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Owing to unexpected circumstances we sincerely regret that this issue of Fluoride is not being published in time to provide further information in advance of the conference. We apologize for any inconvenience that this delay in publication may have caused anyone.

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CONTINUING CONTROVERSY OVER DIETARY FLUORIDE TOLERANCE FOR DAIRY CATTLE

In an earlier Editorial (1), attention was called to disturbing new data assembled by Cornell University veterinary scientists from field investigations of cattle fluorosis caused by industrial fluoride emissions in an upstate New York-Canada area (2,3). Their findings strongly suggested that the present National Academy of Sciences-National Research Council (NAS-NRC) recommendation (4) of a 40-ppm tolerance standard for fluoride in the dry ration does not protect dairy cattle against the destructive biological and economic impacts of severe chronic fluoride poisoning.

Chemical analyses of representative forage and bone samples indicated that the mean dietary fluoride levels of these herds, at least at the time of the studies, were well within the "threshold" values proposed by the NAS-NRC for chronic fluoride toxicosis in dairy cattle. Yet many of these animals, both young and old, had become afflicted with debilitating skeletal as well as dental fluorosis. Moreover, subsequent generations exhibited stunted growth, increased numbers of stillbirths, premature mortality, and a significant decrease in milk production. In further studies (5,6), the Cornell investigators confirmed and amplified these findings. Recently, they have reported similar results (7,8) from fluoride-contaminated commercial feed and mineral supplements introduced into a previously highly productive dairy farm in western Pennsylvania (see abstracts, this issue, pp 142-144).

That fluoride-contaminated dairy feed can indeed cause "serious, but very local, livestock problems in many areas" and that "such responses should not be tolerated" (9) is readily acknowledged by a leading University of Wisconsin fluoride researcher, who was one of the five authors of the NAS-NRC subcommittee report (4) that recommends the 40-ppm dietary fluoride tolerance for dairy cattle. On the other hand, in commenting on the two earlier Cornell investigations (2,3), as well as a related Michigan State University study (10), this same Wisconsin scientist has argued that these "reports . . . cannot be considered to have sufficient validity to consider altering well-thought-out standards." In these, as in most other, field studies, he contends, "it is impossible to separate effects of fluoride from those of other uncontrolled variables, or to establish the dietary intake of fluoride by the involved animals" (9). For these reasons, and also because various experimental studies with 40-ppm fluoride in the dry ration of dairy cattle were interpreted as not having produced severe adverse effects, he therefore attributes such effects in these field investigations to "other variables of [poor] management and nutrition" (9).

Such a view, however, is difficult to reconcile with the available facts. First of all, it was only after the intrusion of fluoride into the diet of these cattle that the various toxic effects began to appear. Nothing else had changed. Up to that time the herds had all been in good health, were reproducing and growing normally, and had acceptable to outstanding levels of milk production. Examination and testing revealed no serious infectious diseases, but the typical dental and skeletal features of fluorosis gradually became evident and were subsequently accompanied by other deleterious effects such as those already mentioned.

Secondly, although not established for every animal, mean fluoride intake levels were shown by bone fluoride analyses at various ages to correspond to

the NAS-NRC estimates for fluoride ingestion at dietary concentrations in the 10-30 ppm range. This is seen by the fact that monthly fluoride increments in the bone ash of the afflicted young cattle averaged 45 ppm on the New York farm and 33 ppm on the Pennsylvania farm. Thus, although other factors may also have contributed, a substantial fluoride component was unequivocally demonstrated. Typical fluoride toxicity effects were clearly evident, even though the levels of fluoride intake were obviously not excessive according to standards recommended by the NAS-NRC.

Of special concern in this connection is the validitiy of the NAS-NRC position that milk production is not significantly affected unless fluoride ingestion is very high, and that any subsequent decrease in milk output is primarily the result of lower food intake rather than direct impairment of lactogenesis by fluoride. Citing evidence that, normally, "milk calcium is derived in about equal proportions from dietary and osseous sources," the Cornell group contends: "Fluoride is toxic to the bone resorbing cells . . . and less calcium is released. With that source reduced in proportion to the severity of fluoride intoxication, milk production decreases; the cow does not produce calcium-dilute milk" (7). In their view, previously superior milk yields, especially on the Pennsylvania farm, strongly depended on "a high turnover rate in bone tissue" and, because "metabolically active bone tissue is very sensitive to fluoride" (7), even a modest degree of osteofluorosis can therefore exert a direct adverse effect on lactogenesis, as observed in their investigations.

Finally, although discounted or denied by the NAS-NRC author (9) because of the apparent absence of such findings from various controlled and even other field experiments, including his own (11), the presence of what are unquestionably dental fluorosis and fluorotic bone lesions in newborn calves in both the Cornell and Michign State studies (2,3,8,12) must be seen as a clear indication of in utero intoxication by fluoride, however much intensified or moderated it might be by other factors. Owing to their high metabolic rate, however, fetal tissues are especially sensitive to toxic agents. Consequently, in the view of the Cornell investigators, even "the seemingly low levels of fluoride in [fetal] teeth and bones are high enough to cause damage" (8).

Obviously, additional studies will be necessary to settle these disagreements. But because of the very weighty and important scientific and economic issues that are at stake, it is vital that this controversy over appropriate dietary fluoride tolerance standards for dairy cattle be fully and fairly resolved as soon as possible.

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A.W.B.

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ROLE OF COPPER IN SKELETAL CHANGES IN FLUOROSIS: AN EXPERIMENTAL STUDY IN RABBITS

by

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SUMMARY: The influence of traces of copper on the normal skeleton is well known. In certain endemic fluorosis areas in India, Krishnamachari and Krishnaswamy (1) observed osteoporotic changes which were considered due to copper deficiency. The present study provides the experimental basis for this theory.

KEY WORDS: Experimental rabbits; Fluorosis, Role of Copper; Skeletal changes.

Introduction

Endemic fluorosis caused by ingestion of fluoride (2,3) has been reported from various parts of the world. Bone changes are characterized by osteosclerosis, calcification of interosseous membrane and tendonous insertions. In India, endemic fluorosis has been reported from many parts of the country by various workers (4-6); both osteosclerotic as well as osteoporotic changes have been seen.

In 1974, Krishnamachari and Krishnaswamy, (1) who reported genu valgum in fluorosis, observed likewise that osteoporosis in extremities was seen where the copper level in water was less than 0.01 ppm. According to Doesthale and Gopalan (7) high dietary molybdenum may cause increased urinary excretion of copper, and Gallagher (8) showed that copper deficiency leads to osteoporosis. In spite of circumstantial evidence that fluorosis is manifested as osteoporosis due to copper deficiency, actual proof is not available in the literature. In view of this, the present study was undertaken.

Material and Methods

Eighty rabbits aged 4-6 months, each weighing about one kilogram, were divided into eight groups (Table 1). Seventy-five out of the eighty were males. All received a similar diet and tap water which contained 0.6 ppm of fluoride.

The first group, controls, received distilled water. Half of the animals in each group were sacrificed after four months (Batch A); to the other half injections were continued for four additional months after which the rabbits were sacrificed (Batch B). Each animal was weighed every fortnight. Dosage was adjusted accordingly.

Radiographs of all long bones of both A and B batches were taken at the

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Table 1

Solution Injected and Doses

Group	Solution Injected	Doses
I	Distilled water	1 cc/kg body weight
II	Sodium fluoride	2 mg/kg body weight
III	Copper sulphate	0.25 mg/kg body weight
IV	Copper sulphate	0.5 mg/kg body weight
V	Copper sulphate	1.0 mg/kg body weight
VI	Fluoride + copper sulphate	2.0 mg/kg body weight + 0.25 mg/kg body weight
VII	Fluoride + copper sulphate	2.0 mg/kg body weight + 0.5 mg/kg body weight
VIII	Fluoride + copper sulphate	2.0 mg/kg body weight 1.0 mg/kg body weight

end of the study. Ground and paraffin sections of the right femur, which was preserved in 10 per cent formalin, were prepared and studied histologically.

Radiological Changes

Batch A, Group II, showed evidence of osteoporosis compared to controls (Figures 1 and 2). Group VII manifested much less osteoporosis (Figure 3). In

Table 2

Mean Weight per Unit Length (mgm/mm) of Long Bones of Rabbits in Each Group

Bone	Groups							
	I	II	III	IV	V	VI	VII	VIII
	Batch A (After four months)							
Femur	42.0	31.9	29.2	32.8	37.8	26.9	34.5	33.9
Tibia/fibula	36.6	25.1	27.9	26.2	30.6	16.5	29.5	28.1
Humerus	28.6	20.5	19.3	21.1	25.3	17.4	27.6	27.1
Ulna	11.4	5.3	5.1	9.7	10.3	9.4	10.6	11.1
Radius	10.3	5.4	3.6	8.4	9.3	9.6	9.7	7.6
	Batch B (After eight months)							
Femur	34.4	33.4	33.5	42.5	42.2	40.9	39.4	39.8
Tibia/fibula	30.6	28.3	30.6	30.2	30.8	29.4	32.5	32.8
Humerus	23.8	21.4	22.2	22.2	30.3	21.4	24.5	30.6
Ulna	9.8	6.1	6.6	11.6	10.4	11.7	10.9	10.9
Radius	8.4	5.6	4.9	9.7	9.3	18.2	8.5	9.6

Batch B, Group VII osteoporosis was absent (Figure 4). Comparison of Groups VI, VII, VIII revealed evidence of increasing osteosclerosis; Group VII was, however, nearest to Group I (control).

Figure 1 Figure 2 Figure 3 Figure 4

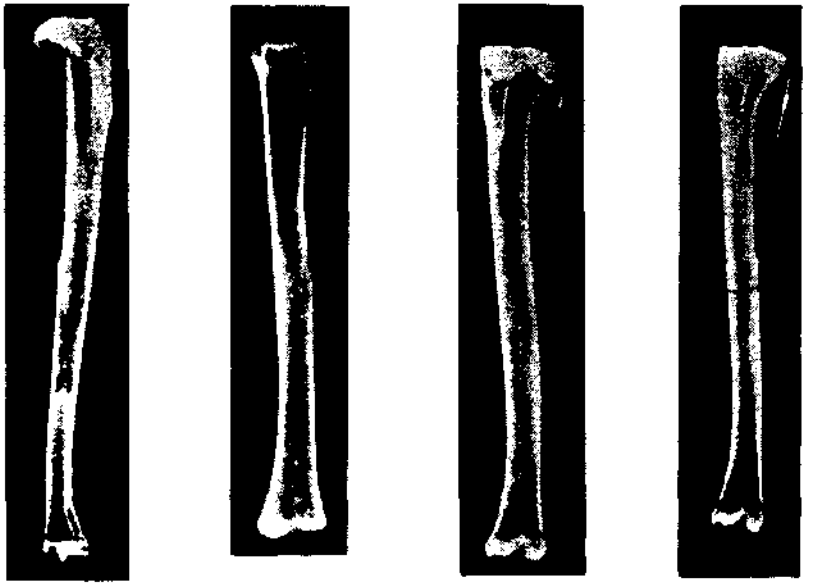


Figure 1: Control Tibiofibular skiagram. Figure 2: Tibiofibular skiagram. Fluoride alone after four months of exposure. Figure 3: Tibiofibular skiagram; copper in dosage of 0.5 mg/kg body weight in combination with fluoride after four months. Figure 4: Tibiofibular skiagram; copper in dosage of 0.5 mg/kg body weight in combination with fluoride after eight months.

Batch A: Weight in groups II-V was less than in controls (Group I) whereas in Group VII, the decrease in weight of the long bones was still less. On comparing Group VI, VII and VIII, Group VII showed the least decrease in weight.

Batch B: There was evidence of osteosclerosis in Group II to V compared with controls. When Groups VI, VII, and VIII were compared with controls, osteosclerosis was less in the majority of bones in Group VII.

Statistical Analysis

F-test, applied between all groups, was significant in all long bones in both A and B Groups.

Histopathological Study

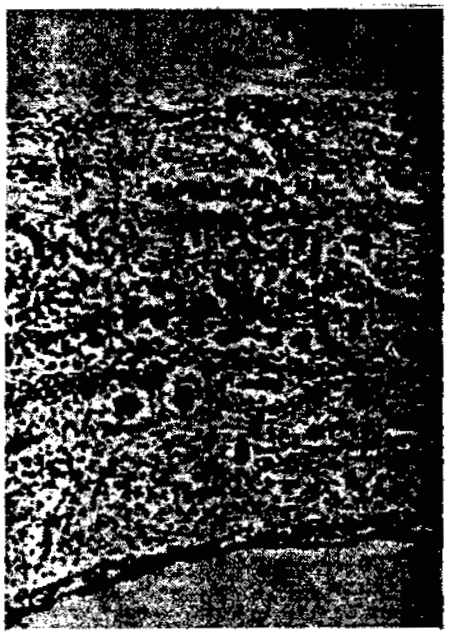
In Batch A, osteoporosis was observed in Groups II to V; it was much less in Groups VI to VIII; it disappeared in Batch B (Figures 5, 6 and 7).

