fluorine compounds in industry, agriculture and medicine. In addition, fluoridation of public water supplies for reduction of the incidence of dental caries in some western countries has exposed a large section of the population to fluoride continuously for prolonged periods. Signs fo acute high dose fluoride poisoning in mammals are severe gastro-intestinal distress including nausea, vomiting, diarrhea and cramps. Neurological disturbances, skin irritations, heart disorder and respiratory complications (1).

The signs and symptoms of muscle involvement in acute fluoride toxicity, namely hyperactive reflexes, painful muscle spasms, extreme weakness and tetanic contractions have been related to fluoride-induced hypocalcemia (2). Studies are lacking on the direct effect of an acute dose of fluoride on muscles.

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This paper was presented by Dr. Shashi at the **XIIth International Congress for Tropical Medicine and Malaria**, held in Amsterdam, The Netherlands, September, 18-23, 1988.

The current study aims to assess the histo-pathological changes in skeletal muscle during acute and chronic fluoride intoxication.

Materials and Methods

Animals: Sixty albino rabbits of both sexes weighing 450-650 g, were procured from Kaila Scientific Corporation, Agra (India). Rabbit pellet chow and water were provided ad libitum.

Experimental Procedure: The animals were divided into five groups of 12 each. The four groups were administered subcutaneous injections of fluoride (as sodium fluoride) in the doses of 5, 10, 20 and 50 mg/kg body weight/day for 100 days. 1 cc distilled water/kg body weight/day was injected into controls. All animals were weighed weekly. Groups of control and treated animals were sacrificed after 100 days, and skeletal muscle from leg and thigh was taken out for histopathological studies.

Histopathology: Small portions of the skeletal muscle were excised immediately and fixed in Bouin's fixative and Carnoy's fluid. The material was washed with 70% alcohol, dehydrated in tertiary-butyl alcohol, cleared in amyl acetate, infiltrated, and embedded in paraffin. The embedded tissue was serially sectioned at 7 µm and stained with iron hematoxylin and eosin. Photomicrographs were taken with an Olympus camera fitted on a traincular microscope (Olympus).

Results

The morphology of the skeletal muscle of the control animals was first studied (Figure 1). In animals treated with 5 mg fluoride/kg body weight, the muscle fibres appeared as rounded or polygonal areas. They showed slight atrophy or hypertrophy and retraction of muscle fibres from perimysial sheaths (Figure 2).

In the 10 mg fluoride group, atrophied and hypertrophied muscle fibres

Figure 1

Section through skeletal muscle of rabbit showing normal structure. H&E x 400.

Figure 2

Skeletal muscle showing retraction of muscle fibres from perimysial sheaths in 5 mg fluoride treated group. H&E x 400.

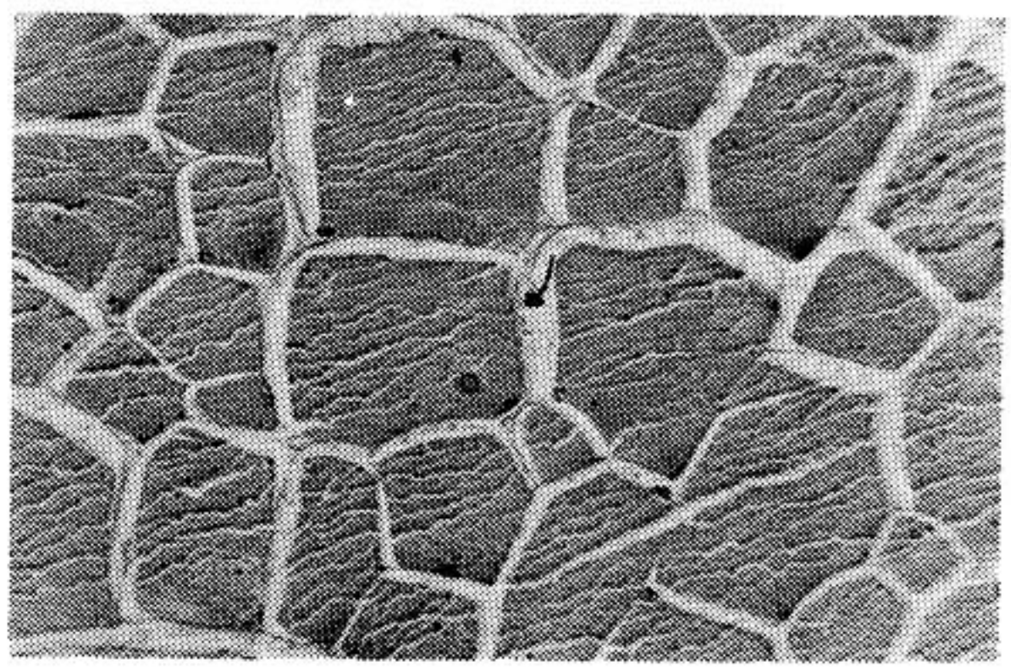


Figure 3

Atrophied and hypertrophied muscle fibres in 10 mg fluoride-treated group. H&E x 100.

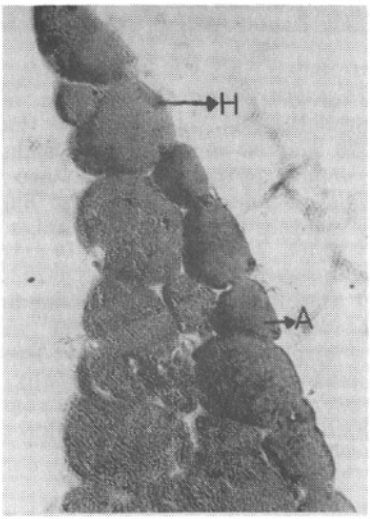


Figure 4

Retraction of muscle fibres from perimysial sheaths in 10 mg fluoride-treated group. H&E x 400.

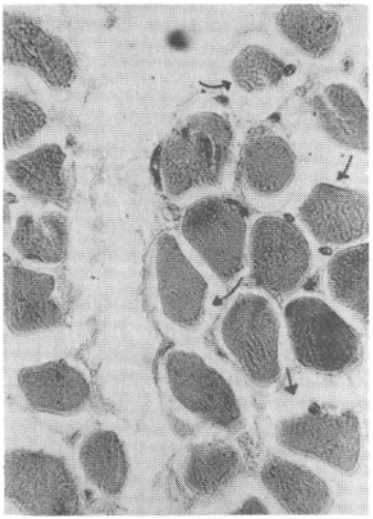


Figure 5

Hypertrophied muscle fibres in animals treated with 20 mg/kg NaF. H&E x 100.

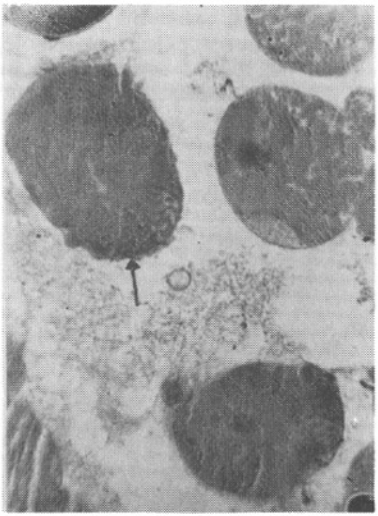


Figure 6

Hypertrophied muscle fibres splitting and disintegration in 20 mg fluoride-treated group. H&E x 100.