Figure 5

Histopathology: Control

Figure 6

Histopathology: Fluoride (Group II after eight months)



Discussion

From weight per unit length of long bones, it was concluded that fluoride induces osteoporosis in the early stages; later, however, it induces osteosclerosis, as reported by Makhni et al (9) and Mittal et al (10). In Groups III, IV and V copper, by itself, produced osteoporosis in lower doses; as the dose increased, as well as the period of exposure, weight per unit length increased progressively in all bones. When copper was combined with fluoride, increase in dose prevented osteoporosis; with continued use of this combination more osteosclerosis was produced than by fluoride alone. The effect of copper in 0.5 mg dosage in combination with fluoride was statistically significant in all bones of Batch A and B (Table 3).

Figure 7

Histopathology: Copper 0.5 mg/kg body weight with fluoride after eight months.

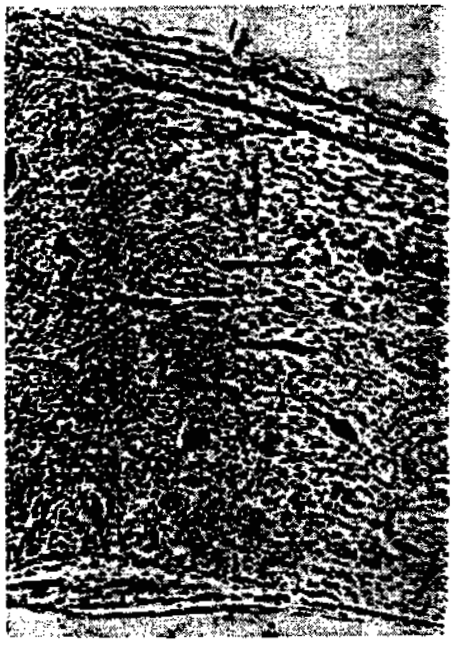


Table 3

Bone	Batch A	Batch B
Femur	+	++
Tibia and Fibula	++	++
Humerus	+	+
Ulna	++	++
Radius	++	++

++ Significant at one percent level
+ Significant at five percent level

This study documents the hypothetical observation of Krishnamachari and Krishnaswamy (1) that copper deficiency is responsible for osteoporosis and the genu valgum snydrome in Andhra Pradesh (India). Jolly et al (11) suggested that sufficient copper in the diet of people living in the fluorotic belt explains why osteoporotic changes are not seen in the State of Punjab (India). They also found more copper in food articles of fluorotic villages than in non-fluorotic areas.

Conclusion

Copper prevents the osteoporotic type of fluorotic manifestation. However, fluorotic changes (osteoscleotic type) are enhanced with prolonged exposure to and by the synergistic effect of copper, as indicated by this study.

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BINDING OF FLUORIDE WITH TAMARIND GEL

by

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SUMMARY: The binding of fluoride with tamarind gel has been studied by adopting equilibrium dialysis technique. Application of Klotz method to this heterogeneous system with r values, i.e., moles of bound fluoride per 10^5g of tamarind gel, ranging from 15-22, yielded the first binding constant (nK) 1 x 10^4. Protein constituent of tamarind has been concluded to be the active component interacting with fluoride.

KEY WORDS: Fluoride binding; Tamarind gel.

Introduction

The inclusion of tamarind (Tamarindus indica) in diet has been shown to reduce the severity of fluorosis and the ability of tamarind pulp to entrap fluoride has also been demonstrated by Sriramachari (1). Tamarind pulp swells in water to form a gel and this tamarind gel, a biocolloid, has large fluoride entrapment capacity. The fluoride content could be brought down from 10 ppm to 2 ppm by tamarind gel, i.e., 80% fluoride entrapment (1) and even to 0.05 ppm in the presence of added sodium chloride (99.5% fluoride entrapment). It has also been shown that none of the individual constituents of tamarind has the fluoride entrapment capacity. In the pH range, 2.8 to 8.0, no change in the ability of tamarind gel to bind fluoride occurred. Substitution of tomato in place of tamarind in the diet is not advantageous which is clear from the analysis of severity of fluorosis as a function of human food habits (1).

The present study is aimed at giving a more quantitative picture for interaction between tamarind gel and F^-. A quantitative determination of the binding constant and number of binding sites is attempted making use of the Klotz method (2). The application of the Klotz method to this heterogeneous system appeared reasonable since the Klotz equation itself is derived on the basis of Langmuir adsorption isotherm. Computing r values (3) for 10^5g of the tamarind pulp,

$$r = \frac{\text{moles of bound fluoride}}{10^5\text{g of the biocolloid}} \quad \frac{[F^-]_{\text{bound}}}{10^5 \text{ of tamarind pulp}} \qquad \ldots 1$$

and substituting into the Klotz equation,

$$\frac{1}{r} = \frac{1}{n} + \frac{1}{nkc} \qquad \ldots 2$$

where c is the free equilibrium concentration of fluoride ion, $[F^-]_{free}$; the intrinsic binding constant, k; and the number of binding sites, n; can be evaluated for the system.

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Materials and Methods

In the present investigation 0.2 to 0.8 g of a commercially available whole ripened tamarind pulp, after purification from pericarp and seeds, was used. An aqueous stock solution (100 ppm) of sodium fluoride (BDH, AR), diluted to 10 ppm was used in the equilibrium dialysis experiment. Dialysis bags were obtained from Wilson Laboratories, USA.

An aqueous suspension of tamarind pulp (0.4 g in 20 mL of water) is acidic with a pH of 2.9. Addition of sodium fluoride (10 ppm) alters the pH only slightly (pH = 2.74). No buffer was employed for the adjustment of pH because the buffer anions would compete with F^- for binding to tamarind gel. Binding of F^- to the cellophane dialysis bag is negligible.

The details of the equilibrium dialysis method are the same as described by Alexander and Block (4). A typical experiment is briefly illustrated below. A known quantity (0.4 g) of tamarind pulp was taken inside the dialysis bag, 8 mL of 526.3 μm (or 10 ppm) fluoride solution was added and dialysed against the outer solution of 20 mL of the same initial fluoride concentation, $[F^-]_o$. After dialysis for 24 hours at constant temperature (32°C), the final concentration of fluoride, $[F^-]_{final}$, of the outer solution was determined using fluoride ion-selective electrode (JAS Chemical Corporation, India) after adjusting the pH to 5.35 with the buffer solution called total ionic strength adjustment buffer.

The concentration of bound fluoride, $[F^-]_{bound}$, was evaluated making use of the procedure illustrated earlier (5). r values corresponding to 10^5g of the biocolloid were used to evaluate the binding parameters by the Klotz method.

Results and Discussion

Generally, antidotes for fluoride intoxication (6) are based on calcium, aluminium, magnesium or boron. Harmless normal dietary ingedients like tamarind pulp appear more promising as antidotes especially for endemic fluorosis whereas the chemical compounds can be recommended for acute fluorosis. Our study on the interaction of fluoride with tamarind gel is a step in the direction of research by Sriramachari (1). Such physicochemical study is a relevant one that should be coupled with concurrent clinical field trials for the development of successful antidotes.

Binding data on the interaction of fluoride with tamarind are given in Table 1.

Table 1

Binding of F^- with Tamarind Gel

$[F^-]_o$ = 52.63 x 10^{-5}M Temperature = 32°C

Weight of tamarind pulp (g)	$[F^-]_{bound} \times 10^5$ mol/dm^3	C x 10^5 mol/dm^3	r	Binding parameters
0.2100	4.631	47.999	22.05	nk = 1 x 10^6
0.3984	7.000	45.630	17.57	
0.6060	9.210	43.420	15.20	
0.7920	13.789	38.841	17.41	

The concentration of bound fluoride increases with increase in the weight of tamarind pulp (Table 1); r values range from 15 to 22 under the experimental conditions. The data do not permit the evaluation of the individual binding parameters n and k. Binding is indicated with a value of 1×10^4 for the product, nk, the first binding constant. The deviation in the value of r at higher dosage of tamarind (0.8g) may be due to cooperative interaction in binding. The first binding constant (nk) for tamarind gel–F^- system lies in between those observed for us for poly(N-vinyl-2-pyrrolidone)–fluoride system (5) and bovine serum albumin-fluoride system (7) as shown below:

System	nk	Reference
Poly(N-vinyl-2-pyrrolidone)-F^-	3.16×10^3	5
Bovine serum albumin-F^-	1.5×10^6	7
Tamarind gel-F^-	1×10^4	Present work

Among differences between experimental and in vivo conditions of tamarind-F^- binding is the acidic pH (≈2.7) of the dialysis experiment in contrast to the pH of the intestine (pH ≈7), the site of protein absorption. One would, therefore, expect a decrease in the amount of F^- found at the intestinal pH. However, in the range 2.8 to 8.0 binding was negligible but the presence of Cl^- and HCO_3^- anions in the intestine affect fluoride binding to a certain extent.

Conclusion

Regarding the possible constituents of tamarind which interact with fluoride: protein content of tamarind pulp is very low, i.e., 1.4 to 3.3%, others are tartaric acid – 8.4 to 12.4%, total sugars – 21.4 to 30.8%, cellulose – 1.8 to 3.2%, fats and oils – 0.71 to 0.81%, pentoses – 4.2 to 4.8%, ash – 1.16 to 1.72%, and moisure – 62.5 to 69.2%. Low protein content in diets leads to enhanced deposition of fluoride in bones (9); hence, in skeletal fluorosis the interaction of protein in the diet with fluoride reduces the amount of free fluoride. It is reasonable to consider the protein-bound fluoride as non-absorbable in analogy of phytates on the absorption of calcium ion and tamarind gel on the absorption of fluoride ion (1). Since it has also been suggested that tamarind-bound fluoride is non-absorbable (1), it is probable that protein in tamarind is the component interacting with fluoride. Protein analysis of the pulp of the tamarind fruit (10) shows proline to be the major amino acid, pipecolinic acid (piperidine-2-carboxylic acid) and serine coming next in the order of concentration. Proline and pipecolinic acid can bind fluoride via hydrogen bonding interactions of the type,

$$= N - H \ldots F^-$$

and serine also by hydrogen bonding interactions of the type,

$$- CH_2 - O - H \ldots F^-$$

Even though protein is present in tamarind pulp in relatively small amounts, it is assumed to be the component responsible for the fluoride binding property of tamarind. The non-absorbable nature of tamarind-bound fluoride

explains the action of tamarind as an antidote for fluorosis. A similar kind of non-absorbable fluoride bound to the natural constituents of tea has also been suggested (1).

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INHIBITION OF LIPID METABOLISM IN GERMINATING MUNG BEAN SEEDS BY FLUORIDE

by

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SUMMARY: The effects of NaF on the fatty acid composition and lipase activity were studied in germinating mung bean (Vigna radiata) seeds. Palmitic, linoleic, and linolenic acids were found to be among the major fatty acids in cotyledon extracts. Fluoride caused a marked reduction in palmitic and linoleic acids. In the presence of 1.0 mM NaF, the activity of lipase prepared from the cotyledon was inhibited by about 50%.

KEY WORDS: Fatty acids; Germination; Lipase; Mung beans; Vigna radiata.

Introduction

Following radicle emergence, a rapid breakdown of stored reserves takes place in the germinating seed so that the breakdown products are supplied to the growing axis. Triacylglycerols are major storage lipids in seeds. The initial degradation of triacylglycerol involves lipases which catalyze the stepwise hydrolysis of triacylglycerol to glycerol and fatty acids. The fatty acids released may then be utilized for the synthesis of sugars.

Although fluoride has been known to cause adverse effects on seed germination (1-4), studies on the influence of fluoride on plant fatty acids and lipid metabolism are limited (5,6). Harwood and Stumpf (2) investigated fatty acid synthesis by germinating pea and safflower seeds by following acetate incorporation in vivo. They reported that fluoride reduced elongation of stearic acid in peas whereas in safflower, fluoride reduced linoleate formation without affecting the very long chain fatty acids. Simola and Kiskimies-Soininen (3) studied the effect of KF on the fatty acid composition of lipid fraction from Sphagnum finbriatum gametophytes and showed that fluoride caused an increase in palmitic acid and a decrease in linoleic acid in all lipid fractions, whereas linolenic acid decreased in glyco- and neutral lipids. The authors suggested that fluoride inhibited desaturation and elongation of the fatty acid chain.

In this study we investigated the effects of fluoride on the fatty acid composition and lipase activity in germinating mung bean (Vigna radiata) seeds.