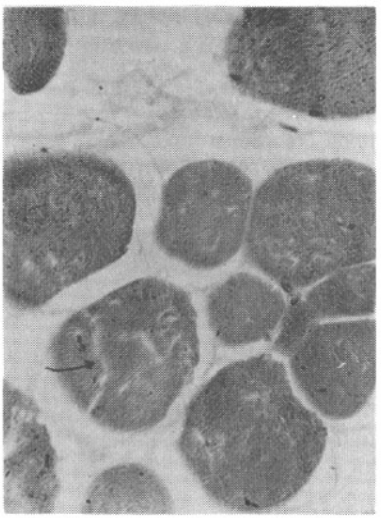


Figure 7

Muscle fibres showing retraction from perimysial sheaths. Focal areas of necrosis also present. 20 mg/kg NaF. H&E x 400.

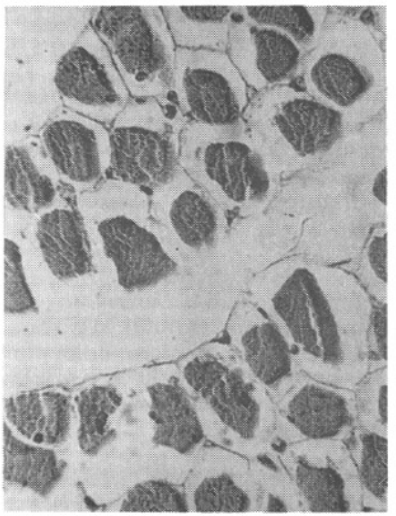


Figure 8

Nuclear hyperplasia in endomysial connective tissue in skeletal muscle: 20 mg fluoride-treated group. H&E x 400.

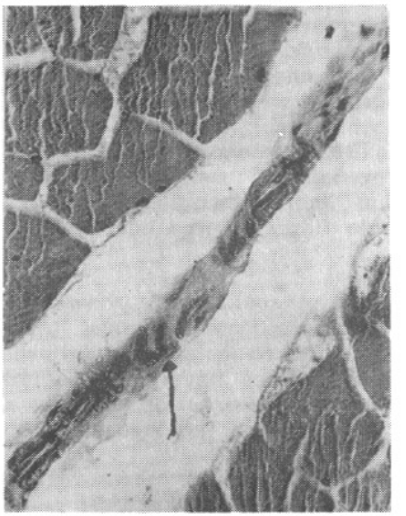


Figure 9

L.S. of skeletal muscle showing fragmentation and disintegration of muscle fibres in animals treated with 50 mg/kg NaF. H&E x 400.

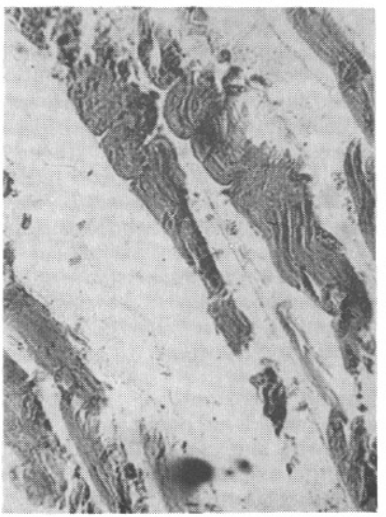
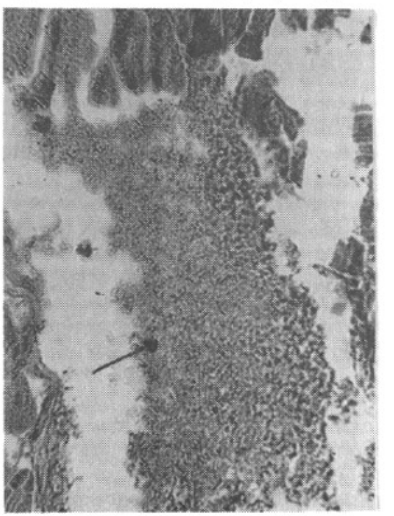


Figure 10

Acute Necrosis of muscle fibres in 50 mg fluoride-treated group. H&E x 400.



were present (Figure 3). The groups of muscle fibres showed retraction from perimysial sheaths (Figure 4). Some muscle fibres showed vacuolization and appeared necrotic.

In animals treated with 20 mg fluoride/kg body weight the majority of muscle fibres were hypertrophied (Figure 5). Some were found spitting and disintegrationg (Figure 6). The sarcoplasm of muscle fibres showed focal areas of necrosis. The muscle fibres exhibited retraction from perimysial sheaths and contained sarcoplasm in various stages of disintegration (Figure 7). Nuclear hyperplasia appeared in endomysial connective tissue (Figure 8).

The degenerative changes were most pronounced in animals treated with 50 mg/kg body weight of sodium fluoride. The dominating change was fragmentation and disintegration of muscle fibres (Figure 9). At some places, the muscle fibres showed acute necrosis.

Discussion

In fluoridated animals, the muscles were reduced in bulk and changed their color to a much paler shade compared to controls. The color changes in the muscles have been recorded by Hogan et al. (3) in their experimental studies on denervation atrophy of muscles, and were believed to be due to the reduction in the myoglobin content of the muscles.

During present experimental investigations, the muscles were fluorosed to a considerable extent. They revealed most of the pathological characteristics such as reduction of muscle fibres, vacuolization, necrosis and deterioration of nuclei. These changes have been recorded earlier by Kaul and Susheela (4) who reported changes in size and shape of muscle fibres and deterioration of muscle fibres after feeding 50 mg fluoride/kg body weight to rabbits for 45 days. In patients with skeletal fluorosis, Kapila et al. (5) reported infiltration of lymphocytes and macrophages in connective tissues, nuclear proliferation with chain formation of nuclei. The sarcoplasm of muscle fibres showed focal areas of necrosis with loss of striation in three patients who have neurological complications. There was moderate increase in fibrous tissue with ring fibres.

More or less similar changes in muscles occurred during the present experimentation on rabbits. Vacuolization and necrosis of muscle fibres, various degrees of disintegration of sarcoplasm and retration of muscle fibres from the perimysial sheath have also been reported by Adam et al. (6) in Meyer-Betz disease (acute necrosis with myoglobinuria) of skeletal muscle. The process of atrophy and hypertrophy of the fibres in a "fluorosed" muscle has followed a definite pattern similar to the changes observed in peripheral neuropathies. The fascicular atrophy, which is the most characteristic feature in muscle undergoing neurogenic atrophy (7) has been observed in severely "fluorosed" muscles also. The process of atrophy found in most of the fibres could be attributed to the inhibition of protein synthesis by fluoride.

Our data indicate that in chronic fluoride intoxication, structural and functional alterations are produced in muscles which accounts for muscular atrophy and weakness. Fluorides act directly on the muscles. Since the fluoride ion has great affinity for calcium (8,9) the high calcium content of skeletal muscle renders it uniquely susceptible to the toxic effects of fluoride.

Conclusion

The results of the present investigation indicate that fluoride can cause extensive damage to the skeletal muscle in fluorotic rabbits which is directly proportional to the dosage of fluoride administered. Furthermore, these exprimments provide a plausible explanation for the marked muscular weakness

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The results of the present investigation indicate that fluoride can cause extensive damage to the skeletal muscle in fluorotic rabbits which is directly proportional to the dosage of fluoride administered. Furthermore, these experiments provide a plausible explanation for the marked muscular weakness and fibrillation of the muscles reported earlier in pre-skeletal fluorosis (1).

Acknowledgement

The material in this paper is a part of the work undertaken for a Ph.D. degree by Dr. Shashi. This work was supported by a grant from Indian Council of Medical Research, New Delhi, India. The author is grateful to ICMR for providing a research fellowship.

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FLUORIDE INDUCED BIOCHEMICAL CHANGES IN REPRODUCTIVE ORGANS OF MALE MICE

by

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SUMMARY: Adult male albino mice were given 10 mg and 20 mg/kg body weight of NaF for 30 days. NaF caused a decrease in body weight, but no change in organ weight, except for the prostate gland and seminal vesicles. No significant change in testis cholesterol and serum testosterone levels occurred. However, in the testis succinic dehydrogenase levels decreased, in the epididymides sialic acid and ATPase levels decreased; in the vas deferens glycogen levels increased, seminal vesicles fructose levels increased and in the prostate glands acid phosphatase and total protein levels increased. After withdrawal of treatment for a period of two months the levels of these substances returned to normal.

KEY WORDS: ACP; ATPase; Cholesterol; Fluoride; Fructose; Glycogen; Mice; Protein; Serum testosterone; Sialic acid; Succinate dehydrogenase.

Introduction

Fluoride is one of the elements in the earth's crust and is distributed ubiquitously throughout nature. It was reported earlier from our laboratory that ingestion of 10 and 20 mg sodium fluoride (NaF) per kg body weight by mice for 30 days caused alterations in the histology of testis, epididymides and vas deferens. The cauda epididymal spermatozoa were rendered non-motile leading to loss of fertility. The sperm density was also reduced. The sperm acrosomal integrity and morphology was altered and some were deflagellated (1,2). NaF-induced effects were tansient and reversible. A microdose of NaF when directly injected in retrograde direction in distal vas deferens of rats also caused alterations in reproductive organ structure and metabolism as well as reduction in fertility (3). The present study is an attempt to investigate the effects of fluoride ingestion for 30 days in the same doses as used earlier on the metabolism and function of reproductive organs of mice. The reversibility and recovery of these organs was also investigated.

Materials and Methods

Adult male albino mice (20-30 gm) of Swiss strain were maintained on standard chow and water was given ad libitum. The first group of 40 mice was given the control diet. The second and third group of animals (40 in each group) were fed sodium fluoride (NaF) at doses of 10 and 20 mg/kg body weight/day respectively for 30 days. In the fourth and fifth groups, treatment (10 mg NaF/kg body weight) was then withdrawn for one and two months respectively. The control and treated mice were weighed and were sacrificed. The testis, caput and cauda epididymides, vas deferens, seminal

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vesicles and prostate gland were carefully dissected out, blotted free of blood and weighed on a torsion balance to the nearest milligram and utilized for various determinations as follows.

Cholesterol: The estimation of cholesterol in testis of control and treated mice, was carried out by the method of Pearson et al., (4) and expressed as mg/100 mg fresh tissue weight.

Succinate dehydrogenase (SDH): The activity of SDH was assayed by the method of Beatty et al. (5) and expressed as µg formazan/100 mg fresh tissue weight.

Sialic Acid: Sialic acid in the epididymis of control as well as treated animals was determined by the method of Jourdian et al. (6) and was expressed as µg/mg fresh tissue weight.

Adenosine Triphosphatase (ATPase): The ATPase activity in epididymides was assayed following the method of Quinn and White (7). The enzyme ATPase hydrolyzes the substrate adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (Pi). The Pi formed at the end of incubation was assayed to determine the rate of reaction.

Glycogen: Glycogen levels were determined in vas deferens of control, treated and withdrawal groups of animals by the method of Seifter et al. (8). The concentration was expressed as µg/100mg fresh tissue weight.

Acid Phosphatase (ACP): Prostate gland ACP activity was assayed by employing the method of Bessey et al. (9) and was expressed as µmoles of p-nitro phenol liberated/mg of fresh tissue weight/30 mins.

Protein: Protein estimation in prostate gland of control, treated and withdrawal group of mice was carried out by the method of Lowry et al. (10) and expressed as mg/100 mg fresh tissue weight.

Table 1

Summary of Treatment

Group	Treatment	Duration (days)	Day of Autopsy
Control	——	—	along with treated
NaF treated	10 mg/kg b.w. (eqiv. to) 230 ppm/animal/day	30	31
NaF treated	20 mg/kg b.w. (equiv. to) 400 ppm/animal/day	30	31
NaF treated	treatment withdrawn for one month	30	31
NaF treated	treatment withdrawn for two months	60	61

Fructose: Fructose was determined in the seminal vesicles by the modified method of Foreman et al. (11) and its concentration was expressed as µg/mg fresh tissue weight.

Testosterone levels from blood serum of control and treated animals were determined by radioimmunoassay (RIA) using RIA kits from Serono Laboratory (Italy).

Results

The body weight decreased after the treatment for 30 days in comparison to control mice, but recovery occurred after withdrawal (Table 2).

Organ Weights: The weights of testis, epididymis and vas deferens were not altered by treatment but those of seminal vesicle and prostrate were increased significantly ($p < 0.001$). Withdrawal of treatment resulted in only partial recovery (Table 2).

Cholesterol level in the testis was not affected by NaF treatment but activity of succinate dehydrogenase (SDH) was significantly ($p < 0.001$) reduced (Table 3). However, recovery was noted after withdrawal of treatment for 60 days (Table 3).

ATPase activity was reduced in both caput and cauda epididymides but recovered significantly after withdrawal (Table 3). The levels of sialic acid were also decreased in both regions of epididymides and subsequently recovery occurred upon withdrawal of treatment (Table 3).

The prostatic protein and acid prosphatase were significantly increased ($p < 0.001$) after treatment. However, recovery was noted after the withdrawal of treatment (Table 4). Similarly, seminal vesicle fructose and vas deferens

Table 2

Body and Organ Weights of Control, NaF Treated and Withdrawn Groups of Mice

Parameter	Control	NaF Treated		NaF Withdrawn	
		10 mg/kg body weight	20 mg/kg body weight	30 days	60 days
Body weight (gm)	25.7 ±0.8	20.5 ±0.4	21.4 ±0.82	24.0 ±0.6	25.9 ±0.6
Testis (mg)	84.0 ±8.0	85.0 ±7.0	84.0 ±1.5	73.3 ±6.0	82.6 ±2.0
Caput Epididymis	14.0 ±0.4	14.8 ±1.0	14.3 ±0.3	15.6 ±0.8	14.6 ±0.6
Cauda Epididymis	10.0 ±1.0	10.0 ±1.0	10.0 ±0.2	10.0 ±0.0	10.6 ±0.6
Vas Deferens	12.0 ±1.0	13.5 ±0.9	13.5 ±0.4	14.3 ±0.8	14.0 ±1.1
Seminal Vesicles	54.0 ±2.0	71.0 ±6.0	74.0 ±4.7	73.6 ±0.8	68.0 ±3.0
Prostate	13.7 ±0.3	20.6 ±1.1	22.6 ±0.8	15.6 ±0.4	17.3 ±0.6

Values are mean ±S.E.

Table 3

Cholesterol; SDH in Testis; Protein, ACP in Prostate; ATPase, Sialic Acid in Epididymis in Control, NaF Treated and NaF Withdrawn Groups of Mice

Tissue	Parameter	Control	NaF Treated		NaF Withdrawn	
			10 mg/kg body wt.	20 mg/kg body wt.	30 days	60 days
Testis*	Cholesterol (mg/100 mg tissue wt.)	0.41 ±0.01	0.43 ±0.01	0.43 ±0.02	—	—
Testis*	Succinate dehydrogenase (µg/100 mg tissue wt.)	836 ±16	400 ±20	415 ±20	560 ±36	761 ±16
Caput Epididymis	ATPase**	13.9 ±0.86	9.26 ±0.47	5.2 ±0.32	—	11.4 ±1.01
Cauda Epididymis	ATPase**	13.94 ±0.23	8.95 ±0.25	6.75 ±0.39	—	12.2 ±0.4
Caput Epididymis	Sialic Acid (µg/mg tissue wt.	4.35 ±0.16	3.83 ±0.15	2.79 ±0.08	—	3.9 ±0.08
Cauda Epididymis	Sialic Acid (µg/mg tissue wt.)	5.77 ±0.1	4.29 ±0.21	3.21 ±0.15	—	5.12 ±0.14

Values are mean ±S.E.
* Data on NaF treatment alone taken from Sequeira and Chinoy (34).
** µmoles ip/30 min/100 mg tissue.