Materials and Methods

Germination of seeds. One-day-old mung bean seedlings were packed in four petri dishes lined with filter paper. The seedlings in each of the dishes were treated with 0 (control), 0.1, 1.0, and 5.0 mM NaF, respectively, and maintained in an incubator at 25°C for 72 h. Additional treatment solutions were added at 24 and 48 h. At the end of the experiment the seedlings were harvested and the cotyledons were used as tissue samples.

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Extract of lipids and analysis of fatty acids. The cotyledons were died in an oven at 75°C under reduced pressure. Crude fat was extracted in a Soxhlet extraction apparatus. The fat extract was then taken into n-hexane. An aliquot of the hexane extract was used for methylation using boron trifluoride-methanol. The resulting methyl esters of the fatty acids were analyzed by gas chromatography. A Perkin-Elmer gas chromatograph, Sigma 300, equipped with an FID detector was employed. A stainless steel column (0.3 cm x 200 cm) packed with 10% SP 2330 on chromosorb-WAW (Supelco, New Jersey, USA) was used, and nitrogen was the carrier gas. The analysis was carried out isothermally at 200°C. Authentic samples of methyl esters of different fatty acids were prepared from the corresponding acids (obtained from Sigma Chemical Co., N.J., USA). Identification of the methyl esters was done by retention times and co-chromatography. Quantitative analysis was carried out by comparison of relative areas obtained by use of an integrator (Spectra Physics, model SP 4290, San Jose, CA, USA).

Preparation and assay of lipase. Enzyme extracts for lipase study were prepared from seedlings germinated for 24, 48 and 72 h, respectively. The cotyledons were separated and ground in cold water in a mortar and pestle and the homogenate was passed through six layers of cheesecloth. The extract was then centrifuged at 8000 x g for 8 min. The supernatant was re-centrifuged at 22,000 x g for 25 min and the resulting supernatant was fractionated by ammonium sulfate. The fraction precipitated at 45-60% saturation was collected. Enzyme assay mixture normally contained 1 mL enzyme extract, 1 mM NaF, and 0.46 mmol tributyrin (Sigma Chem. Co.) in a total of 2 mL. The assay was carried out in a radiometer pH stat (Copenhagen, Denmark), with the initial pH being set at 7.5. The titrant used was 0.005 N NaOH. Enzyme reaction was initiated with the addition of the substrate. Normally, the reaction was allowed to continue for 5 min or until a straight titraction curve was obtained on the recorder chart. Protein content of the enzyme preparation was determined by the method of Lowry et al. (7). Enzyme activity was expressed as n mol of acid produced/mg protein/5 min.

Analysis of data. The experimental data were analyzed by use of t-test for the differences between two independent means.

Results

Chromatograms of the methyl esters of fatty acids extracted from seedlings treated with water (controls) and with 5.0 mM NaF are shown in Figures 1 and 2, respectively. Palmitic, linoleic, and linolenic acids, plus a few unidentified compounds, were found to be the major fatty acids in mung bean cotyledons. Fluoride caused both qualitative and quantitative changes in these components. A marked reduction in both palmitic and linoleic acids was observed in extracts from the experimental seedlings. As shown in Table 1, seedlings treated with 5.0 mM NaF showed decreases of 77%, 35%, and 15% in palmitic, linoleic, and linolenic acids, respectively. Differences in palmitic and linoleic acids between the control and F-treated seedlings were found to be statistically significant ($p < 0.01$). The concentrations of stearic and oleic acids were much lower than the other three fatty acids identified. No attempt was made to quantify them. Changes in the concentration of several other fatty acids with lower carbon number were also noted (Figures 1, 2).

Lipase activity of cotyledon extracts increased with the age of the seed-

Figure 1

Chromatogram of methyl esthers of fatty acids isolated from mung bean seedlings treated with water (control) for 72 h. Peaks represent methyl esthers of myristic [tentative] (1), palmitic (2), stearic (3), oleic (4), linoleic (5), and linolenic (6) acids.

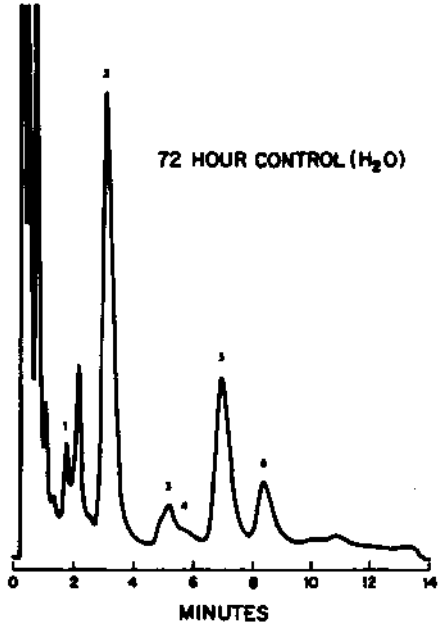

Figure 2

Chromatogram of methyl ethers of fatty acids isolated from mung bean seadlings treated with 5 mM NaF for 72 h. Peaks represent methyl esthers of myristic [tentative] (1), palmitic (2), stearic (3), oleic (4), linoleic (5), and linolenic (6) acids.

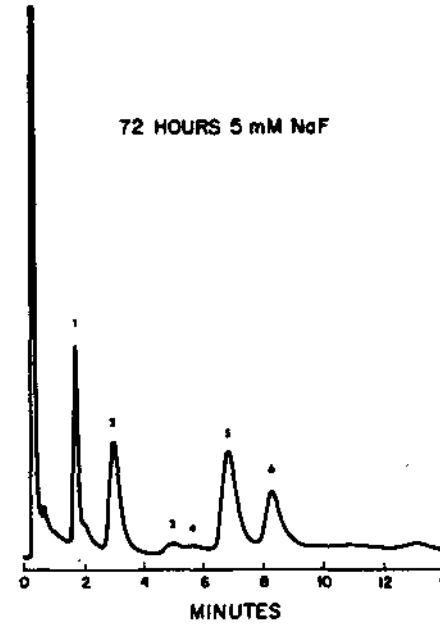

Table 1

Effect of NaF on Fatty Acid Content in Mung Bean Seedlings

Fatty Acid	F Concentrations, mM 0	0.1	1.0	5.0
Palmitic (16:0)	67797 ±16120.4	27519 ±3621.7**	35717 ±10551.4*	15487 ±2969.8**
Linoleic (18:2)	37902 ±2139.1	30647 ±6347.4	28009 ±5871.3*	24708 ±3157.5**
Linolenic (18:3)	15201 ±771.4	16191 ±2940.6	15343 ±4444.1	12709 ±3093.1

Seedlings treated with water or NaF for 72 h were used. Values are mean ±S.D., based on relative peak areas printed out by an integrator (Spectra Physics, model SP 4290).

* $p < 0.05$.

** $p < 0.01$.

lings. The activity of the extract from the 72-hour seedlings was 40-50% higher than those of the 24-hour and 48-hour seedlings (Table 2). To study the influence of fluoride on lipase activity, extracts prepared from control seedlings were tested in the presence of 1 mM NaF. The results showed fluoride to be highly inhibitory (Table 2).

Table 2

Effect of NaF on Lipase Activity

Treatment	n	Enzyme activity (n mol acid produced/ mg protein/5 min)	Percent of control
24 hr			
Water	8	41 ±7.4[1]	100
1 mM NaF	2	22 ±2.4**	53
48 hr			
Water	7	43 ±5.4	100
1 mM NaF	2	20 ±1.7**	46
72 hr			
Water	6	61 ±6.2	100
1 mM NaF	2	27 ±3.1**	44

[1] Values are means ±S.D.

** $p < 0.01$.

Discussion

As mentioned previously, Simola and Koskimies-Soininen (3) observed an increase in palmitic acid and a decrease in linoleic acid in all lipid fractions while linolenic acid decreased in glyco- and neutral lipids in the gametophytes of Sphagnum fimbriatum. Khan and Malhotra (8) also showed that SO_2 treatment of Pinus banksiana needles produced a marked reduction in the content of linolenic acid and an increase in the content of palmitic acid. These authors suggested that SO_2 inhibited both the elongation and desaturation processes. By contrast, no palmitate accumulation was observed in mung bean seedlings treated with NaF (Table 1). In fact, reduction in palmitic acid by fluoride treatment was most pronounced among the five fatty acids identified. The finding on palmitic acid is similar to that reported by Tomlinson and Rich (9) on Nicotiana tabacum leaves affected by ozone. Our observations are consistent with earlier reports that fatty acid pattern in plant tissues may vary with plant species and different environmental factors (2, 10-12). Different organelles are also known to produce characteristic patterns of fatty acids (2).

It is important that the partially purified enzyme preparation used in this study was markedly inhibited by NaF at a concentration as low as 1.0 mM (Table 2). Giannini et al. (13) reported inhibition of biochemical processes of photosynthesis in isolated soybean chloroplasts at 10 mM NaF. According to Ballantyne (14), the threshold concentration of KF for inhibiting chloroplast activity was approximately 2 mM.

Because lipids are important constituents of biological membranes, in

addition to being a concentrated energy source, inhibition of lipase by F could be detrimental to the overall metabolism of germinating seeds. The F-treated seedlings showed an impaired radicle formation and development. This may be attributed, in part, to F-induced inhibition of lipase in the seedlings. It is interesting that, while lipase activity as well as fatty acid content of the tissues treated with 0.1 mM NaF was significantly reduced (Table I), little morphological changes were manifested in the seedlings.

Acknowledgement

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INDUSTRIAL FLUORIDE POLLUTION IN THE METALLURGICAL INDUSTRY IN CHINA

by

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SUMMARY: The hazard of airborne fluoride pollution in 63 plants in the metallurgical industry in China was studied. Fluoride injuries to plant workers were most severe in the electrolysis works in aluminum plants and iron smelters. The incidence of fluorosis among workers was 3.2%, and the symptoms were systemic. For diagnosis, both the effects of airborne fluoride pollution and fluoride content in water must be considered, because some workers come from areas where fluoride content in water is high and fluorosis is endemic. Anti-air-pollution devices are needed to reduce the hazard of industrial fluoride pollution.

KEY WORDS: China; Dual effect; Hazard; Industrial fluoride; Industrial fluorosis.

Introduction

Because fluoride is used extensively in industry, airborne fluoride has not only polluted the air and water supply but also adversely affected humans and cattle as described earlier by Roholm (1). Waldbott states: "Since fluoride is one of the most prevalent air pollutants, contaminated air and regionally contaminated food are likely to play an important role in soft tissue storage . . . ." (2). Reports on the hazard of industrial fluoride are available from many countries (3). To evaluate fluoride problems in China, we investigated industrial fluoride pollution in the metallurgical industry in China between 1980 and 1984.

Materials and Methods

Air samples were taken from inside workshops of 63 fluoride-emitting plants in the metallurgical industry and their surroundings. Altogether, 9624 factory workers participated in the study, which included 3500 workers who underwent dental examination, electrocardiograms (2939 workers), skeletal x-rays (6224 workers), urinary F analysis (9422 workers), and hair F analysis (839 workers). For controls, 400 non-fluoride workers were randomly selected. Industrial fluorosis was diagnosed among workers according to Diagnostic Criteria (4).

Results

Fluoride pollution monitoring data (Table 1) indicate that pollution from combined iron-ore, which contained 5-11% fluoride, was the most hazardous to plant workers and the surroundings because numerous cattle suffered from

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Table 1

Fluoride Pollution at 63 Industrial Plants

Types of Plant	Workplace		Outside	
	Range of Mean F Level (mg/m^3)	Times Above Criteria*	Range of Mean F Level (mg/m^3)	Times Above Criteria**
Electrolysis of Alumina (Large Scale)	1.48-8.53	0.48-7.53	0.05-0.44	6-62 (Leeward of workshop 100-500 m)
Electrolysis of Alumina (Small Scale)	0.40-3.36	0-2.36	0.02-0.09	2-12 (Leeward of workshop 100-500 m)
Iron Ore mixed with Fluoride	0.40-9.94	0-8.94	——	—— (Pollution reached 50 km)
Steel Making (Fluorspar is used)	0.63-1.58	0-0.58	0.004-0.05	0-6 (within a radius of 100 m)
Special Steel Making (Fluoride is used)	0.03-18.12***	0-6.25	0.006-0.05	0-6 (within a radius of 100 m)
Cryolite Synthesis	1.40-4.95	0.40-3.95	0.014-0.58	1-82 (Leeward of workshop 500 m)
Special Steel Making (HF is used)	4.78-5.64***	0.91-1.23	0.004-0.007	0
Manufacture of Phosphate Fertilizer	0.10-28.0***	0-10.21	——	——
Manufacture of Monocrystalline Silicon (HF is used)	0.04-0.46	0	0	0
Lead Electrolysis	0.25-4.17	0-3.17	——	——
Electric Welding	13.1-26.9	4.25-9.76	——	——

* Criteria levels in workshops: HF 1 mg/m^3; Total Fluoride, 2.5 mg/m^3 (5).
** Criteria levels in atmosphere: HF 0.007 mg/day, Mean Value
*** Showed by total fluoride.

fluorosis within 50 km of the plant. Pollution in the electrolysis of alumina was mostly due to the use of open "Vertical Stud Soderberg Cells." In particular, high amounts of HF and other fluorides were emitted from large-scale electrolysis plants. In some small scale electrolysis plants, scrubber and exhaust gas retrieval systems were used to reduce F emission. Hydrofluoric acid is used in the synthesis of cryolite. Since the facilities where fluoride emission occurred were insufficiently sealed, pollution of the surrounding area was as serious as that with large-scale electrolysis. Although manufacture of phosphate fertilizer and of special steel causes F levels to rise to 28.0 and 18.12 mg/m^3, respectively, the period of production and workers' exposure to fluoride was shorter, the effect was less damaging. Except for the above-cited, because the number of affected workers was smaller and/or the pollution was less severe, the hazard of industrial fluoride was less marked in lead electrolysis, special steel, and monocrystalline silicon maufacturing plants. In electric welding, fluoride was emitted because of the use of welding rods which contained 8-40% CaF_2. Since F exposure was intermittent and since F containing welding rods were only selectively used in the steel making processes, the effect on workers were reduced. Table 2 shows the clinical data obtained from 9624 workers and 400 non-fluoride controls.