Table 4

Fructose in Seminal Vesicles; Protein, ACP in Prostate and Glycogen in Vas Deferens in Control, NaF Treated and NaF Withdrawn Groups of Mice.

Tissue	Parameter	Control	NaF Treated		NaF Withdrawn	
			10 mg/kg body wt.	20 mg/kg body wt.	30 days	60 Days
Prostate	Protein (mg/100 mg tissue wt.)	4.03 ±0.21	6.16 ±0.2	6.43 ±0.3	5.01 ±0.19	4.39 ±0.02
Prostate	ACP*	0.14 ±0.01	0.24 ±0.07	0.25 ±0.005	0.23 ±0.02	0.18 ±0.02
Seminal	Fructose (µg/mg tissue wt.)	43.00 ±2.7	50.00 ±1.5	57.00 ±2.6	48.1 ±1.0	44.0 ±0.8
Vas Deferens	Glycogen (µg/100 mg tissue wt.)	573 ±9	932 ±9.7	943 ±22	901 ±11	661 ±24

Values are Mean ±S.E.
* µmoles of p-nitro phenol released/mg fresh tissue weight/30 min.

glycogen were also increased after NaF treatments with both doses. The increase was more significant ($p < 0.001$) in case of glycogen. Recovery was obtained on withdrawal of treatment.

The serum testosterone level of treated group was about 40% lower than that of the control group (Table 5).

Table 5

RIA of Serum Testosterone

Tissues	Parameter	Control	NaF treated 10 mg/kg b.w.
Serum	Testosterone (ng/mL)	1.5 ±0.41	0.884 ±0.072*

Values are mean ±S.E.
* Data from Sequeira and Chinoy (34).

Discussion

Treatment with 10, 20 mg NaF/kg body wt. for 30 days resulted in a decrease in the body weight. Schwartz and Milne (12) reported that much lower levels of fluoride (1-2 µg F/gm of diet) stimulates their growth when fed a highly purified amino acid diet and maintained in trace element controlled isolators. However, other workers were not able to confirm these findings (13,14). Saralakumari et al. (15) observed that supplementation of drinking water with 100 ppm of fluoride for two months resulted in reduction in growth rate.

Fluoride is known to disturb carbohydrate metabolism (16). In fluorotic rats the levels of glucose-6-phosphate dehydrogenase was decreased and glycogen turnover depressed (17,18). Rats consuming 450 ppm F in the diet were unable to metabolize glycogen normally due to some effect at the liver enzyme level (19,20). These observations are in agreement with the data of the present study wherein a signifcant increase in glycogen levels in vas deferens was obtained in 10, 20 mg/kg body weight NaF treated mice. This might be related to increase in activity of some enzymes of carbohydrate metabolism as reported by Strochkova and Zhavoronkov (21), or to reduced utilization of glycogen in vas deferens related to the decline in sperm density (2). Macuch et al. (22) have reported that fluoride interferes with binding of amino-acyl-t RNA adducts to the ribosomal RNA template, which is responsible for the impaired polypeptide formation. In the present study, the prostatic protein levels were increased significantly. Tsunoda et al. (23) observed that the total protein in serum in the control goats and those subjected to air-borne fluoride were about the same.

Underwood (16) has reviewed enzyme changes in chronic fluorosis in animals. The testis succinate dehydrogenase (SDH) activity decreased significantly with both doses of NaF treatment which suggests that the oxidative metabolism of testis was affected. Similar data has been obtained for muscle SDH (24). The decrease in SDH might be similar to that of isocitrate dehydrogenase (25), another tricarboxylic acid cycle enzyme which leads to accumulation of citric acid. Bogin et al. (20) reported declines in LDH and isocitrate dehydrogenase levels in the livers, kidneys, hearts, and skeletal muscles of mice treated with 100 ppm NaF. Similarly, Chitra et al. (26) have found decreased LDH in muscle and liver of NaF-treated Channa punctatus. Sullivan

(27) found that animals receiving drinking water supplemented with fluoride (F^-) (around 100 ppm) showed a marked lowering of hepatic SDH activity after continued administration. Fasske (28) and Androsov (29) also reported interference of fluoride with enzymes like SDH in heart and in the liver.

Distinct hypercholesterolemic effects in the serum were observed in the animals after exposure to fluoride (24,25,30,31). Fluoride and zinc increase blood cholesterol and may constitute a predispostion to atherosclerosis (32). However, testis cholesterol levels remained constant throughout treatment. It has been reported elsewhere (1) that Leydig cell and nuclear diameter were not affected by NaF ingestion in mice. Hence it follows that androgenesis by testis might not be altered. Levels of ATPase, sialic acid in epididymides, ACP, protein in prostate, fructose in seminal vesicles, glycogen in vas deferens as well as the histolgy of these organs was altered by NaF treatment. It is also likely that these changes might be the outcome of reduced end-organ response to androgens. According to Hodge and Smith (33) NaF toxicity involves inhibition of enzyme activity particularly those in which divalent metal cations act as cofactors. In the present study too, the alterations especially in ATPase, SDH and ACP activities might be due to the fact that they either Mg^{2+}, Ca^{2+}, or Zn^{2+} metalo-proteins. The altered membrane integrity of spermatozoa by NaF ingestion in mouse as reported earlier (2) might result from a decrease in ATPase in epididymis leading to sperm structural and functional changes and finally to infertility.

Acknowledgement

One of the authors (E.S.) is grateful for financial assistance from R.J. Cama Research Project for carrying out this research work and to St. Theresa's College for granting the necessary permission to carry out doctoral work.

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MONITORING OCCUPATIONAL FLUORIDE EXPOSURE THROUGH URINARY AND SALIVARY TESTS

by

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SUMMARY: To monitor individual fluoride exposure urine and saliva of workers exposed to fluoride contamination at their work place in an enamel factory in Kecskemét (Hungary) were analyzed. The authors recommend testing of renal function and determination of salivary fluoride concentration to complement the currently accepted determination of urinary fluoride and fluoride creatinine ratio.

KEY WORDS: Hungary; Occupational fluoride exposure; Urinary, salivary fluoride.

Introduction

To monitor fluoride metabolism, also in case of occupational fluoride exposure, the most widely accepted test – indeed almost the only test used for this purpose all over the world – is still the determination of the urinary fluoride concentration.

In recent years several papers (1,2,3) have drawn attention to the determination of plasma fluoride level because it gave more adequate information about fluoride exposure, even when renal function was normal. However, obtaining blood samples is difficult on ethical and technical grounds.

Fluoride excretion may significantly decrease when renal function is impaired (diabetes, pregnancy, nephritis, nephrosis, etc.) (4). In other words, urinary fluoride values themselves of persons with impaired renal function fail to provide information with any degree of precision of actual fluoride exposure.

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Kono and coworkers (3) pointed to the importance of plasma fluoride concentration in persons with occupational fluoride exposure. In case of impaired renal function, urinary fluoride fails to indicate the probability of higher fluoride retention, whereas elevated plasma concentrations do so.

Fluoride concentration, fluoride creatinine ratio of urinary spot samples and renal function were determined instead of plasma fluoride and salivary fluoride concentration because sample collection was thereby facilitated. According to the literature, salivary fluoride correlates with the plasma fluoride level (5,6).

Materials and Methods

The investigated persons were smelter workers in an enamel factory in Kecskemét, Hungary, aged between 21 and 49, employed in the factory 1 to 24 years. They declared themselves healthy. During the test period, in the parts of the factory where workers faced fluoride exposure, the fluoride concentration of the air varied widely, 0.15-6.06 mgF/m^3 as a function of the production level. HF was 60% of the total fluoride in the air (7).

Urine and saliva were collected twice, first after a five week period of non-exposure, involving 10 subjects; 3 weeks later, 12 subjects were involved. Preshift urine creatinine was determined according to methods described elsewhere (8).

Saliva collection was performed a minimum of two hours after the last meal and 18 hours after the last toothbrushing. Paraffin stimulated whole saliva was collected for five minutes. Salivary fluoride concentrations were measured according to the Ekstrand method (9).

Renal function was tested using "Hema-Combistix" (Ames, England). Persons with positive blood and/or protein urine results were separated from those whose urine tests were negative.

During the statistical evaluation mean and S.D. were calculated; significance analysis was performed with the two sample t-test.

Results

No significant difference in urinary fluoride and fluoride creatinine ratio between the urinary negative and positive groups was observed either during the non-exposure period (Figure 1a) or the exposure period (Figure 2a).

Salivary concentrations differed significantly ($p < 0.05$) between urinary positive and negative groups in both non-exposure and exposure periods (Figure 1b and Figure 2b).

Discussion

According to international and Hungarian air standards 2.5 and 2.0 mg F/m^3 are maximum acceptable levels of fluoride in exposed areas (NIOSH, 1975; Msz. 2146178). Osteosclerosis is not anticipated if preshift urinary concentration fails to reach the 210 µmol/L and postshift 368 µmol/L. (See ref. 1). Our previous investigation in the same factory (10) showed that pre- and

Figure 1

Urinary and Salivary Fluoride Data in Workers During the Period of Non-exposure

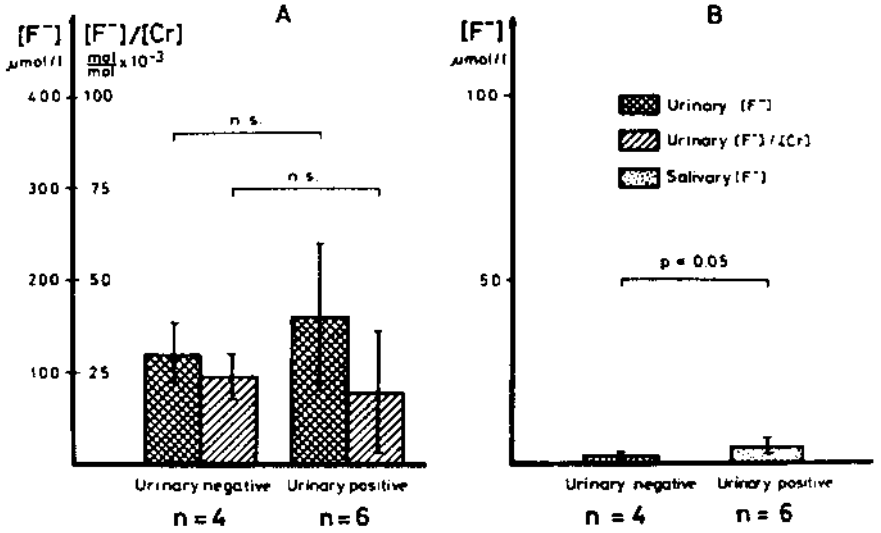
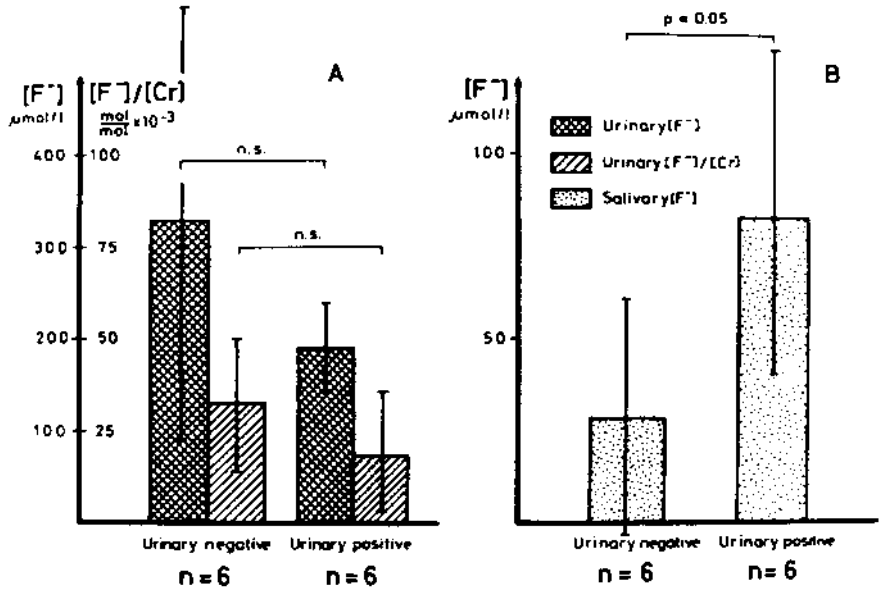


Figure 2

Urinary and Salivary Fluoride Data in Workers During the Period of Exposure



post-shift urinary concentrations were below, but very close to, the maximum acceptable level.

In our present study during the non-exposure period the urinary negative group represented the normal salivary fluoride level. These salivary concentrations indicated that fluoride exposure was the same as that anticipated on the basis of urinary fluoride data. Salivary fluoride concentrations in each of the other three groups clearly suggested a higher fluoride exposure than that which could be derived from the calculations of urinary data.

This discrepancy in the urinary negative group during the period of exposure is probably due to the fact that the renal-function test which was utilized was not the most sensitive one; therefore, presumably that group included "pseudo-negative" cases as far as fluoride excretion is concerned. This presumption is suggested by the fact that in two of the six persons investigated in that group, salivary fluoride concentration was extraordinarily high.

Regarding urinary positive groups, the finding that salivary fluoride values were significantly higher than in urinary negative groups during both non-exposure and exposure periods shows that higher fluoride retention should be anticipated than that based on urinary fluoride data. The literature (11,12,13) adequately explains the alterations in renal function: Decreased renal fluoride excretion leads directly to a rise in plasma fluoride level, and indirectly to increased salivary fluoride concentration.

According to our investigations in controlling individual fluoride exposure, it is justified to test renal function together with urinary fluoride concentration; furthermore it is advisable to examine fluoride concentration of saliva simultaneously, when collecting blood samples is not feasible. Our results, in line with other similar investigations (1,3), suggest that, for adequate information about fluoride exposure, urinary fluoride values alone seem to be unsatisfactory.

Acknowledgements

The authors gratefully acknowledge the assistance of Prof. Jan Ekstrand for help in determination of salivary fluoride concentrations as well as Mrs. Márta Puruczki and Mr. Can Yurdunuseven for their technical assistance.

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REPORT ON THE FOURTH FLUORINE SYMPOSIUM IN SZCZECIN
September 1-2, 1988

by

Z. Machoy
Szczecin, Poland

The fourth Fluorine Symposium in Szczecin was attended by scores of local participants and by invited guests from other countries (GDR, FRG, Hungary). During the two-day symposium under the theme "Previous Achievements in Studies of Fluorine Compounds" 11 papers and 30 experimental reports in the form of posters were presented.