Table 2

Clinical Manifestations of Plant Workers and Controls

Clinical Manifestations	Percent[1]		p
	Fluoride Workers (9624 Cases)	Controls (400 Cases)	
Neurasthenia syndrome[2]	34.9	21.8	*[3]
Cough	27.4	17.6	*
Abdominal pain	28.4	12.4	*
Backache	40.5	18.6	*
Restricted joint movement	11.0	2.7	*
Chronic nasopharyngitis	33.5	19.3	*

[1] Percent of population with positive manifestations of symptoms.
[2] Includes headache, dizziness, fatigue, insomnia, etc.
[3] * $p < 0.01$

Length of employment of the 9624 workers, aged 18-70 years (average 34), was 3 mo. to 36 yrs, with the majority ranging from 10-20 years. Clinical manifestations were significantly different between the two groups. Analysis of clinical data on 1020 workers revealed that prolonged exposure was associated with increased frequency of clinical manifestations. For example, restricted joint movement was revealed in 4.5%, 27.7% and 32.2% of those employed for 5, 15 and 25 years, respectively. Frequency of chronic nasopharyngitis in some potmen (large scale) was as high as 60.5%, due to prolonged irritation of the nasopharynx mucosa. In contrast, frequency shown by some HF acid washing workers was as low as 20%, possibly because of the lower F levels maintained in the workshop.

Regarding the dental examination, in 22% of the 3500 workers the

corroded appearance on the surface of the teeth, might have been caused by HF acid erosion. In aluminum plants located in areas where fluoride content in water was high enamel mottling occurred in 65.9% of the 1500 native workers, compared to 61% among non-fluoride controls from the same area. Enamel mottling in controls may be the result of exposure to fluoride prior to employment (6,7).

F levels in urine and hair: In the past, many have believed that the extent of fluoride poisoning can be determined by the level of 24-hour urinary fluoride or spot samples. In a study of individual cases, however, urinary fluoride cannot be used as a function of fluoride intake (8). In this study 9422 workers were sampled from 36 assorted plants; urinary F content in pre-shift samples were compared with those in 1200 non-fluoride controls. Mean values of fluoride workers (0.3-7.5 mg/L) were higher than those in non-fluoride controls (local mean values 0.25-1.8 mg/L) which indicates that urine excretion was the main route for eliminating excess fluoride from the body and reflected the body burden of fluoride. In addition, some individual's post-shift urinary F content was as high as 21 mg/L, suggesting that post-shift urinary F could indicate the extent of fluoride exposure (9). By use of Spearman's Method, the correlation between the F level in workshops and urinary F content in operators was significantly positive for 2373 workers from 19 plants ($r = 0.69$, $p < 0.01$). Thus urinary fluoride may be used to determine individual body burden and appraise exposure of workers. Waldbott earlier reported that fluoride-induced injury could not be reliably determined on the basis of the level of urinary fluoride and that spot samples or single 24-hour samples of urine could be very misleading (10).

The F content in the hair of 839 workers (range 15-3884 ppm) was compared with that of 330 non-fluoride controls (range 20-85 ppm), and the difference between the two groups was significant ($p < 0.01$). However, no correlation was found between clinical findings, skeletal damage and F content in the hair samples. In chronic industrial exposure, the fluoride content of hair is likely to be a useful indicator of fluoride absorption (11).

Electrocardiogram changes in workers: Routine ECG check-up (using 9 leads) was carried out on 2939 workers and the abnormal frequency excluding primary heart and vessel diseases, was 46.6%. The frequency for the 150 non-fluoride controls, was 33.3% ($p < 0.05$). Analysis of abnormal ECG features revealed that over a third of the population showed sinus arrhythmia and/or bradycardia, the remainder had various conductive blocks, T wave changes (V_3,V_5), premature beats and myocardial ischemias. Whether the heart and arteries are damaged by fluoride or not (12-14), should be further investigated. Results of the analysis of radiograms of pelvis, forearms and lower legs are shown in Table 3.

With exception of the frequency of occurrence in coxarticular degeneration (Table 3: pelvis), significant differences ($p < 0.01$) in x-ray skeletal changes were found between the two groups. On the basis of x-ray films, osteosclerosis was the main change in industrial skeletal fluorosis, in agreement with Franke et al (3). Various degrees of increased density and trabeculae proliferations were observed in the pelvis. In order to determine the ossifiction of osteomembrane or interosseous membrane at forearm and lower leg, we used slightly underexposed photographs similar to the kind of radiography used for soft tissues (3); the existence of periosteal appositions could be established.

Table 3

Positive Radiological Findings in Skeletons of Workers Compared to Controls

Radiological findings	Fluoride Workers (N = 6224) %	Non-fluoride Controls (N = 845) %
Pelvis:		
Density increase	10.57	0.39
Trabeculae gauze-like[1]	4.8	0
Trabeculae linen-like[2]	1.04	0
Trabeculae marble-like[3]	0.08	0
Ossification on Obturatoria membrane	25.40	16.60
Ossification of iliolumbar ligament	11.54	5.79
Ossification of sacrospinosum ligament	2.38	0.39
Coxarticular degeneration	5.27	8.88
Tibia:		
Increase in density	5.91	0
Trabeculae coarse	12.35	2.33
Ossification of osteomembrane or interosseous membrane	21.19	8.67
Knee articular degeneration	14.93	2.67
Radius and Ulna:		
Increase in density	4.88	0
Trabeculae, coarst	8.74	1.05
Ossification of osteomembrane or interosseous membrane	11.02	4.20
Articular degeneration of elbow	4.72	0.35

[1] Traceculae slightly coarse
[2] Trabeculae obviously coarse
[3] Trabeculae no longer discernible, bone structure white and marble-like.

In this investigation, a considerable number of workers suffered from endemic skeletal fluorosis because they came to us from an area where water F content as high. Dual effects from fluoride, which they suffered have been reported previously (6).

Diagnosis of industrial fluorosis: Based on Diagnostic Criteria (4), the total incidence of fluorosis in the studied population was 3.2%, 80% of which was in stage I; the age of the population ranged from 26-70 years (average 44.8 years); the period of occupational exposure ranged from 3-30 years (average 17.1 years).

Occurrence of the disease was related to the period of occupational exposure (Table 4); increased exposure period and increase in degree of fluorosis were directly related; after more than 20 yrs. of employment 47.7% of the workers were in stage 1, 73.3% in stage 2, and 100% in stage 3. After labor protection measures were adopted in 6 plants (Table 5), airborne F in workshops fell to permissible levels and fluorosis decreased to 0.7%. The highest incidence was 7.0% (potmen, large-scale pots), the lowest was less than 1% in furnace workers where fluorspar was used in steel making.

Table 4

Correlation Between Incidence of Industrial Fluorosis and Employment Period

Employment Period (yrs.)	Incidence (%)
5	5.0
10	23.8
15	31.5
20	43.7

Table 5

Correlation Between Incidence of Industrial Fluorosis and F Levels in Workshops

No. of Plant	No. of Workers	Times above Critical Level	Incidence (%)
9	2710	3.5-8.5	6.8
6	1637	0	0.7

Discussion

Fluoride exposure by metallurgical workers was surveyed for the purpose of preventing the fluoride hazard. New technology in production processes, namely increased sealing of fluoride-emitting facilities as well as installation of ventilation and cleansing systems, tends to reduce fluoride pollution. Kaj Roholm (1), originator of modern fluoride research, outlined in detail the clinical manifestations of industrial fluorosis. Moreover, a vast amount of research (88 professional publications on fluoride from 1955 to 1983) was carried out by Waldbott on how fluoride affects the human organism.

The current investigation revealed that the total incidence of fluorosis was high because many of our workers were exposed in polluted workshops where F levels were above permissible limits for a prolonged period. For the purpose of diagnosis, occupational exposure must first be established. Some clinical symptoms are associated with the non-skeletal phase. Restriction of joint movements was frequently associated with abnormal findings in skeletal films. However, the classical symptoms of the non-skeletal phase of fluorosis were first delineated by Roholm. Walbott encountered the same symptoms prior

to the onset of bone changes (15). According to our survey, clinical manifestations of fluoride injury were systemic (Table 2). A wide variety of vague, subtle symptoms occurred either prior to or simultaneously with the development of bone changes similar to those reported previously (16). Nonskeletal symptoms, therefore, are important for early diagnosis.

On the other hand, the dual effects of endemic and industrial fluoride should not be disregarded when the plant is located in an endemic area or the worker had been residing prior to employment in an area where the F level in water was high. Mottled enamel, a predominant sign, which develops only if the individual is exposed to fluoride early in life, contributes to differential diagnosis.

The diagnosis of industrial fluorosis cannot be established on the basis of urinary F data. No definite correlation was observed between urinary F levels, clinical stage, and neurological sequlae (17). Even among healthy workers we observed, as did Girakaja (18), F levels in urine could be higher than those in workers suffering from fluorosis. Factors affecting urinary F excretion include age, nutritional status, diet, kidney function, types of fluoride compounds, previous fluoride exposure, and many others (8). Thus, in the diagnosis urinary fluoride only indicates exposure to fluoride.

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EFFECT OF FLUORIDES IN AMBIENT AIR ON GRASS SPECIES GROWING IN ARTIFICIAL GRASSLAND COMMUNITIES

by

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SUMMARY: Much fluoride research has been done on pure grass cultures. However, to determine how grass species behave in a grassland community exposed to ambient fluorides, two mixtures of grass species, mixture A consisting of Lolium perenne, Dactylis glomerata, Phleum pratensis and mixture B consisting of the same species without Dactylis glomerata were sown in containers (48 x 30 cm; h. 20 cm) filled with a sandy arable soil, equipped with a semi-automatic watering system.

Containers were placed in a polluted area and in a control area; grass was clipped 3-5 times a year for 3-4 years, 4-5 cm above ground level.

Each harvested grass mixture (400-500 shoots per container) was divided into the different species and the dry matter production and the number of tillers per container were determined as well as the fluoride content of each species.

The daily average fluoride concentration during the growing season was 1.4 to 2.2 $\mu g/m^3$. Peak concentrations up to 14 $\mu g/m^3$ (daily average) occurred. Immission measurements were done with a single filter method. Lolium perenne appeared to be the least sensitive species whereas Phleum pratense was the most inhibited. After 1-2 years, Lolium started to dominate the community (mixture B) in the polluted area in contrast to the control area. Dactylis glomerata (mixture A) was also rather sensitive but under the applied mowing cycle it was dominating on both areas in spite of a strong growth inhibition in the polluted area.

KEY WORDS: Ambient air fluorides; Grassland community, growth inhibition, reduced tillering.

Introduction

In a meadow, the plant community consists of different species of grasses and weeds in a more or less stable proportion. When a mixture of seeds of different grass species is sown, the initial botanical composition of the association is strongly related to the composition of the seed mixture. The composition is not stable and the relative proportions in number of tillers changes under the influence of a number of parameters related to soil conditions such as humidity, availability of nutrients; fertilization (high or low nitrogen input,

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...) (1); utilization for grazing or mowing; climatic conditions (winter stress, drought); influence of air pollutants.

Drastic changes in a plant community under the influence of atmospheric fluoride pollution, are possible in industrial areas shown by Kotrisova and Zdenek (2). Changes in the botanical composition as a result of fumigation with SO_2 has been shown by Guderian (3).