The inaugural lecture by Z. Machoy (Szczecin) entitled "Is Fluorine Essential to Humans?" dealt with accumulation of fluoride in man based on analyses of bones and nails. Storage of fluorine is highest during the period of intensive juvenile growth of the organism when the systemic pool is lowest.

Chemical studies of fluorine compounds in industry, farming and medicine were discussed by U. Glabisz (Szczecin). His paper was entitled "New Trends in Fluorine Chemistry and Technology." K. Jacyszyn (Wrocław) reported on fluorine compounds in medicine. His paper, "Biological Role of Fluorinated Nucleosides and Nucleotides" illustrated the hitherto scored achievements in applying fluorine derivatives in control of diseases, mainly neoplastic during the last 30 years.

The three papers which followed by J. Markiewicz and J. Sadlik (Kraków) were concerned with the toxicology of fluorine compounds. Under the title "Fatal Cases of Poisoning with Fluorine Compounds in the Practice of the Institute of Forensic Research" they discussed the course and circumstances of seven lethal cases of poisoning caused by fluorine compounds. Certain details of the poisoning triggered a lively discussion among symposium participants. The following report by M. Gumińska (Kraków) entitled "Chronic Exposure of Humans to Fluorine Compounds in Industrial Regions and Some Selected Enzymatic Changes and Health Consequences" evaluated chronic industrial poisoning in humans which occurs frequently on a large scale. The consequence of these poisonings was interpreted on the basis of metabolic processes with regard to changes in the activity of the respective enzymes as well as alterations in the mineral composition of the electrolytes in man. Elaboration by W. Wardas and U. Mazurek (Sosnowiec) under the title "Utilization of Unicellular Algae in Biotoxicological Studies and the Valuation of F^- Ion Toxicity in Chlorella Cultures" incorporated biotoxicological studies encompassing various representatives of aquatic plants.

The second day sessions which opened with the report by H. Runge and J. Franke (GDR) entitled "Different Sensitivities to Fluoride in Humans and Animals: An Overview" provided interesting data on sodium fluoride therapy for osteoporosis. Although some patients failed to respond to fluoride therapy, a group was exceptionally sensitive to it. In the next report E. Czerwiński (Kraków), whose subject was "Clinical Diagnosis of Industrial Fluorosis" stated that, in order to establish the correct diagnosis, fluorosis, the following should be taken into account: data depicting the degree of risk, the case history,

as well as physical, radiological and analytical examinations. The outcome, resulting from the effect of industrial emission containing fluorides, was exemplified by people inhabiting a small township in Saxony in a report entitled "Effects of Fluoride Emissions in the Vicinity of Industry" by Ch.W. Schmidt (GDR).

Research by M. Bély (Hungary) under the title "Experimental Fluorosis in Rats: Sodium Fluoride Induced Changes in Bone and Cartilage" encompassed his own studies in this field and summed up results established earlier by others.

The report under the headline "Fluoride Accumulation in Antartic Krill and Consequences to Its Natural Predators" by D. Adelung (FRG) showed that krill, in contrast to humans, accumulate colossal amounts of fluorine. Therefore krill constitute a harmful natural source of nourishment for many predators including penguins. The author discussed the mechanism of absorption, deposition and excretion of fluorine which stimulated many participants to contribute additional facts from the floor.

For the poster-session 30 reports were arranged under the following subjects: six posters related to problems of biochemistry (interaction of fluorides with leukocytes, erythrocytes and enzymes); eight posters presented interaction of fluorides and hard tissues (bones, krill armour); six, mineralization processes in dental caries control; the remaining posters dealt with the level of fluorides in plants and algae, in milk and other foodstuffs.

The research orginated in nine different scientific centers. The poster-session was terminated by summing-up hitherto recorded achievements. The symposia in Szczecin, held regularly every three years, are known to be an important platform for presentation of scientific research in Poland. They are attended by representatives of various scientific disciplines, and lecturers are invited regularly from the neighboring countries. The unfavorable ecological situation existing in these countries renders it indispensible to perform relevant studies on the noxious effect of fluorine compounds which are transmitted primarily through the atmosphere and originate from both local and more remote sources that steadily pollute the environment.

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THE FLUORIDE CONTENT OF FINNISH HONEY

by

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(Abstracted from J. Agric. Sci. Finland, 59:379-385, 1987)

The fluoride content in 59 samples of honey from 47 localities in Finland, studied with an ion-selective electrode, ranged from 0.025 to 0.550 ppm. The mean for all localities was 0.086 ppm of the fresh weight of honey. Although fluoride concentrations in honey mainly correlated with amounts in bedrock and groundwater, fluoride introduced into the environment by man's activity may have affected some cases. The average fluoride content of honey was about 20% of that in the groundwater.

The fluoride content of honey is so low that it seems to have no significant effect on human nutrition. Even the highest concentrations in honey were lower than the level of fluoride in drinking water recommended by WHO for teeth. Average concentrations of fluoride in honey in Finland were the same as in milk and vegetables.

KEY WORDS: Finland; Fluoride; Honey.

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DENTAL CARIES, FLUOROSIS, AND FLUORIDE EXPOSURE IN MICHIGAN SCHOOLCHILDREN

by

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(Abstracted from J. Dent. Res. 67:802-806, 1988)

This study relates the prevalence of caries and fluorosis among Michigan children residing in four different areas, to the various concentrations of F in communal water supplies. Demographic information, details of F history, and dental attendance were supplied by parents in response to a questionnaire. Six to 12-year-olds were screened for caries by means of the NIDR criteria and for fluorosis by means of the TSIF index. Results pertain solely to continuous residents and permanent dentition. The prevalence of both caries and fluorosis was significantly associated with the F concentration in community water supply. Approximately 65% of all children were caries-free, ranging from 55.1% in Cadillac (less than 1 ppm) to 73.7% in Redford (1.0 ppm F).

About 36% of all children had dental fluorosis, ranging from 12.2 in Cadillac to 51.2 in Richmond (1.2 ppm). All fluorosis was very mild. The prevalence of caries was significantly assocated with age, dental attendance, and use of 1 ppm fluoridated water.

The odds of experiencing fluorosis increased at every F level above the baseline (Cadillac), with the use of topical F rinses, and with age. Results suggest that children in the four communities may be ingesting a similar level of F from sources such as dentifrices, dietary supplements, and professional applications, but the factor that differentiates them with respect to the prevalence of caries and fluorosis is F concentration in community water supplies.

KEY WORDS: Caries; Fluoride; Fluorosis, dental; Michigan.

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DENTAL FLUOROSIS IN THE PRIMARY AND THE PERMANENT DENTITION IN FLUORIDATED AREAS WITH CONSUMPTION OF EITHER POWDERED MILK OR NATURAL COW'S MILK

by

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(Abstracted from J. Dent. Res. 67:822-825, 1988)

The aim of the present study was to describe the patterns of dental fluorosis in primary and permanent dentition of children born and reared in two different fluoridated areas; in one powered milk was commonly suspended in tap water (Narssaq, Greenland: 1.1 ppm fluoride in water) and in one cow's milk was provided (Vordingborg, Denmark: 1.4-1.6 ppm fluoride in water). Dental fluorosis was recorded according to Fejerskov's classification.

In both locations, the prevalence of dental fluorosis increased the later in life the tooth type was formed. The prevalence of dental fluorosis in the teeth formed earliest was higher where powdered milk was suspended in fluoride-containing tap water than where pasteurized cow's milk was used. In the first permanent molars, the maxillary incisors and canines, the prevalence was similar in the two areas. In the latest formed teeth, premolars, the level of fluorosis was higher in Vordingborg. The pattern of dental fluorosis suggests that with frequent use of powdered milk the children were exposed to a higher fluoride intake earlier in life than were those consuming cow's milk during infancy and childhood.

KEY WORDS: Cow's milk; Fluorosis, dental; Powdered milk.

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EFFECTS OF pH ON EXPRESSION OF SODIUM FLUORIDE RESISTANCE IN STREPTOCOCCUS MUTANS

by

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(Abstracted from J. Dent. Res., 66: 1594-1596, 1987)

The aim of this study was to determine the effects of pH on levels of fluoride resistance in stable, spontaneously fluoride-resistant mutants of Streptococcus mutans GS-5.

Administration of fluoride results in the formation of fluoro-apatite, which resists acid dissolution. Fluoride can also promote remineralization of early caries lesions. The cariostatic effect of fluoride seems to be associated, to some degree, with reduction in growth and metabolism of the microbial flora of the oral cavity.

Inhibitory effects of fluoride in solution are more pronounced at low pH values. Growth of streptococci is limited when the incellular pH falls below an average threshold value of about 5.7.

Metabolism of cells, held in low pH buffer with fluoride, will be inhibited to a greater extent than if the same cells were exposed to fluoride in a buffer at neutral pH.

The generation times for first-step mutants ranged from 47 to 58 minutes (average 50 min.). Second-step mutant generation times ranged from 61 to 94 minutes (average 71.5 min.). Analysis of variance showed no significant difference between generation times of first-step mutants and that of the parent strain. However, the difference between generation times of second-step mutants and first-step mutants as well as between second-step mutants and the parent strain was statistically significant. No statistical correlation was observed between generation time and maximal level of resistance for either first-step or second-step mutants.

KEY WORDS: Fluoride resistance; Generation times; Growth inhibition; Streptococcus mutans.

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IS FLUORIDE A MUTAGEN?

by

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(Abstracted from Sci. Total Environ., 68:79-96, 1988)

Recent studies suggest that fluoride may be genotoxic. While the concentration of fluoride in artificially fluoridated water (1 mg F/L) is generally considered "safe" in this respect levels of fluoride present in a number of widely used dental health products, such as fluoride-containing toothpaste, appear to be potentially mutagenic. Since fluoride is being used increasingly in medication and contamination of the total environment by fluoride emissions and solid wastes from industry is a growing problem, reassessment of the evidence regarding the potential mutagenicity of fluoride may be required.

KEY WORDS: Environmental fluoride; Genotoxic effects; Mutagenic effects.

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FLUORIDATION: FORTY YEARS ON

by

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(Abstracted from **Endeavour**, New Series 11:16-20, 1987)

Since fluoridation was first introduced over 40 years ago, a number of new factors have emerged namely: 1.) Large quantities of fluoride emissions and solid wastes increasingly contaminate the total environment which may enter the food and beverage chain, particularly in developed countries, 2.) Fluoride-containing dental health products are widely used both in dental surgery and in the home: fluoride compounds are now incorporated in toothpastes, mouthrinses, and a variety of dental filling materials; gels, varnishes and paints are used in the surgery and sometimes in the home, to apply fluoride to teeth; fluoride tablets, drops, and F-containing vitamin supplements are regularly prescribed; and even fluoride-impregnated tooth-picks and dental floss have been manufactured. Some individuals, particularly children, may ingest significant amounts of fluoride from certain preparations, such as fluoride toothpastes, gels, and mouthrinses. 3) Tooth decay rates have declined in developed countires whether or not fluoridation is practiced.

Since overall fluoride intake has been increased, and the average ionic plasma fluoride level of the population has been raised, individuals who ingest submilligram amounts of fluoride (for example from fluoridated toothpaste)

run a greater risk of having their ionic plasma fluoride concentration peak above the threshold level, a situation which may precipitate dental fluorosis or other ill effects.

In view of the fact that the incidence of dental fluorosis appears to be increasing in fluoridated areas, "it is naive to argue, as some do, that the subject of fluoridation is closed forever, and therefore is not debatable."

KEY WORDS: Dental fluorosis; Fluoridation review; Fluoride dentifrices; Fluoride intake.

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COMPARISON OF THE CROWN SIZE IN TEETH IN CHILDREN FROM A HIGH AND AN OPTIMUM FLUORIDE AREA IN SOUTH AFRICA

by

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(Abstracted from **Community Dent. Oral Epidemiol.**, 15:329-331, 1987)

The morphology of teeth in children, in Garies (1.06 ppm) and Klipfontein (2.5 ppm), South Africa, was studied to test the hypothesis that increasing the level of fluoride in drinking water from 1 ppm to higher levels would result in a reduction in crown size. When the mesio-distal dimension of the maxillary and mandibular central incisors were measured no significant differences were found between contralateral teeth. The difference between the pooled means for the maxillary second premolars and for the madibular second premolars were significant ($p < 0.05$). For the second molar bucco-lingual dimension, however, the difference between means at 1 ppm and at 2.5 ppm fluoride was significant for the maxillary ($p < 0.05$) but not for the madibular.

According to the present study a higher level of fluoride tends to produce a correspondingly greater reduction in tooth dimensions compared to the 1 ppm level. Maxillary central incisors and second premolars showed significant reductions in mesio-distal dimenstions in the high-fluoride sample. However, the maxillary second molar bucco-lingual dimension was significantly smaller in the high-fluoride sample.

Despite having 1.06 ppm fluoride in the drinking water, in a high proportion of 14-15-yr-olds in Garies first permanent molars were extensively carious or missing. High sugar intake was a possible factor.

KEY WORDS: Fluorides; Odontometry; Tooth crown size.

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DIETARY FLUORIDE INTAKE OF 6-MONTH AND 2-YEAR-OLD CHILDREN IN FOUR DIETARY REGIONS OF THE UNITED STATES

by

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(Abstracted from the Amer. J. Clin. Nutrition, 42:701-701, 1985)

In view of the fact that dental fluorosis, which is one of the earliest and most sensitive overt indications of fluoride intoxication, has increased in both fluoridated and nonfluoridated communities, dietary fluoride intake of infants and young children was assessed from birth to age 16 at critical times during periods when crowns of permanent teeth are undergoing calcification.

Based on 44 basket food collections, average daily dietary fluoride intakes were determined for 6-month and 2-year-olds residing where drinking water contained 0.05 to 1.04 ppm fluoride. In cities with < 0.7 ppm fluoride in drinking water, dietary fluoride intake for a 6-month infant and a 2-year-old toddler was 0.418 mg/day (0.052 mg/kg body weight) and 0.621 mg/day (0.050 mg/kg body weight), respectively. Dietary fluoride intake, which ranged from 0.54 to 0.65, was highest in fluoridated cities. Daily fluoride intake from foods, namely total dietary fluoride intake less fluoride intake from water and beverages [average 0.17 ±0.01 (SE) mg/day], did not correlate with fluoride in drinking water.

A daily fluoride intake of 0.1 mg/kg body weight during the period of crown calcification is sufficient to cause mild dental fluorosis in several species of animals, including humans. The percentage of dietary fluoride intake contributed by drinking water ranged from 7.6 to 13.6% for infants and toddlers residing in cities with < 0.3 ppm fluoride in drinking water. It increased to between 50.6 and 53.8% in cities where drinking water contained > 0.7 ppm fluoride. An additional 17.8% of dietary fluoride intake by 2-year-old children in the fluoridated cities (> 0.7 ppm) was provided by beverages other than water. These data illustrate the important contribution made by drinking water and beverages to total fluoride intake of infants and young children.