Changes in the botanical composition of a grassland community is due to different reaction of single species; the occurrence of one or more air pollutants can cause growth reduction. Grass species, least reduced in growth by air pollution, will dominate in the community after a certain time. Indirectly, root growth can be depressed so the effects only occur after prolonged exposure (4); inhibited tillering under pollution circumstances, species where tillering is more reduced than others will be dominated in the community; a reduced vitality, which occurs most frequently after winterstress, combined with air pollution. Plants grow much slower after winter (5) and grass species are much more sensitive during winter than during summer (6), which is linked to a low light intensity and short photoperiods (7), a reduction of seed production and bad seed quality. Plants which produce seeds can be eliminated when they no longer produce fertile seeds (2); indirect effects are likely to occur when soil is polluted because of accumulation of heavy metals, soil acidification etc. The occurrence of SO_2 might depress the frost hardiness of Perennial rye grass (8).

In this work, the evolution of two different artificial meadow populations in a polluted and non-polluted area has been followed during 3-4 years. The soil conditions, mowing cycles and fertilization were equal for both areas, and the climatic conditions were closely related because the two sites were located about 20 km from each other.

Materials and Methods

A container system with semi-automatically water supply has been used to expose the artificial grassland populations. Two containers were placed on top of each other, the bottom one was used as a water reservoir, the upper one was filled with an arable sandy soil in which the mixture of grass seeds were sown. To secure the water supply, two filter candles were placed in the soil of the upper container. In dry periods the water was sucked up from the bottom container via a silicone rubber tube (Figure 1).

The container systems were put in a groove surrounded by wooden boarding so that the grass grew at ground level. A control of the water level and addition of water in the bottom container was possible. Turfs, with a comparable composition of grass species, were placed around the containers so that the grass in the containers was an integrated part of the turf.

Figure 1

Container System

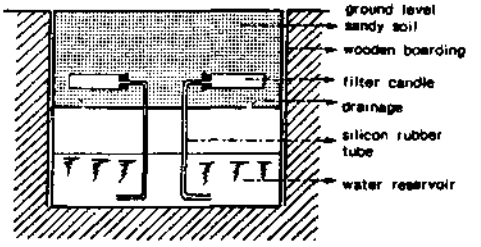


Table 1

The Grass Species Used in the Experiment

Common name	Scientific name and abbreviation	cultivar	mixture A	mixture B
Perennial rye grass	Lolium perenne (Lp)	Melino R.V.P.	5 kg/ha	10 kg/ha
Timothy grass	Phleum pratense (Phl)	Erecta R.V.P.	5	10
Cocksfoot	Dactylis glomerata (D)	Lemba R.V.P.	0.05	-
Meadow fescue	Festuca pratensis (Fp)	Merbeem R.V.P.	8	12

Each spring, a combined NPK fertilizer (9-9-15) was applied (140 kg N/ha; 140 kg P_2O_5/ha; 230 kg K_2O/ha). The grass was harvested 3-5 times a year; after each harvest ammonium nitrate (50 kg N/ha) was added.

In most cases, the harvested grass was split up under the different species using their vegatative characteristics as an indicator. The number of tillers was counted and the dry weight production of each species determined. All the fluoride determinations were carried out by using a specific fluoride electrode.

Fluoride determinations in plant material were carried out on dried (75°C, 16 h) and ground material. The fluorides were extracted with nitric acid and measured after addition of a buffer solution (9).

Total F^- content of the soil was determined by the Jagner and Pavlova (10) method. A dried and seived soil sample was ashed at 900°C after addition of Na_2CO_3 and ZnO, the residue dissolved in a Na_2CO_3 solution and the fluoride content measured, after addition of HCl and a complexing solution. The fluoride content of the water extract of the soil was determined on the filtrate after addition of a buffer solution (11).

Immission concentrations were measured by using the single filter method of Elfers and Decker (12), while deposition measurements were carried out with lime papers. After 28 days' exposure, the lime papers were ashed followed by extraction of the ash by HCl; fluoride content was determined after addition of a buffer solution.

Air Pollution Climate in the Polluted and Control Areas

Gaseous fluorides are the main pollutants in the industrial area where a fertilizer plant is the main source of ambient fluorides. Daily averages for SO_2, NO and NO_2 are low for an industrial area but high peak concentrations may occur for a short time. The measured concentrations are listed in Table 2 for SO_2, NO and NO_2 (13) and in Table 3 for fluoride.

The evolution of the immission concentration (weekly averages) together with the evolution of the deposition measurements, with lime papers and the fluoride content of the grass mixtures (average of 6 replicates) are shown in Figure 2.

The data for grass and lime papers are summarized in Table 4 for the industrial as well as for the control area.

Table 2

Air Pollution Levels in the Industrial Area

Pollutant	daily average	maximum	short time peaks
SO_2	15-50 µg/m³	120 µg/m³	1500 µg/m³
NO	0-13 µg/m³	13 µg/m³	590 µg/m³
NO_2	37-137 µg/m³		rare

Table 3

Immission Concentrations of Fluorides in the Industrial Area

year	n	$\bar{x}$	min.-max.	Percentiles			
				50 pct.	75 pct.	95 pct.	99 pct.
1979	157	2.20	0.11-14.0	0.85	3.07	8.59	10.56
1980	195	1.51	0.11-12.4	0.50	2.07	6.47	8.12
1981	196	1.44	0.14-9.24	0.57	1.98	5.38	6.57

Table 4

Fluoride Content of Grass and Deposition Rate of Fluorides on Lime Papers

	F^- content of grass µg/g (dry matter)	F^- deposition on lime papers µg/dm²/day
Industrial area $\bar{x}$	132	13.3
min.-max.	38-293	2.8-46.5
Rural area $\bar{x}$	3.1	0.6
min.-max.	1.1-7.3	0.4-1.3

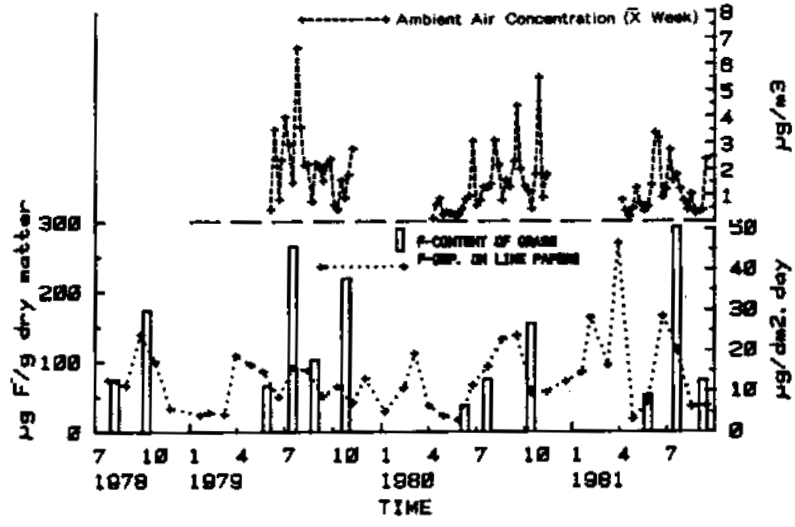
Results and Discussion

Evolution of global production of grass mixtures. As shown in Figure 2 production in the industrial area almost during the whole observation period is higher than in the control area. (Control area = 100%). A decrease in production is sometimes observed which is roughly linked to periods of high fluoride concentrations in ambient air, high deposition and high fluoride accumulation in grass. The higher production level in industrial areas is the effect of an undesired fertilizer application due to gaseous and particulate nitrogen compounds released by the fertilizer plant. At high polution levels, however, production in the industrial area declines; growth is reduced more through pollution than strengthened because of surplus fertilization.

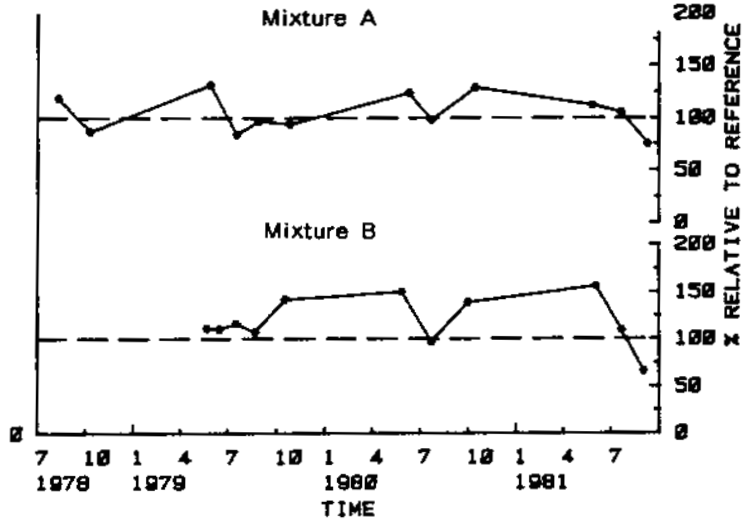
Evolution of number of tillers and the dry weight yield per grass species. The evolution of the composition of the experimental grass communities is shown in Figures 3 and 4. The relative proportion of different grass species is not stable as a function of time. The figures show a marked difference in the

Figure 2

A. Evolution of the Fluoride Content in Grass and Ambient Air



B. Dry Matter Yield of Grass in Industrial Area



evolution of the relative proportions of the different grass species in the industrial and rural areas. In the very beginning of the experiments, the evolution of the grass mixtures in the two experimental sites was similar but after approximately one year distinct differences appear.

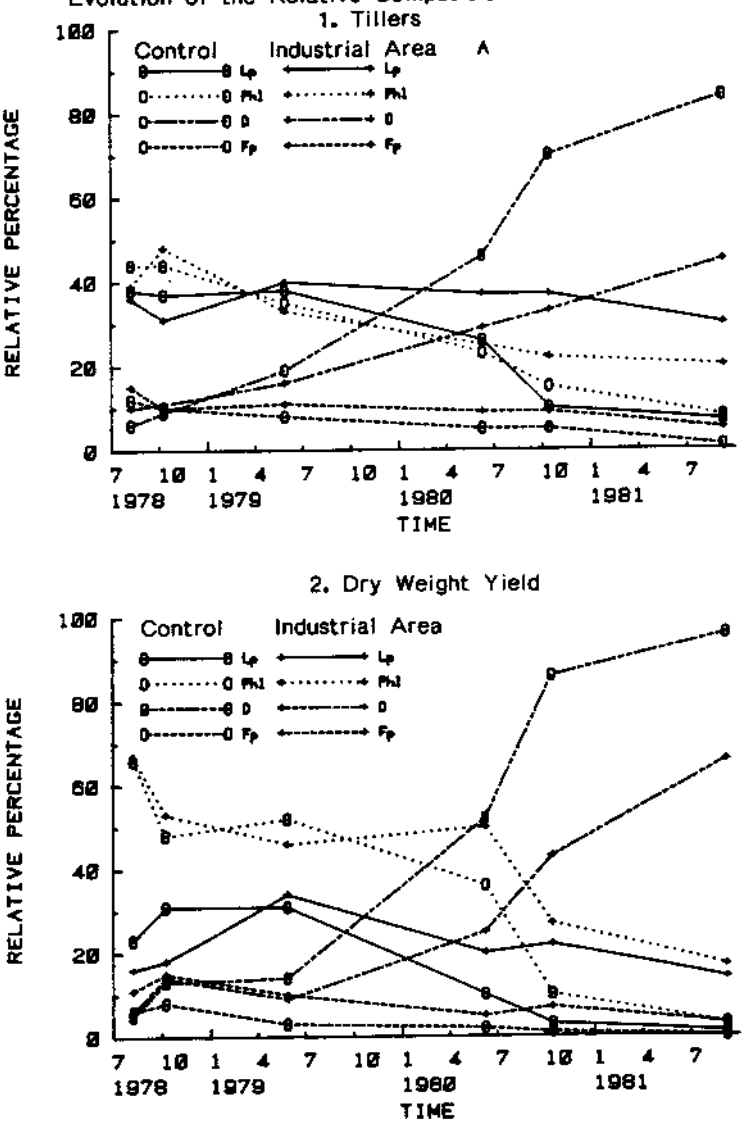
In the first mixture, Dactylis glomerata strongly dominates the grass

community in both areas, a normal evolution under the mowing cycle used in the experiment. However, domination of Dactylis glomerata is stronger in the control area; it is likely to be inhibited in the polluted area, which is also clear when the relative 100 shoot weight (SW_{100}) is plotted against time (Figure 5.)

$$SW_{100} = \frac{\text{dry weight (g)}}{\text{number of shoots}} \cdot 100$$

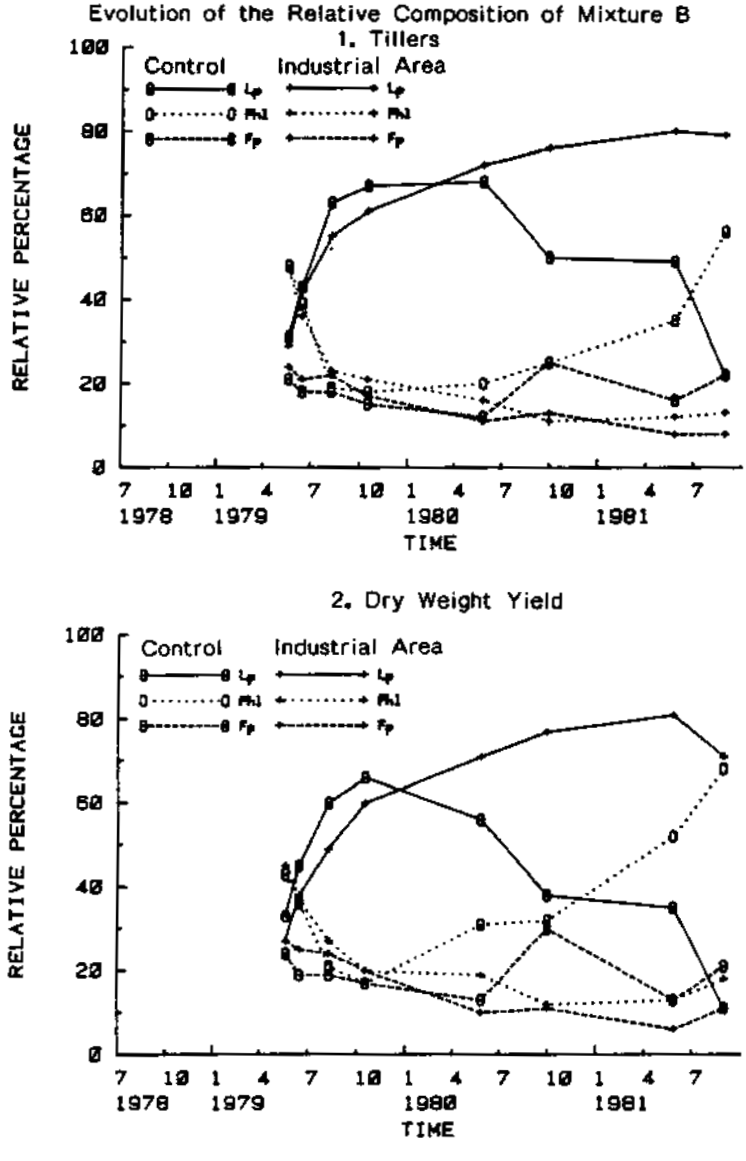
Figure 3

Evolution of the Relative Composition of Mixture A



Throughout the duration of the experiment and in comparison to the control area, the cocksfoot plants are always smaller in the industrial area which indicates that the plant is inhibited by ambient air pollution. In mixture B where cocksfoot doesn't occur, timothy grass is the dominant species in the control area which is normal in a good fertilized meadow under mowing conditions (1).

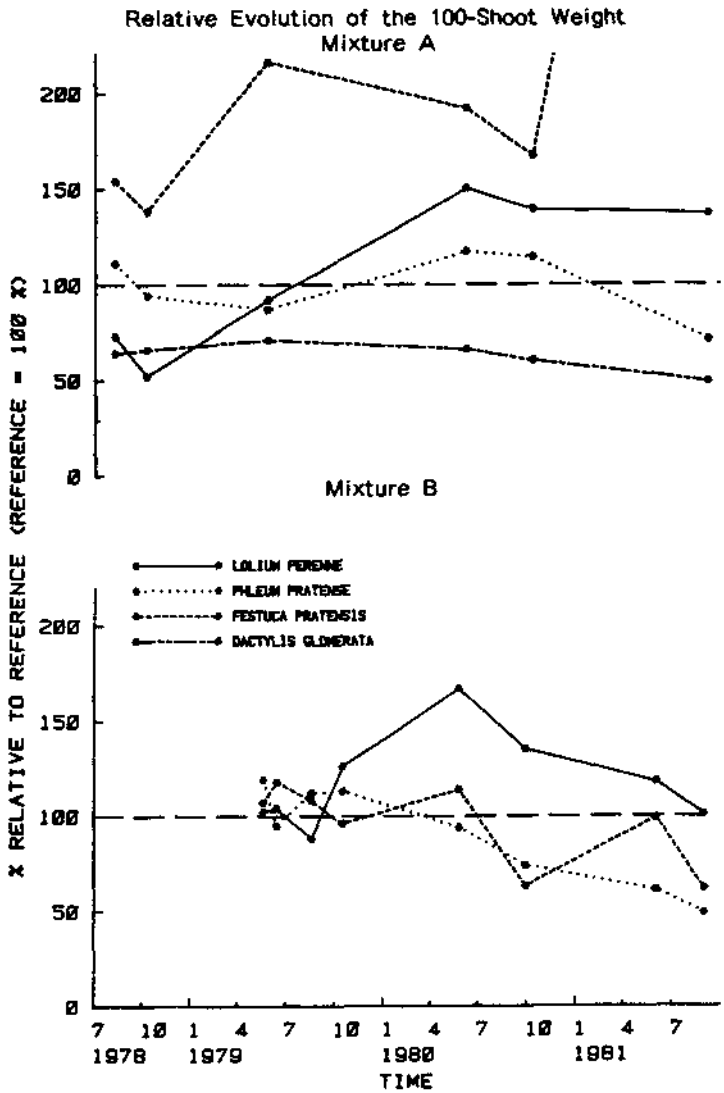
Figure 4



In the polluted area, however, this species is much more inhibited than cocksfoot in mixture A; perennial rye-grass even becomes dominant.

In both mixtures, the most distinct differences between areas occur after the winter periods 1979-80 and 1980-81. In both, a high fluoride load during wintertime has been registered. The influence of air polution during this period must have been especially strong. Figures 3 and 4 show that dry weight production is the most sensitive indicator of pollution. Growth reduction occurs much earlier than a decrease in the number of shoots. Reduced tillering is a secondary effect.

Figure 5



Fluoride

The relative SW_{100} indicates that cocksfoot and timothy grass are the most inhibited grass species. Perennial rye-grass is far less affected by air pollution; this species is dominated by other species under mowing conditions in Spring plants such as cocksfoot and timothy grass have the ability to reach their maximal development due to larger leaves which catch more light. Festuca pratensis seems to be less inhibited by air pollution; it plays a secondary role when it occurs together with cocksfoot and/or timothy under mowing conditions.

Evolution of acidity and fluoride content of the soil filled in the containers is given in Table 5.

Table 5

Evolution of Soil Acidity and Fluoride Content Of Soil Used in the Experiment (average of six replicates)

May, 1978	Acidity				µg/g F^- in Air Dry Soil			
	pH H_2O 6.7		pH KCl 5.6		H_2O-extract 1.8		total F^- 340	
	Cont.	Indus.	Cont.	Indus.	Cont.	Indus	Cont.	Indus.
Oct., 1981								
top layer 0-10 cm	5.5	5.4	4.4	4.4	6.1	10**	293	413
bottom 0-10 cm	5.8	6.15	4.9	5.3	4.7	5.6	383	378

** significantly different at the 1% probability level. The other results do not show a significant difference between the control and industrial area. Except for total fluoride, the difference between 1978 and 1981 was significant at the 1% level for all parameters.

Acidification of the soil between 1978 and 1981 has been clearly observed in both experimental sites, but acidification was not significantly higher in the industrial area. The acidification process induced an increase in the fluoride concentration in the soil water phase in both areas. In the polluted area, however, accumulation of water extractable fluoride was significantly higher. Enrichment of total fluoride was also probable, but was not significant.

Conclusions

In comparison to Lolium perenne and to a minor extent to Festuca pratensis, cocksfoot (Dactylis glomerata) and timothy grass (Phleum pratense) have clearly shown growth reduction in the polluted area which has consequences for the botanical composition of a meadow in polluted areas.

The cause of growth reduction and the shift in relative proportion of the meadow populations is probably due to air pollution, more likely to gaseous fluoride in ambient air, the most important pollutant in the studied area. Comparing the different grass species during fumigation tests, timothy grass was the most sensitive of the four species in the field experiment, and showed the greatest growth reduction. Cocksfoot was less sensitive than timothy grass but its growth was far more inhibited than that of perennial rye-grass and meadow fescue (14).

In addition, experiments with hydroponic cultures showed that timothy grass was also the most inhibited of the studied species when growing in a mutrient solution containing 10 ppm fluoride at a pH of 5.0 in comparison with a nutrient solution without fluoride. Growth reduction of cocksfoot was negligible but root growth was reduced (14).

As soil conditions at the end of the experiment approached closely conditions of the hydroponic experiment, a fluoride accumulation in the root zone may influence negatively. The most inhibited species (cocksfoot and timothy grass) are also the most sensitive to SO_2 and NO_2 (15-17). These pollutants in the polluted area may have an additional effect on the grass species. However, the average concentration of SO_2 and NO_2 is lower in experiments than reported in the literature.

The relative SW_{100} indicates that cocksfoot and timothy grass are the most inhibited grass species. Perennial rye-grass is far less affected by air pollution; this species is dominated by other species under mowing conditions in Spring when plants such as cocksfoot and timothy grass have the ability to reach their maximal development due to larger leaves which catch more light. Festuca pratensis seems to be less inhibited by air pollution; it plays a secondary role when it occurs together with cocksfoot and/or timothy under mowing conditions.

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EFFECTS OF INHALED HF ON CHOLESTEROL, CARBOHYDRATE AND TRICARBOXYLIC ACID METABOLISM IN GUINEA PIGS

by

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SUMMARY: HF inhalation produced a significant increase in plasma cholesterol levels in guinea pigs. This increase may be related to an enhanced activity of glucose-6-phosphate dehydrogenase. The NADPH and H^+ produced in the shunt serves as hydrogen and electron donors in cholesterol biosynthesis catalyzed by 3-hydroxy-3 methylglutaryl Co A reductase. On the other hand, HF inhalation caused a reduction in isocitrate dehydrogenase activity, leading to an accumulation of citric acid, which is a positive effector of acetyl Co A carboxylase, key enzyme in fatty acid biosynthesis.

KEY WORDS: Fluoride, cholesterol; HMG CoA reductase; Isocitrate dehydrogenase; Glucose-6-phosphate dehydrogenase.

Introduction

In a previous work, we demonstrated a significant increase of plasma cholesterol levels in guinea pigs exposed to HF (1). On the other hand, in a HF inhalation study, the activity of cholesterol 7-hydroxylating system containing cytochrome P_{450} was not shown to be decreased in hepatic microsomes (2). The increase in cholesterol, therefore, cannot be related to an acceleration of its catabolism.

Comparative study of acetate and mevalonate incorporation suggests that β-OH, β-methylglutaryl Co A reductase (E.C.1.1.134), the enzyme catalyzing mevalonate synthesis, could be activated by HF (1). The present study was undertaken to investigate which mechanism might be involved in the activation of this enzyme.

Materials and Methods

Animals and experimental design: Male albino guinea pigs weighing 350 g were used at the beginning of exposure. They were fed with commercially available pellets (purchased from Usine d'Alimentation Rationnelle "UAR," France) containing 20 ppm fluoride and with a fresh supply of carrots and vitamins.

To study the effects of HF on metabolism of carbohydrate, tricarboxylic acid and cholesterol, the animals were placed in a cylindrical plexiglas cage as described previously (1).

Gaseous HF was produced by use of an aqueous solution of HF, by means of a peristaltic pump, into a vaporization oven at 150°C. The desired level of HF in the atmosphere (5 mg HF/m^3) was obtained by varying the concentration and volume of the solution and by modifying the amount of purified air

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used to dilute the HF vapor. The level of fluoride was checked every 3 hrs for 84 hrs with an automatic captor by trapping the HF in a known volume of air on a dry caustic soda-impregnated filter and assaying with a specific electrode (3). During the study the average room temperature was 20°C and the humidity 72%.

All animals had free access to water and food during exposure and were fasted overnight before the blood samples were taken. Plasma cholesterol and enzymes of guinea pigs were determined prior to (control) and after exposure to a constant fluoride atmosphere at 5 mg HF/m^3 for 4 days.

Total plasma cholesterol was determined by the method of Röschlau et al. (4). D-Isocitric acid was determined by UV method (5), whereas plasma fluoride by analyzed by the method of Hall et al. (6).

The dose factor is considered qualitatively (each day the animals received a supplementary dose of HF). The homogeneity of the averages is tested (analysis of variances with repetition). If the averages differ significantly, the monotony of the response (amount of cholesterol) according to the dose of HF inhaled was studied. The response is supposedly linear. The linearity of the curve and its slope were tested.