In cities with > 0.7 ppm fluoride in the drinking water, toddler intake was significantly higher (0.621 mg/day) than that by infants (0.418 mg/day), due mainly to a greater consumption of water and beverages by older children. On a body weight basis, mean dietary fluoride intake for both groups of children was approximately 0.05 mg/kg. Since daily fluoride intake from foods was similar for infants and toddlers, the difference in daily dietary fluoride (mg/day) became less as drinking water fluoride decreased. Since 1979, however, prepared ready-to-eat formulas contained < 0.3 ppm fluoride.

Diluted with 1 ppm fluoridated water, they contain 0.5-1.1 ppm, the maximum fluoride intake for infants.

In metabolic balance studies with 5 infants (8-17 weeks of age), the fluoride in formula diluted with fluoridated water had a bioavailability of 95-100%. Consequently, during the first few months of life, significant numbers of infants are ingesting biologically available fluoride at a level greater than

that associated with the mildest form of dental fluorosis (0.10 mg/kg). In addition, nondietary sources of ingested fluoride such as a fluoride-containing dentifrice, contributed to the total fluoride intake of young children. Barnhart and co-workers found that children 2-4 years of age used an average of 0.86 g of dentifrice at each brushing; due to a limited ability to control their swallowing reflex, they ingested an average of 0.30 g/brushing. Thus, a 2-year-old child brushing his teeth twice daily would ingest nearly as much fluoride from dentifrices as from food and fluoridated drinking water combined. This nondietary source of fluoride may exert a major effect on the incidence of dental fluorosis.

Clearly, additional work is needed to establish the relationship, if any, between various sources of ingested fluoride and the incidence of fluorosis.

KEY WORDS: Children, F intake; Fluoride intake, dietary, nondietary; Infant F intake.

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FLUORIDE INTAKE FROM BEVERAGE CONSUMPTION

by

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(Abstracted from Community Dent. Oral Epidemiol., 16:11-15, 1988)

Children in grade six were invited to participate in recording of beverage intake in two cities in the province of Alberta, Canada: Wetaskiwin (with water supplies fluoridated at 1.08 ppm F, and Camrose (non-fluoridated) where water supplies contain 0.23 ppm F. Three-day beverage intake records — "Drink Diaries" — were collected from 179 children in Wetaskiwin and 230 children in Camrose. Fluoride in beverages over than the water in non-fluoridated Camrose ranged from 0.03 ppm in milk to 0.80 in juice and in carbonated beverages commercially prepared from fluoridated water. Fluoride in fluoridated Wetaskiwin ranged from 0.03 ppm in milk to 2.18 ppm in tea.

Available beverages and actual consumption should be considered when fluoride supplementation is recommended for children with minimal fluoride in their drinking water.

KEY WORDS: Beverages; Cariostatic agents; Dental caries; Drinking; Fluorides.

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UNSUITABILITY OF WORLD HEALTH ORGANISATION GUIDELINES FOR FLUORIDE CONCENTRATIONS IN DRINKING WATER IN SENEGAL

by

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(Abstracted from **The Lancet**, 1:223-225, 1988)

A survey of dental fluorosis in children aged 7-16 yrs and skeletal fluorosis among adults 40-60 yrs old in Senegal where fluoride in water ranged from 0.1 to 7.4 mg/L revealed that dental fluorosis in its milder form was 68.5% at 1 ppm. Where fluoride concentrations exceeded 4 mg/L the prevalence of dental fluorosis reached 100%.

Kyphosis was very prevalent in a community where drinking water contained 7.4 mg/L fluoride. Radiographs of the vertebral column, hand, and wrist of three adults with kyphosis confirmed the diagnosis of skeletal fluorosis. About 30% and 60% of the children in Guinguineo and Darou Rahmane Fall, respectively, had severely discolored brownish-black teeth.

The mean (SD) fluoride concentrations in the morning urine of the children were 0.7 (0.6) mg/L in Nioro du Rip, 12.0 (6.2) in Guinguineo ($p < 0.025$). In three adults from Darou Rahmane Fall with Kyphosis X-rays of the vertebral column, hand and wrist confirmed the diagnosis of skeletal fluorosis. Urinary fluoride concentrations in children were about 2.5 times higher than the fluoride concentration in drinking water. X-rays taken in Senegal confirmed that crippling skeletal fluorosis occurs at 7.4 mg/L fluoride in drinking water. The disfiguring brownish-black discolorations in the enamel of the teeth are an especially serious esthethic problem in Senegal because the locals aggravate the fluorotic damage to the teeth by trying to file or scrape off the stains.

These findings strongly indicate that the WHO guidelines on the fluoride concentration in drinking water should not be applied to countries where the climate is hot and dry as in Senegal. Studies should be carried out to determine whether around 0.6 mg/L fluoride as an upper limit is more suitable.

Where fluorosis is due to excessive fluoride from drinking water a shift to alternative water sources where levels of fluoride are lower or defluoridation of water is recommended.

KEY WORDS: Dental fluorosis; Drinking water standards; Kyphosis; Senegal; WHO guidelines.

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RHEUMATOID ARTHRITIS IN ASSOCIATION WITH SKELETAL FLUOROSIS

by

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(Abstracted from J. of Assoc. of Physicians of India, 34:296-299, 1989)

A case of skeletal fluorosis combined with rheumatoid arthritis is described: R.S., a 50 year old policeman from the state of Haryana, was admitted to the hospital for the first time in February 1985 for evaluation of joint symptoms. Ten years earlier pain and swelling in his right wrist had begun with morning stiffness for 2-3 hours. For 9 years the pain was confined to the right wrist — occasional spontaneous remission lasted for a few months.

One year prior to admission, similar symptoms had started in his left wrist along with stiffness. There was a history of occasional heel pain and pain in both ankles. Both wrists were swollen and tender with marked synovial hypertrophy; mild swelling and tenderness was noted in the right ankle, as well as limitation in extension and flexion of the spine.

Rheumatoid factor (latex) was positive in a titer of 1:80, x-ray of both wrists showed advanced changes of rheumatoid arthritis. A skeletal survey showed dense bones with calcification of the interosseous membrane of the forearm diagnostic of skeletal fluorosis. Urinary fluoride content was 3.40 ppm (normal 0.10 ppm)* with blood serum level of 0.085 ppm (normal 0.02). Synovial biopsy from the left wrist showed focal areas of intense inflammation with plasma cells and lymphocytes which was compatible with R.A. A diagnosis of advanced skeletal fluorosis, a radiological surprise, was confirmed by elevated fluoride levels in serum and urine. Rheumatoid arthritis (RA) was diagnosed on the basis of history of morning stiffness, high ESR, presence of rheumatoid factor in significant titres, the radiological appearance of the carpal bones and the synovial biopsy.

The patient had three features which would be considered unusual for RA, namely total sparing of small joints of hands, the asymmetry of arthritis and oligoarticular involvement. The author asks, is it possible that fluorosis had in some way modified the course of RA in this patient?

It appears that fluoride displaces the hydroxyl ion on the hydroxyapatite from the bones and forms fluorapatite which results in formation of crystals that are more resistant to the resorptive process than normal bone.

KEY WORDS: Clinical manifestations; India; Rheumatoid arthritis; Skeletal fluorosis.

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EDITOR: F in urine is ca 0.4 - 0.6 ppm in people drinking low F in water.

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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.

6. **Results** – should contain the *direct conclusions* of the experimental work.

7. **Discussion** – should deal with the *general conclusions*. Reference should be made to other work on the subject with an indication whether the experimental results agree or disagree with previous work. In short papers, results and discussion can be combined.

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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