Isocritrate dehydrogenase (E.C.1.1.1.42) and glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) were determined by the method of Walfson et al. (7) and Kornberg et al. (8), respectively using Boehringer Kits.

Results and Discussion

Fluoride is a well-known inhibitor of many enzyme systems in vitro. The effects of fluoride on glycolysis and Krebs cycle have been well documented. Despite the plethora of in vitro studies on the effects of fluoride on enzymatic activity, there is a paucity of information concerning the in vivo metabolic lesion caused by sublethal doses of fluoride.

The present investigation focuses on the relationship between cholesterol synthesis, carbohydrate metabolism and Krebs cycle "in vivo." Therefore, the effect of HF inhalation on enzyme activities and the level of isocitric acid (Figure 1) in relation to increase of cholesterol were studied in guinea pigs. HF inhalation produced a significant increase in plasma cholesterol and glucose 6-phosphate dehydrogenase activity (Tables 1 and 2).

Enhanced activity of glucose 6-phosphate dehydrogenase, an enzyme

Table 1

Effect of HF inhalation on plama cholesterol levels (mMole/L)

	Before exposure	After exposure
n	14	14
$\bar{m}$	1.076	1.584
S.D.	±0.527	±0.710

The difference between means of pair cases is statistically significant ($p < 0.01$).

Table 2

Effect of HF inhalation on plasma glucose-6-phosphate dehydrogenase activity (u/L)

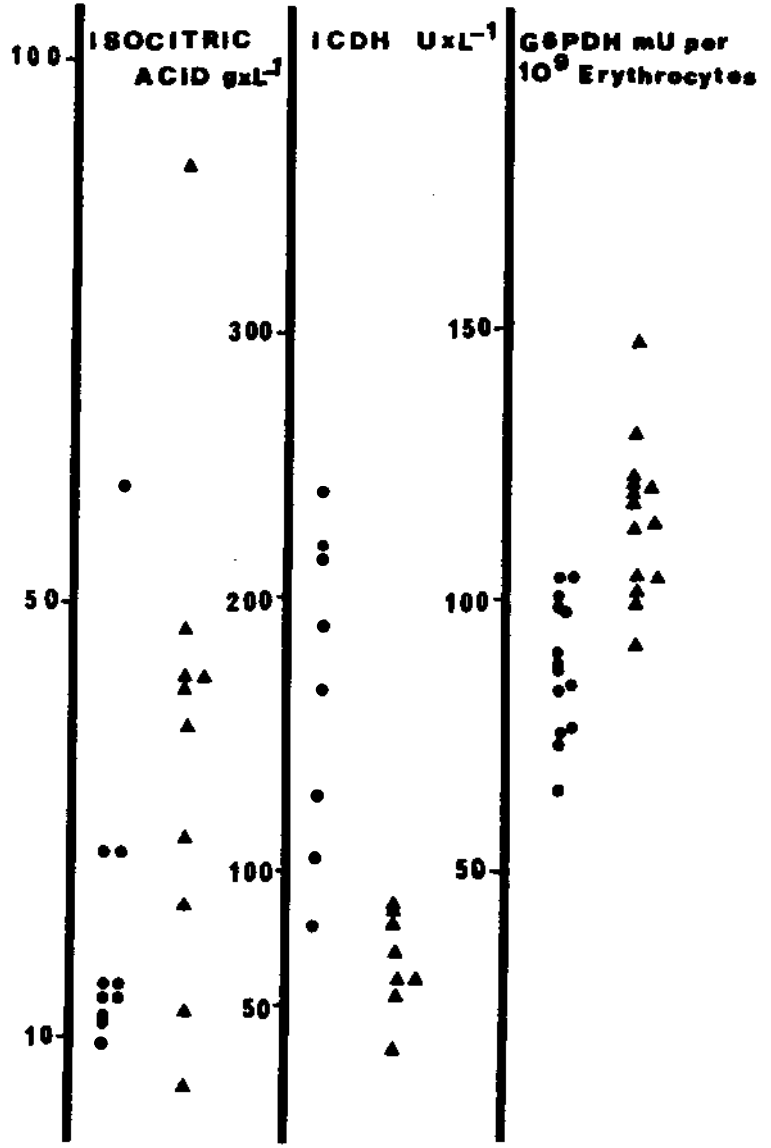
	Before exposure	After exposure
n	14	14
$\bar{m}$	85	114
S.D.	12	14

The difference between means of paired cases is statistically significant ($p < 0.001$).

Figure 1

Comparative effects of HF on plama isocitric acid, glucose-6-phosphate dehydrogenase, and isocitrate hydrogenase.

- ● controls
- ▲ exposed guinea pigs



highly specific for $NADP^+$, will result in increased levels of H^+. The reduced nucleotide is required by 3-hydroxy-3 methylglutaryl Co A reductase which catalyses the conversion of 3-hydroxy-3 methylglutaryl Co A to mevalonate, an intermediate in cholesterol biosynthesis.

The increase in glucose-6-phosphate dehydrogenase activity permits an increased pentose phosphate production.

Table 3

Effect of HF inhalation on plasma isocitrate dehydrogenase activity (u/L)

	Before exposure	After exposure
n	8	8
$\bar{m}$	165	67
S.D.	55	15

The difference between means of paired cases is statistically significant ($p < 0.001$).

Table 4

Effect of HF inhalation on plasma D-isocitric acid (mg/L)

	Before exposure	After exposure
n	10	10
$\bar{m}$	21	34
S.D.	±16	±23

The difference between means or paired cases is not statistically significant.

Transketolase and transaldolase creating, a reversible link between the pentose phosphate pathway and glycolysis, increase the activity of glucose-6-phosphate dehydrogenase and balance inhibition of the glycolytic pathway. The ability of fluoride to affect several enzymes involved in carbohydrate metabolism is well known. Fluoride acts as an inhibitor of many enzymes whose activity is linked with magnesium, especially in the presence of phosphorus. The mechanism of enolase inhibition by fluoride has been elucidated by Warburg and Christian (9,10), who showed that fluoride, in the presence of phosphorus, forms an undissociating magnesium-fluoride-phosphate complex.

On the other hand, decreased isocitrate dehydrogenase and citrate accumulation were observed in the present investigation (Tables 3 and 4). Isocitrate dehydrogenase is known to require magnesium for its activity. Allosteric stimulation of this enzyme is well established. The binding of magnesium, ADP and NAD^+ on the enzyme is mutually cooperative.

Citrate that accumulates is a negative effector for phosphofructokinase. Inhibition of this enzyme may lead to decreases in glycolysis. The increase of cholesterol biosynthesis can be related to the acceleration of the pentose phosphate pathway due to the enhanced production of NADPH consecutively to the key-enzyme inhibition of the glycolysis by HF.

Acknowledgement

We are grateful to Mr M. Giroux for the statistical analysis of the results.

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FLUORIDE INTOXICATION IN DAIRY CALVES

by

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(Abstracted from Cornell Vet. 77:84-98, 1987)

Chronic fluoride intoxication, which has been previously described in dairy cattle, was caused by feeding a dairy herd fluoride-contaminated commercial feed. Dental fluorosis and a catastrophic decrease in milk yield were the foremost findings. The present report is concerned with congenital fluorosis in calves born to these fluoride-intoxicated dairy cows.

The calves manifested congenital fluorosis by brown discoloration of enamel, enamel hypoplasia, brown mottling of bone, severe retardation of cartilage cell differentiation, atrophy of osteoblasts and of bone marrow cells, osteopenia, serious atrophy of bone marrow fat and severely stunted growth.

Bone lesions in calves were severe, more so than in advanced fluorosis in heifers in a previous report. Severe retardation of differentiation of cells in the growth plates — the morphologic basis of stunted growth — must be considered in utero toxic effects of fluoride. Since, following birth, the calves were fed milk ad libitum, starvation cannot be blamed for the lesions. Severe depletion of bone marrow is likewise to be considered an in utero effect of fluoride.

Dental lesions (enamel damage), the main external signs of fluoride intoxication, is initiated only during the formative stage, early in fetal development. Skeletal fluorosis, on the other hand, can be induced at any time during fetal development. Our findings of no or minimal dental fluorosis in 13/36 of the calves under study by no means excludes skeletal fluorosis in the 13 calves without dental fluorosis. Brown mottling of enamel is, obviously, an expression of transplacental transmission of fluoride.

The existence of dental fluorosis in calves has been denied by some investigators who base their claim upon too small a sampling. Actually the recorded incidence (0/4) does not differ statistically from that in the calves under study (23/36). The adjusted χ^2 value is 1.64 ($P > 0.1$) the fetuses were not exposed to the highest levels of fluoride during amelogenesis. In addition, these investigators assume that fetal tissues are reflective to the toxic action of fluoride. However the fetus is exposed to the same blood fluoride levels that cause dental fluorosis in cow or heifer. Fetal tissues grow more rapidly and are more, not less, sensitive to fluoride.

KEY WORDS: Calves; Dental fluorosis; Fluoride intoxication.

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MILK PRODUCTION OF COWS FED FLUORIDE-CONTAMINATED COMMERCIAL FEED

by

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(Abstracted from Cornell Vet. 76:403-414, 1986)

This report deals with the way commercial feeds with excessive amounts of fluoride influence milk production in dairy cattle and the discrepancies between National Academy of Sciences (NAS) standards and the reality of field conditions.

A commercial feed concentrate and a mineral mix (AB40 and AMM) with excessive amounts of fluoride, when given to a Holstein dairy herd, caused the average milk production which was well above national standards to decrease drastically. During the following 6 years, the deficit in milk production in the herd, which ranged from 52 to 120 milking cows, was 1.5 million Kg (3¼ million lbs.). Tolerance levels established by the NAS for fluoride ingestion by a lactating cow proved to be inadequate.

Severely misshapen hooves in a few animals and chronic decubital sores on rear legs were a common problem. Samples from 3 bags of AB40 from 1982 contained 78, 102, and 112 ppm fluoride on a dry matter basis, respectivley (average 97.33 ppm). The fluoride concentration in one 1982 AMM sample was 1415 ppm. Levels in 2 barn water samples were 0.52 and 0.49 ppm fluoride. The farm under observation was a successful dairy operation until 9/1978.

Introduction of fluoride-contaminated commercial feeds was the only change that could have been responsible for the drastic decrease in herd health and milk production which began in September, 1978. Management cannot be blamed for the disaster because it did not change; only fluoride can. The average milk yield before introduction of excessive fluoride in feed was 7,235 kg with a peak at 9,976 kg over the standard lactation period of 305 days. This remarkable production achievement is possible only be excellent management, breeding and feeding. Milk yield of the cows of another farm, when converted to a 305-day lactation period, was only 59% of that of the average cow on the farm under study.

The high milk yield by the studied cows prior to 9/1978 requires a high turnover rate in bone tissue. Such metabolically active bone tissue is very sensitive to fluoride with disastrous negative consequences on milk production. The lowest values were recorded about 4 years later. Fluoride gradually accumulates in bone tissue in a herd of cattle exposed to it. Fluorotic heifers and cows during pregnancy transmit fluoride transplacentally to the developing fetal skeleton; the calf is therefore born with some degree of fluorosis, which is prepetuated in the next generation, etc. The full impact of fluoride intoxication in such a field situation, which becomes manifest after many years, is called the generation effect.

The NAS "tolerance level" for fluoride ingestion as it relates to milk

production is, obviously, erroneous. Another serious NAS mistake is the Academy's position that the "tolerance level" for cattle is 40 ppm in total dry matter ration. A dry cow needs about 9.25 kg dry matter feed per day. At 40 ppm of fluoride, 370 mg fluoride is ingested per day. If the cow is expected to yield 27.2 kg of milk per day (60 lbs.), the dry matter feed requirement is 18.5 kg. At 40 ppm of fluoride, 740 mg fluoride would be ingested, or twice the amount of the dry cow! Thus the NAS claim that a lactating cow, with very high demand of calcium release from bone tissue, can tolerate twice the amount of dietary fluoride compared to a dry cow with minimal demand on bone resorption is completely out of line.

KEY WORDS: Dairy cattle; Fluoride Intoxication; Fluoride tolerance standards; Milk Production.

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THE EFFECTS OF DIFFERENT FLUORIDE CONCENTRATIONS IN DRINKING WATER ON DENTAL FLUOROSIS AND DENTAL CARIES INCIDENCE AMONG 12-14 OLD ADOLESCENTS IN WUHAN

by

Wang Fuyuan et al
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(Abstracted from Chung-Hua Kou Chiang Ko Tsa Chi 21:20-22, 1986) [in Chinese]

The total intake of fluoride in 350 school boys and girls aged 12-14 in Wuhan was 1.106 mg/day in one group of 200 persons drinking East Lake water, and 0.618 mg/day in the other group of 150 persons drinking Yangtze River water. The fluoride content of East Lake water was 0.381 mg/L, Yangtze River water was 0.143 mg/L. The former is 2.6 times as much as the latter. Although the difference in dental caries incidence between the 2 groups was not statistically significant, the difference in the dental fluorosis and urine fluoride was statistically significant. Dental fluorosis in the population drinking East Lake water was severe and moderate. Therefore, in addition to increased intake of protein and calcium, adolescents should drink Yangtze River water instead of that from East Lake.

KEY WORDS: China; Dental caries; Dental fluorosis; F in water; F in urine.

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FLUORIDE, THE ENVIRONMENT, AND HUMAN HEALTH

by

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(Abstracted from Perspectives in Biology and Medicine 29:560-572, 1986)

In this review of the literature on fluoride, the author identifies important areas in need of further research: the pathogeneses of the osseous changes in skeletal fluorosis, the question of the lower limit for a toxic fluoride content of bone, the possible existence of preskeletal fluorosis in some individuals, and the precise mechanism by which dental fluorosis or "mottled enamel" is caused, whether it is the incorporation of fluoride in the hydroxyapatite lattice of the enamel or the presence of fluoride in the fluid environment of teeth that protects against caries.

Potential sources of fluoride emissions include industrial plants concerned with phosphoric acid and superphosphate production; aluminum smelters; foundries; glass, brick, and tile works; plastics and fluorinated hydrocarbon production; coal burning, both industrial and domestic. In 1971, in the United States alone, the estimated total fluoride emissions from major industrial and commercial operations was between 120,000 and 155,000 tons per year. In 1977, the Canadian National Research Council estimated the global figure to be around 500,000 tons per year.

Fluoride is the most phytotoxic of pollutants; it may cause injury to susceptible plant species at atmospheric concentrations (less than 1 ppb or about 0.8 µg fluoride/m^3). Depending on plant species and concentrations, hydrogen fluoride can be 10-1,000 times more harmful than sulphur dioxide. The application of 1,000 pounds of superphosphate fertilizer to an acre adds approximately 17.5 pounds of fluoride. Plants such as spinach appear to be fluoride accumulators. In Japan, fluoride content of some common foods rose markedly as a result of increased use of supersphosphate fertilizers. The fluoride content of pumpkins, green tea, watermelons, and lotus rhizomes increased 429 percent, 575 percent, 831 percent and 976 percent, respectively. Of all pollutants that affect farm animals, fluorides have caused the most severe and widespread damage; cattle are especially susceptible.

Individuals can ingest fluoride from a multiplicity of every day sources including water, foods, processed beverages, some medicines and dental health products, as well as certain pesticide and fertilizer residues. In addition, some people may inhale fluoride in the air they breathe. With so many possible sources of intake, it is difficult, if not impossible, to control the amount of fluoride individuals receive. In low-fluoride areas, fluoride intake from all sources is 2 mg or more per day; in areas with fluoridated water, 5 mg or more a day.

Fluoride retention is variable, not only among individuals and groups but also within individuals over time. Urinary excretion of fluoride is markedly reduced in patients with diminished renal function; children have lower renal clearance rates than adults.

Veterinarians, horticulturists, and environmental scientist are all aware that low concentrations of fluoride can damage livestock and vegetation. Biochemists, toxicologists, and clinical pharmacologists all recognize that fluoride is a potent enzyme poison. It has long been recognized that the earliest warning — and the most sensitive indication of overexposure to fluoride — is dental fluorosis.

Because the overall intake of fluoride has increased and because the average ionic plasma fluoride level of the population has risen, individuals who ingest submilligram amounts of fluoride run a greater risk of having their ionic plasma fluoride concentration peak above the threshold level, a situation that can precipitate dental fluorosis or other ill effects. Can people ingest increasing amounts of fluoride, from a growing number of everyday sources, with impunity? Because of its strong reactivity, the ever-increasing presence of fluoride in the environment could be far-reaching.

KEY WORDS: Environmental fluoride; Fluoride distribution; Health effects; Review.

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DENTAL FLUOROSIS IN AN AREA OF KENYA WITH 2 PPM FLUORIDE IN THE DRINKING WATER

by

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(Abstracted from J. Dent. Res. 65:659-662, 1986)

In a previous study of the prevalence and severity of enamel changes in populations living in a rural area of Kenya with water fluoride concentrations in the range 0.1-1.0 ppm, an unexpectedly high prevalence and severity of dental fluorosis was found.

In the current study a total of 102 children born and reared in an area of rural Kenya with 2 ppm fluoride in drinking water, the prevalence of dental fluorosis was 100%; in 95% of the children more than 50% of all teeth exhibited TFI* scores of 4 or higher, and 50% of the children had pitting or more severe enamel damage in at least half the teeth present. For more than half of their teeth about 5% of the children received TFI* scores from 7 to 9.

* TFI = Thylstrup and Fejerskov Index reported in 1978 "Clinical Appearance of Dental Fluorosis in Permanent Teeth in Relation to Histologic Changes," Community Dental Oral Epidemiol. 6:315-328.

The first molars were the most severely affected; from 70 to 80% had enamel destruction. On the other hand the lower incisors were the least affected; about 30% enamel destruction was recorded. However, about 50% of the upper central incisors showed loss of surface enamel to a varying extent (TFI scores, 5-9).

The children examined in the two studies originated from very different provinces of Kenya with respect to ecology, mean annual maximum air temperatures, and annual rainfall. Moreover, the children were from different ethnic groups. Nevertheless, the same tendency to higher prevalence and severity of dental fluorosis in relation to water fluoride concentration in both populations was observed. A larger proportion of the upper incisors exhibited post-eruptive changes than would be anticipated from other studies which is explained by the fact that fluorotic changes in the anterior teeth are cosmetically unacceptable to the present population: It was common to find subjects who had ground the buccal surfaces of their teeth to a varying extent. Exact and accurate classification of actual changes were very difficult and often tended to increase the scoring of severity.

The authors are in the process of further investigating the possible variables which may explain the unexpected susceptibility of various populations to the effects of low levels of fluoride.

KEY WORDS: Dental fluorosis; Fluorosis scores; Kenya; Waterborne fluoride.

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FLUORIDES AND FLUOROSIS IN KENYA
PART II: THE OCCURRENCE OF DENTAL AND SKELETAL FLUOROSIS

by

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(Abstracted from Odontostomatol. Trop. 9:71-74, 1986)

In 1949 water authorities called for "a correction of the water supplies" because of the high levels of fluoride in many parts of the country. Among the Masai, 23.7 per cent of those under 20 years of age had mottled enamel, whereas only 2 per cent of those above 20 were thus affected. Fluorosis of the deciduous teeth was also noted. Water sources contained between 1.8 and 3.5 ppm. Severe forms of dental fluorosis have been reported in nearly all provinces of Kenya. Fluorosis was more prevalent in children born and brought up in the area than among those living there for less than a year.

Among Kenya brewery workers and children age 6 to 16 years, Qureshe reported moderate to severe degree of fluorosis, not only in permanent teeth

but also in primary teeth. Clay pots reduced the fluoride concentration of water stored in them. They may remove about 80 mg of fluoride per kg of clay.

In Nairobi primary schools in 1984, 44.1 per cent had fluorosis of the permanent dentition, in 5.6 per cent fluorosis was severe enough to cause pitting of the enamel. This unexpectedly high prevalence of fluorosis in a city in which centralized water supplies contain about 0.1-0.4 ppm, requires further investigation.

In Kenya, anterior tibial deformities were described in nearly all the El Molo of Turkana above 5 years old. Water fluoride levels were about 8.0 ppm in drinking water. In some adults both anterior and lateral tibial bowing was observed. Fluoride may not have been the only contributory factor causing these deformities, although they were remarkably similar to those in Tanzania where no other major etiological factor was identified.

Clinical findings in osteofluorotic patients include pain in back, hips, knees, legs, weakness of legs, wasting, spinal stenosis, poker-back kyphosis, exostoses, as well as tibial and femoral bowing causing the conditions known as "genu valgum" and "genu varum." The brunt of the disease is taken up by the spinal column with calcification and ossification of the interosseous membranes, enlarged vertebral bodies, reduction in the antero-posterior diameter of the spinal canal, fusion of the vertebrae and narrowing of the intervertebral foramina.

Osteoporosity, also reported as characteristic of fluorosis, may be due to secondary hyperparathyroidism. Combinations of demineralization and osteosclerosis produce a spectrum of radiographic changes. Of 251 children under 16 years old in Arusha, 78 percent were afflicted with skeletal fluorosis and deformity of legs. Among 11 year old Tanzanian girls dental fluorosis may be related to skeletal maturity even at levels of 3.6 ppm fluoride; above this level fluoride may retard skeletal maturity.

KEY WORDS: Dental fluorosis; Genu valgum; Genu varum; Osteofluorosis; Osteoporosis; Osteosclerosis.

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CHANGING PATTERNS OF DENTAL CARIES: A SURVEY OF 20 COUNTRIES

by

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Singapore

(Abstracted from Ann. Acad. Med. 15:284-298, 1986)

In non-industrialized countries, Australia, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden, the United Kingdom and the USA, apparent reduction (30-50 percent) of dental caries in 5 to 12 year olds during the past decade appears substantial.

The contribution of improved dental health programs, other than those involving fluoride, could not be adequately assessed.

Inevitably in developed countries along with reduction in dental caries need for dental services and hence the need for dental personnel will decrease. However, the lack of adequate data in most countries makes predictions of future changes in oral health and manpower needs a precarious procedure. Regular monitoring of oral health status in all countries for better personnel planning and production is urgently needed.

KEY WORDS: Caries reduction; Changing caries patterns; Dental personnel needs.

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DENTAL FLUOROSIS DEVELOPED IN POST-SECRETORY ENAMEL

by

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(Abstracted from J. Dent. Res. 65:1406-1409, 1986)

The present controlled study on domestic pigs was designed to determine whether fluoride affects the secretory or the post-secretory (maturation) phases of enamel formation, and whether fluorotic enamel lesions could be produced in porcine fourth pre-molars by administration of fluoride during only the maturation phase of enamel development.

The enamel of pre-molar (P_4) teeth from all the animals in the fluoride group was chalky white and more opaque than was the P_4 enamel from any of the control animals which exhibited normal translucency. The chalky white opacity of the enamel from the fluoride-exposed animals extended over the

entire surface of the crowns of the P_4 teeth in all specimens. Similarly, microradiographs of all P_4 teeth from the fluoride group revealed a sub-surface zone of hypomineralized tissue extending in a band over the enamel of the entire crown. None of the teeth from control animals exhibited this sub-surface zone hypomineralization.

The clinical implications of the present study on pigs are shown by the results of a recent study of prevalence of enamel fluorosis in children who had begun to receive fluoride tablets at various ages. This human study showed that enamel fluorosis can be produced by commencing fluoride administration after the age at which the whole of the form of the tooth crown is completed. Further studies of the pathogenesis of dental fluorosis should be directed toward effects of fluoride on the processes which occur during the maturation phase of enamel.

KEY WORDS: Dental fluorosis; Enamel formation; Pigs.

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SUPERFICIAL FLUORIDE LEVELS AND RESPONSE TO IN-VITRO CARIES-LIKE LESION INDUCTION OF ENAMEL FROM BRISTOL (U.K.) AND BIRMINGHAM (U.K.) HUMAN DECIDUOUS TEETH

by

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(Abstracted from Arch. Oral Biol. 31:201-204, 1986)

Surface enamel fluoride levels were measured in deciduous canine teeth from Bristol with non-fluoridated water supplies, and Birmingham, with fluoridated water supplies. Three populations studied were from Bristol (teeth shed before 1960), Bristol (after 1975) and Birmingham (after 1975). Up to 75 μm from the enamel surface, fluoride concentrations of post-1975 Bristol and Birmingham teeth were, respectively, x1.3 and 3.4 greater than those of pre-1960 Bristol teeth. The increase in the Bristol teeth is presumably due mainly to the increased use of fluoride-containing toothpastes, that in Birmingham to fluoridated water plus the use of fluoride toothpastes. No difference in the rates of penetration into the enamel of acid-gel induced, caries-like lesions were found between the two Bristol populations; Birmingham teeth showed a reduction of 10 per cent in penetration rate. It is suggested that raised fluoride levels in surface enamel do not reduce solubility sufficiently to account, by themselves, for the recent nationwide marked reduction in caries in children. Possibly, the raising of plaque fluoride levels is a more important factor, affecting demineralization, remineralization and bacterial activity.

KEY WORDS: Birmingham, Bristol, (U.K.); Enamel fluorosis; Enamel lesions.

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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.

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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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